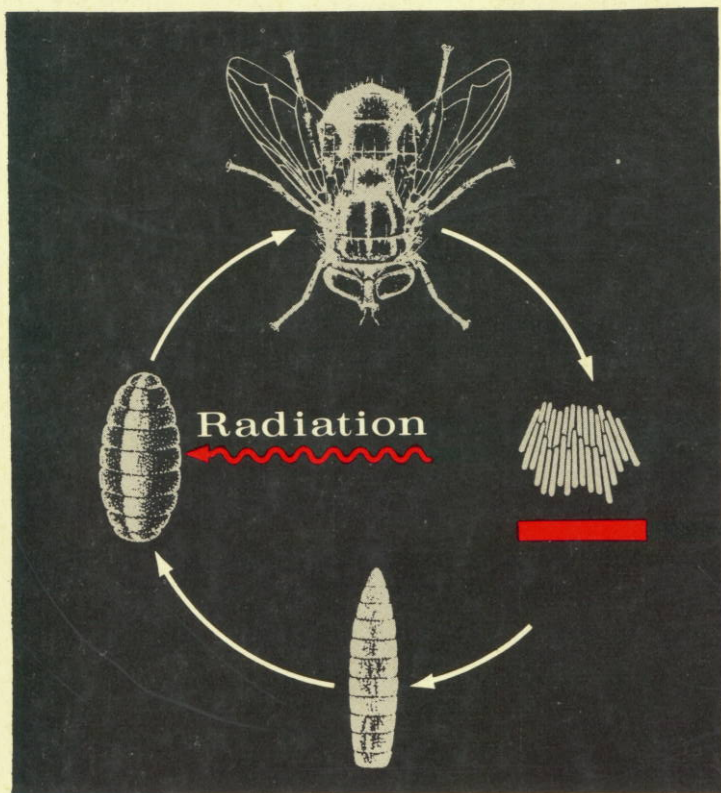


**Bibliographical Series No.36**



**Radioisotopes  
and Ionizing Radiations  
in Entomology**  
**Vol.IV (1966-1967)**



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1969

**RADIOISOTOPES AND IONIZING RADIATIONS  
IN ENTOMOLOGY  
Vol. IV  
(1966-1967)**

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STI/PUB/21/36

## FOREWORD

The present bibliography on Radioisotopes and Ionizing Radiations in Entomology represents the fourth and probably final volume of a series which covers the years 1950 to 1967:

1950-1960	11 years	1577 references
1961-1963	3 years	1585 references
1964-1965	2 years	1074 references
1966-1967	2 years	1800 references

The first three of these volumes were published as Bibliographical Series Nos 9, 15 and 24, respectively. The figures for references indicate the dramatic and continuing increase in published work in this field and explain why it is unlikely that the IAEA will continue this documentation service.

The bibliography is again fully annotated, and abstracts have been included wherever possible. The bibliography is organized in such a way as to permit several different approaches to the individual references. In the first place, the references are grouped together according to a broad classification scheme of different areas of work. Within each section or sub-section, they are listed in alphabetical order of authors. There is a detailed Subject Index which identifies the radioisotopes or radiations used in the particular study cited; a taxonomic index for insects and related arthropods; and special additional tables to identify insecticides studied with radiotracers, both by their chemical and by their proprietary names, with an indication of the system (insect, plant, etc.) used. Finally, there is an Author Index showing each author's affiliation (with date).

The documentation will be of practical use to the specialist requiring a rapid survey of relevant publications in related disciplines, to someone in search of detailed documentation on a particular aspect of the field, and to the scientist in developing countries whose access to the world literature might be somewhat limited.

The bibliography was compiled by Mrs. M. BINGGELI of the Agency's Division of Scientific and Technical Information.

Readers are invited to address their suggestions and other correspondence regarding the "Bibliographical Series" to: The Director, Division of Scientific and Technical Information, International Atomic Energy Agency, Kärrntner Ring 11, P.O. Box 590, A-1011 Vienna, Austria.



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## INTRODUCTION

### COMPILATION OF BIBLIOGRAPHY AND GUIDE FOR ITS USE

#### SOURCES

The bibliography was compiled from the open literature.<sup>1</sup> A first routine search involved scanning selected secondary sources:

##### (a) Abstracting journals

Biological Abstracts (BA)  
Bulletin Bibliographique:  
    Isotopes. Rayonnements. Agriculture.<sup>2</sup> (BB)  
Chemical Abstracts (CA)  
Dissertation Abstracts (DA)  
Nuclear Science Abstracts (NSA)  
Review of Applied Entomology  
    series A: Agriculture (RAE-A)  
    series B: Medicine (RAE-B)

##### (b) Title listings

Atomindex<sup>3</sup> (STI/Doc/12, fortnightly) (LOR)  
Bibliography of Agriculture (BAg)  
Biological and Agricultural Index (AI)  
Bulletin Signalétique Hebdomadaire (BS)  
    - Périodique de Chimie<sup>4</sup>  
Current Contents  
    Chemical, Pharmaco-Medical &  
    Life Sciences<sup>4</sup> (CC)  
Pesticide Documentation Bulletin (PDB)

Subsequently, primary sources were scanned, abstracts being prepared where necessary.<sup>5</sup> Otherwise, sources of abstracts are indicated. References cited in original papers were followed up, this method of documentation search proving particularly productive. Numerous books, conference proceedings, bibliographies, and reports were also scanned, including such review series as A. Rev. Ent. 11 (1966) and 12 (1967) (Smith, R. F.,

<sup>1</sup> With the addition of some title citations for reports.

<sup>2</sup> Includes keywords but no abstracts.

<sup>3</sup> List of references to current literature, including numerous reports, received routinely by the International Atomic Energy Agency.

<sup>4</sup> Lists contents of a variety of journals.

<sup>5</sup> Either because no abstract was available or because the existing abstract proved not sufficiently informative on the technical aspects stressed by the bibliography.

Mittler, T.E., Eds), *Adv. Insect Physiol.* 4 (1967) (Beament, J.W.L., Treherne, J.E., Wigglesworth, V.B., Eds), *Residue Rev.* (Gunther, F.A., Ed.), also periodic bibliographies in *Anz. Schädlingssk.*, *Drosoph. Inf. Serv.*, *Fd Irrad.*, *Entomophaga*, *Mosquito News*, *Pesticide Prog.*, and others.

Papers presented at meetings and published as abstracts only have been cited, usually in toto, since they often give enough information for the interested reader to decide whether he wishes to write to the author; in such cases, he can find the author's address in the Affiliation Index.

Among the selected journals scanned routinely are:

Am. J. trop. Med. Hyg.	Isotopenpraxis
Ann. appl. Biol.	Jap. J. appl. Ent. Zool. (Nihon Oyo Dobutsu)
Ann. ent. Soc. Am.	Kouchugaku Zasshi
Anz. Schädlingssk.	Jap. J. Genet. (Idengaku Zasshi)
Appl. Ent. Zool.	J. agric. Fd Chem.
Archs Biochem. Biophys.	J. Ass. off. analyt. Chem.
Atompraxis	J. biol. Chem.
Aust. J. biol. Sci.	J. Cell Biol.
Biochem. J.	J. econ. Ent.
Biochim. biophys. Acta	J. exp. Biol.
Biol. Zbl.	J. Hered.
Bull. ent. Soc. Am.	J. Insect Physiol.
Bull. ent. Res.	J. Invertebrate Path.
Can. J. Biochem.	J. med. Ent.
Chromosoma	J. molec. Biol.
C. r. hebdom. Séanc. Acad. Sci. D	J. Sci. Fd Agric.
Dokl. Akad. Nauk SSSR - Biological Section	Kernenergie
Drosoph. Inf. Serv.	Life Sci.
Ecology	Mosquito News
Entomologia exp. appl.	Mutation Res.
Ent. Rev. (AEC-tr-Russian)	Nature, Lond.
Experientia	Naturwissenschaften
Expl Cell Res.	Nucleonics
Fd Irrad.	Pesticide Prog.
Genetics	Proc. ent. Soc. Ont.
Genetika	Radiat. Res.
Hereditas	Radiobiologiya (Radiobiology, AEC-tr-)
Insectes soc.	Science, N. Y.
Int. J. appl. Radiat. Isotopes	Z. indukt. Abstamm. - u. Verh. Lehre
Int. J. Radiat. Biol.	

The omission of an abstract for a given reference may be due to one of the following reasons:

(1) the reference had originally been obtained from a title listing or from a citation in another publication, and the compiler had not been able to obtain the original article;

(2) the original article had appeared in interim form intended only for limited circulation, and permission could not be obtained for quoting details; or

(3) the original articles were descriptions of projects or progress reports which would largely be outdated by the time the bibliography appears in print, much of the results having been published in the literature by then.

Such references have, nevertheless, been included as title citations and appropriately indexed since some readers might wish to follow them up by establishing direct contact with the scientists concerned; again the addresses of the authors can be obtained from the Author Index.

## Reports

Numerous reports have been abstracted, and may be considered valuable as indicative of trends in a particular field or institution. The user is thus enabled to contact a particular scientist, even if no conventional publication is (yet) available.

## REFERENCES

References are arranged by subject matter as set out in the table of Contents. Articles reviewing the particular subject or of a general or introductory nature are placed at the beginning of a section. Such reviews are more specific than those reported in the Addendum (3.2. Bibliographies and General Surveys), where broad fields are surveyed.

Where new data (e.g. abstracts) have been obtained on references included in Vols I - III, their original number, preceded by the volume number, are also indicated; an abstract may thus be added to a reference originally cited by title only, e.g. reference 1146 had been cited as reference 1038, without abstract, in Vol. III. This fact is indicated by 1146 = III/1038.

## Cross-References

These are cited at the end of each section, giving the title (in English only), first author, year of publication, and the reference number. Despite the very detailed Subject Index, cross-referencing has been used extensively.

## ADDENDUM

### Techniques

Some selected papers representative of particular techniques (e.g. autoradiography, neutron activation analysis, etc.) have been grouped together.

## TABLES

Three tables have been compiled.

### Table 1. Systematic Listing of Insects and Related Arthropods

The insects and related arthropods cited in the bibliography are grouped together systematically by order, family, genus, and common name.<sup>6</sup> Their place in this table is also indicated in the Subject Index where the systematic code appears next to the scientific name.<sup>7</sup>

### Table 2. Radiotracer Studies on Insecticides

This table gives a digest of radioisotope tracer studies on insecticides. Chemical names and other designations are indicated throughout. The parti-

<sup>6</sup> Largely based on the scientific and common names listed in Bull. ent. Soc. Am. 11, 4 (1965).

<sup>7</sup> Traceable via the common name, if the scientific name is not known.

cular radioisotope used in an analysis or synthesis as well as the animal, plant or particular substrate used in metabolic and residue studies are given. The insecticides have been grouped in certain broad categories, also used in the two Insecticide Indexes.

Table 3. Some Insect and Related Arthropod Pests:  
Guide to Colonization and Mass Rearing

Various people had suggested to the compiler that, in view of the increasing importance of the sterile-male technique for insect control, it would be useful to list the laboratories with experience in the colonization and mass rearing of insects. Although it was not possible to carry out the survey that would be needed in order to produce a comprehensive list, a recently published book does, in fact, provide much useful information. The book is entitled "Insect Colonization and Mass Production" and is noted in the bibliography as reference APX. 11 in the Appendix. No attempt has been made to collect information beyond the period or areas covered there, but a table has been compiled as a guide to the rearing of various types of insect and related arthropod pests, with indications of their common and scientific names, taxonomic code, and some specialists in the particular field with affiliations.

## INDEXES

### Insecticide Indexes

To facilitate checking of an insecticide, e. g. where a synonym has been used, the following two indexes have been compiled:

- (i) Common and Manufacturers' Names Index
- (ii) Letter and Number Index

to be used in conjunction with Table 2.

### Author Index

1. A Corporate Author Index has been compiled.
2. Personal Author and Affiliation Index.

As far as possible, up-to-date affiliations have been indicated for each author. Sole or first authors, in cases of joint authorship, have been indicated by underlining the pertinent reference number.

### Subject Index

A detailed Subject Index is provided. The radioisotopes or radiations used are indicated for each reference. The following convention has been adopted concerning the position in which they are cited with regard to a particular study: When the radioisotopes or radiations represent the cause they precede the phenomenon reported; when used as a tool for analysing existing conditions they follow the phenomenon studied. Irradiation, when combined with other modifying treatments (gaseous environment or temperature at irradiation, etc.) is quoted as radiation/treatment. When a comparison

between radiation and other treatments (e.g. chemosterilant) is made, a comma is used to indicate this, e.g. x, tepa: housefly. Further illustrations are given on page 687. Life stages or tissues relevant to a particular study are indicated, in brackets, whenever possible. A considerable amount of information may thus be obtained at a glance.

## GLOSSARY\*

$\alpha$	alpha radiation
(a)	adult
AET	aminoethylisothiuronium bromide hydrobromide
ATP	adenosine triphosphate
ATPase	adenosine triphosphate phosphohydrolase
att	attached
$\beta$	beta radiation
(c)	cocoon
CNS	central nervous system
(d)	diapause
(dpp)	diapausing pupa
(Dp)	Dauerpupa
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
dopa	$\beta$ -(3,4-dihydroxyphenyl)-L-alanine
DPN <sup>+</sup>	diphosphopyridine nucleotide
e	electrons
(e)	egg
EDTA	ethylenediamine tetraacetate
EM	electron microscop(y) (ical findings)
(emb)	embryo
e. m.	electromagnetic fields
e. s.	electrostatic fields
(h)	hypopial stage (mites)
ir	infrared
i. v.	intravenous
(l)	larva
(l5)	5th larval instar
LD	lethal dose
(m)	metamorphosis
NA	nucleic acid(s)
n	neutrons
(n)	nymph
n <sub>f</sub>	fast neutrons
	[when energy is specified suffix is dropped]
n <sub>s</sub>	slow neutrons
NADPH <sub>2</sub>	nicotinamide adenine dinucleotide phosphate
n. d.	non-dated
<u>OP</u>	organophosph(orus) (ate)
	[underlined to avoid confusion with elements]
P <sub>i</sub>	inorganic phosphorus
p	protons

\* Excluding chemical elements.

(p)	pupa
(dp)	diapausing -
(Dp)	Dauer -
(pp)	pre -
R-	resistant (strain)
RBE	relative biological effectiveness
Rev.	Review
RF	radio frequency
r.i.	radioisotopes (non-specified)
RNA	ribonucleic acid
mRNA	messenger RNA
tRNA	transfer RNA
RNase	ribonuclease
S-	susceptible (strain)
SMT	sterile male technique
T	temperature
TPN <sup>+</sup>	triphosphopyridine nucleotide (oxidized)
uv	ultraviolet
I/ 120	Vol.I, reference 120
III/ 76	Vol.III, reference 76

Note: The following symbols may occur in the Subject Index:

IV	Roman numbers in connection with genetic effects indicate "chromosome, number IV".
QQ.2	After scientific name of insect: <u>systematic code</u> , referring to appropriate position in Table 1.
[PA.3]	After chemical compound: <u>insecticide code</u> , referring to appropriate position in Table 2.

#### ABBREVIATIONS

(Bibliographical Abbreviations for Abstract Sources)

AI	Biological and Agricultural Index
BA	Biological Abstracts
BAG	Bibliography of Agriculture
BB	Bulletin Bibliographique. Isotopes. Rayonnements. Agriculture.
BS	Bulletin Signalétique Hebdomadaire. Périodiques de Chimie.
CA	Chemical Abstracts
CC	Current Contents
DA	Dissertation Abstracts
LOR	Atomindex
NSA	Nuclear Science Abstracts
NSA/J	Nuclear Science Abstracts Japan
PDB	Pesticide Documentation Bulletin
RAE	Review of Applied Entomology

#### ACKNOWLEDGEMENTS

The compiler (MB) is much indebted to Dr. D.L. Lindquist, of the Joint FAO/IAEA Division of Atomic Energy in Agriculture, for helpful discussions on insecticides.

# 1. RADIOISOTOPES

## 1.1. INSECT LABELLING

### 1.1.1. Methods

- 1 Anonymous. THE RADIOACTIVE GRASSHOPPER. Int. atom. Energy Ag. Bull. E, 2 (1966) 14-16.  
"--- They are fed on young wheat containing  $^{192}\text{Ir}$ ; the radioactivity taken up by the grasshoppers can then be observed by a portable scintillation counter. Laboratory tests have shown the biological period of the  $^{192}\text{Ir}$  to be  $\sim 7\text{d}$ , and that  $\sim 1\ \mu\text{Ci/insect}$  is needed to enable them to be traced during two months".
- 2 Arroyo, M., Jiménez, A., Mellado, L., Caballero, F. APLICACION DE ISOTOPOS RADIATIVOS A LA INVESTIGACION DE METODOS SOBRE LUCHA BIOLOGICA CONTRA LAS PLAGAS. I. ENSAYOS SOBRE MARCADO DE ADULTOS DE Ceratitis capitata Wied., CON P-32. (Application of radioactive isotopes to the investigation of methods for the biological control of pests. I. Tests on marking adults of C. capitata Wied. with  $^{32}\text{P}$ .) Boln Patol. veg. Ent. agric. 28 (1965) 233-240. (In Spanish)  
  
The effects of supplying adults of C. capitata (Wied.) with radioactive phosphorus in their diet are described. Newly emerged individuals were given mashed banana, renewed every 2 d, into which  $^{32}\text{P}$  had been incorporated at either 0.01 or 0.1 mCi per 5 g. The eggs laid were collected daily, and the larvae hatching from them were reared on mashed carrot. The  $^{32}\text{P}$  had no influence on the mortality of the flies but appeared to stimulate oviposition, especially at the lower rate, though this may have been due to the addition of phosphorus to the diet and not to the radioactivity. The percentages of the total radioactivity supplied to the adults that could be detected in the eggs were 2.3 and 1.03 for the two rates, respectively. Screening the pupae derived from the two groups of eggs showed that they retained 82.8 and 44.6%, respectively, of the radioactivity present in the eggs; the corresponding percentages for the adults were 46.5 and 34.2. Adults derived from eggs laid 9 or 10 d after the  $^{32}\text{P}$  had first been supplied to the parent flies were paired with each other and with adults derived from material to which no  $^{32}\text{P}$  had been supplied, in all possible combinations, three males being confined with five females in each instance. Increased oviposition (as compared with the completely non-radioactive series) was observed when one sex (either male or female) was derived from the group supplied with the lower concentration and the other was from the untreated series, but when both sexes were of this group or one or both sexes were from the group supplied with the higher rate, there was a reduction in oviposition. Virtually no radioactivity was detectable in the eggs laid. (From RAE-A 55: 1967, ref. 252)
- 3 Arroyo, M., Jiménez, A., Mellado, L., Caballero, F. APLICACION DE ISOTOPOS RADIATIVOS A LA INVESTIGACION DE METODOS SOBRE LUCHA BIOLOGICA CONTRA LAS PLAGAS. II. ENSAYOS DE MARCADO DE LARVAS DE Ceratitis capitata Wied. CON P-32. (Application of radioactive isotopes to the investigation of methods for the biological control of pests. II. Tests on marking larvae of C. capitata with  $^{32}\text{P}$ .) Boln Patol. veg. Ent. agric. 28 (1965) 241-256. (In Spanish)  
  
Larvae of C. capitata in order to obtain labelled adults for studies on dispersion. Eggs were placed on mashed carrot containing 0.2 mCi  $^{32}\text{P}$  per 15 g. The larvae hatched in 2-3 d, and nutritive solution was added daily from the 5th day, which somewhat reduced the radioactivity of the medium. The first pupae were formed on the seventh day. As a result of the progressive decrease in the radioactivity of the medium, the pupae formed on the 12th day were only half as radioactive as those formed on the 7th-9th. Adults were obtained from the 19th day and were supplied with food without  $^{32}\text{P}$ . Their radioactivity was measured on the 19th, 22nd, 24th and 29th days, and it is concluded from the results that by this technique adults can be obtained that are radioactive to a measurable

degree (in the order of 0.01  $\mu\text{Ci}$  per adult) more than three weeks after the eggs are placed on the radioactive medium. Over 80% of the pupae gave rise to adults. Labelled adults could be distinguished easily and rapidly from normal ones in catches in trap-jars containing 2% ammonium phosphate and examined 6-8 d later, even when the contents of the traps had been filtered and stored for two days in test-tubes and the 'normal' adults had become very slightly radioactive as a result of contact with the labelled ones. When further tests were carried out with larvae using the same technique and rates of 0.4, 2 or 20 mCi.  $^{32}\text{P}$  per 15 g food, no pupae were obtained with the highest rate and adults were obtained only with the lowest. When these adults were paired each way with normal adults or with each other, the number of eggs laid was lower than that obtained from normal adults if at least one of the sexes was from the labelled group. As the increase in oviposition obtained by labelling adults (as noted in the first paper) does not appear to occur when the larvae have been supplied with 0.4 mCi, this is considered a safe rate for labelling material for field release. Although pupae were formed when rates of 0.5, 0.6, 1 or 1.2 mCi per 15 g were used, no adults were obtained. Inconclusive results were given in a preliminary experiment to study the distances covered by individuals released in the field, but the radioactivity of the flies recaptured after 8 d was readily detected. (From RAE-A 55; 1967, ref. 253)

- 4 Baldwin, W. F., Allen, J. R., Slater, N. S. A PRACTICAL FIELD METHOD FOR THE RECOVERY OF BLACKFLIES LABELLED WITH PHOSPHORUS-32. Nature, Lond. **212** (1966) 959-60.

The report describes improved methods for tagging blackflies (Diptera: Simuliidae) in the larval and pupal stages, the use of a new trap for collecting large numbers of adults, and an autoradiographic technique for identifying radioactive individuals. A concentration of 1.2  $\mu\text{Ci}$   $^{32}\text{P}$ /ml water was used for labelling. An estimated total of 260 000 larvae and pupae were labelled. Two methods of detecting radioactive flies are described; 37 and 129 flies were detected by Marconi scaler and autoradiographs respectively. Adults giving < 10 cpm above background could be detected. The results at individual traps are tabulated, for a period of 2 weeks after treatment. The largest numbers of radioactive flies were captured in traps immediately above the tagging site, confirming visual observations that emerging flies tended to move upstream.

- 5 Chigarev, G. A. THE PRESENT STATE OF THE PROTECTION OF POTATO AGAINST THE COLORADO BEETLE IN THE USSR AND THE PROBLEMS INVOLVED. Trudy vses. Inst. Zashch. Rast. **17** (1963) 324-343. (In Russian, with English summary)

Mention is made in the report of experiments in which  $^{60}\text{Co}$ -labelled adults were released in the field in the spring of 1960. The numbers were counted on the following day. Only 67% were recovered in three repeats, showing the ineffectiveness of visual inspection.

- 6 Dow, R. P. RADIOACTIVE YEAST FOR TAGGING MOSQUITOES. Bull. ent. Soc. Am. **13**, 3 (1967) 202. Abstr. 331. "New York Meeting of the Entomological Society of America. New York, N. Y., USA, 27-30 Nov. 1967".

Radioactive yeast, prepared by fermenting dried yeast with sugar and  $^{32}\text{P}$ , marked *Culex nigripalpis* mosquito larvae more uniformly and over four times as efficiently as the usual inorganic phosphate. Females so marked showed no reduction of egg production or hatchability at levels near 0.02  $\mu\text{Ci}$ .

- 7 Espinola, H. N., Silva, J. E., da. TAGGING SANDFLIES WITH  $^{32}\text{P}$  (DIPTERA, PSYCHODIDAE). Revta bras. Biol. **25** (1965) 295-304.

A technique was developed for tagging sandflies with  $^{32}\text{P}$  that could be used for studies of the ethology, biology, and ecology of these Psychodidae. Adults of *Lutzomyia longipalpis* (*Phlebotomus longipalpis*) were tagged with  $^{32}\text{P}$  in the form of a  $\text{Na}_2\text{H}^{32}\text{PO}_4$  solution added to the culture media. Two methods were tested for this purpose. The first consisted in tagging the adult sandflies through the 4th instar larvae (prepupal stage) by culture media containing 160, 80, 40, 20, 10, 5, and 1  $\mu\text{Ci/g}$  of  $^{32}\text{P}$ . The second consisted in obtaining tagged adult sandflies by rearing them in the radioactive media from the egg stage. Since the insects in this group were expected to remain in the radioactive media for a longer time, the  $^{32}\text{P}$  doses were reduced to 48, 24, 12, 6, 3, 1.5, and 0.3  $\mu\text{Ci/g}$ . Differences were found between the male and female counts that could be correlated with the inequality of weight and volume between the sexes. The  $^{32}\text{P}$  radiation apparently did not cause any injury, either in morphology or in sandfly viability. The results of tagging adults starting from the eggs suggested that this method was preferable to the first, in view of the homogeneity of the counting results and

because it avoided transfer of larvae from one medium to another. The differences found between the counts of the first and the last sandflies to emerge were probably due to  $^{32}\text{P}$  decay. (From auth. summary)

- 8 Garby, L., Yasuno, M., Phurivethaya, Y. LABELLING MOSQUITOES WITH RADIO-ACTIVE IODINE,  $^{131}\text{I}$ . Trans. R. Soc. trop. Med. Hyg. 60, 1 (1966) 136.

Radioactive isotopes have been used to label insects for studies of their flight range and dispersal rates.  $^{32}\text{P}$ , a  $\beta$ -emitter, has been most commonly employed, but its detection in insects is time-consuming and complicated. The authors report the successful use of  $^{131}\text{I}$ , a  $\gamma$ -emitter, for labelling Culex pipiens fatigans. (From NM)

9. Hickman, C., Gates, J., Edmonds, E. FLY TAGGING: A NEW APPROACH. J. envir. Hlth 28 (1966) 277-282.

In December, 1963, a radioactive materials license was procured for 5 mCi of  $^{32}\text{P}$  (at any one time) "to be used for the isotopic labelling of flies in manure piles on dairy and chicken ranches in Orange County in a study of fly propagation and dispersion". The procedures consisted of three phases. Phase 1 - Laboratory: A. Determine the optimum amount of  $^{32}\text{P}$  to be ingested by larvae (min. - undetectable, max. - lethal), B. Determine the best laboratory media, Phase 2 - Field Experiments: A. Release and capture of laboratory flies to verify identification of laboratory flies, B. Tag larvae on chicken ranches to determine if laboratory tests were reproducible under field conditions; Phase 3 - Legal interpretations: A. Determine if intended procedures are legal, B. Determine admissibility of tagged flies as court evidence. Tagging of Musca domestica larvae for identification of fly breeding sites has produced better-than-expected results. The laboratory portion of the programme was very successful. The field programme can probably be improved by higher concentration of radioactive material, higher larvae concentration, and closer observation of the fly tapes. The experiment will not be complete until a favourable court decision is made using fly tagging as evidence.

- 10 Hickman, C., Gates, J., Edmonds, E. RADIOACTIVE FLY TAGGING. Pest Control 35, 4 (1967) 20-22.

Tagging (larvae) with  $^{32}\text{P}$  was used to pinpoint breeding sites of Musca domestica L. in Orange County. Criteria for legal proof of breeding site to permit prosecution have not been established.

- 11 James, H. G. LOCATION OF UNIVOLTINE Aedes EGGS IN WOODLAND POOL AREAS AND EXPERIMENTAL EXPOSURE TO PREDATORS. Mosquito News 26, (1966) 59-63.

$^{32}\text{P}$  was used as tagging agent to determine whether aedine eggs are subject to predation in the field. Two series of experiments were carried out; in June 1959 activated eggs of Aedes aegypti (L.) were tested, in June 1960 eggs of A. trichurus. In the 1st experiment ~100 000 eggs were immersed in a solution of 250 mCi  $^{32}\text{P}$ /ml for 4 d at 2°C until individual eggs had reached 500 cpm. 1300 activated eggs were then put out in lots of 100 at random near pool margins. After 24 h insects and other arthropods were collected within the area and tested. In general eggs tended to be deposited by the mosquitoes at or near the margins rather on pool bottoms than that had little or no plant cover. Few eggs were present in soil above the high water contours though these were often well covered with vegetation subsequently. Predator feeding potential was tested in the laboratory. Adults of the beetle Bembidion frontale disposed of 15.7 of A. aegypti per day compared to 15.3 by B. muscicola and 5.1 by the ant Myrmica 1. fratricornis. Species of Bembidion may therefore be more important than ants as predators.

- 12 Lee, W.R. FEEDING RADIOACTIVE ISOTOPES TO SPECIFIC LARVAL STAGES OF Drosophila melanogaster. Drosoph. Inf. Serv. 40 (1965) 101.

For a number of reasons it has proved desirable to pulse label ( $^{32}\text{P}$ ) larvae during the first 24 h of the 3rd instar. The method is described in detail. Larvae are allowed to feed on labelled yeast for 24 h. Radioautographs of sperm from females inseminated by males treated in the manner described in the paper with either  $^{32}\text{P}$  or  $^3\text{H}$ -thymidine showed heavy uniform labelling during the first 3 d of mating yet the male germ cells had been subjected to radiation from radioactive media for only 1 d and during the relatively insensitive spermatogonial stage.

- 13 Peleg, B. A., Nadel, D. J. A METHOD OF  $^{32}\text{P}$  LABELING OF THE ARMORED AND SOFT SCALE PREDATOR *Chilocorus bipustulatus* L. *Kivim* 36, 2 (1986).

Citrus fruits infested with immature (white-cap stage only) Egyptian black scale, *Chrysomphalus aonidum* (L.) (Diaspididae), were briefly dipped in a  $^{32}\text{P}$ -solution (as orthophosphate in an isotonic solution containing phosphate buffer with pH 7, and a phosphorus content of 1 mg/ml). Only in this initial stage of its development will the total scale, dorsal armour and all, be consumed in great quantities daily by the lady beetle, *C. bipustulatus* L. (Coleoptera: Coccinellidae). The beetle is thus effectively marked. (A less permanent marking was achieved when radioactive material adhered to the beetle body.) The beetles were exposed to scale-infested fruit which had been dipped in  $^{32}\text{P}$ -solution, with a specific activity of 0.01 mCi/ml and 0.05 mCi/ml for 48 and 96 h. Feeding upon  $^{32}\text{P}$ -contaminated young scales did not affect beetle longevity, fecundity or egg viability. An effective half-life of 4.5 to 5.0 d was found in beetles fed on young scales treated with 0.01 mCi/ml, and 5.5 d when a 0.05 mCi/ml solution had been used. Most normal field studies could thus be carried out. With slight adaptations the method should prove suitable for labelling most scale insect predators.

- 14 Raimundo, A. C., Santos, L. R. COMBATE AOS INSETOS. 1. A MARCAÇÃO DA MOSCA DA FRUTA (*Ceratitis capitata* Wied.) COM FOSFORO RADIOACTIVO NO METODO DOS MACHOS ESTERILIZADOS. (Insect control. 1. Tagging the fruit fly *Ceratitis capitata* Wied. with radioactive phosphorus for the sterile male technique.) *Estudos agron.* 6, 3 (1965) 105-112. (In Portuguese)

In a preliminary study concerned with population analysis prior to attempting eradication of the sterile male technique, tagging was effected by  $^{32}\text{P}$  mixed added to the larval medium. Four different concentrations (0.1, 0.3, 0.5 and 1  $\mu\text{Ci/g}$ ) were used.

- 15 Rieffel, S. M., Crouse, H. V. THE ELIMINATION AND DIFFERENTIATION OF CHROMOSOMES IN THE GERM LINE OF *Sciara*. *Chromosoma* 19 (1966) 231-276.

The germ line chromosomes of *S. coprophila* have been followed from the time of origin of the germ cells up to the time of meiosis in the male and up to first larval moult in the female. The mechanism which prevents the accumulation of L (limited) chromosomes in the germ line is a unique process of chromosome elimination; it occurs in male and female embryos after the germ cells have migrated from the pole plasm to the definitive gonad site, and it involves the movement of whole L-chromosomes through the nuclear membrane into the cytoplasm. The extra paternal X-chromosome is eliminated from the germ cells at the same time and in the same manner. Following this elimination there is a cytological differentiation of the chromosomes remaining inside the nucleus. First, the four paternal homologues of the regular complement undergo a loosening of coils and become light-staining whereas the maternal homologues remain condensed like the L's. Next, the L-chromosomes undergo a process of extreme attenuation and dispersion following which they return to the condensed state.  $^3\text{H}$ -thymidine autoradiography\* on gonial and premeiotic cells in the testis reveals that the L-chromosomes undergo DNA replication at the end of the S period, also that there are asynchronies in DNA synthesis among the regular chromosomes. The phenomena of differential chromosome staining and asynchronous DNA replication are considered in the light of current theory regarding heterochromatinization and gene inactivation, also in relation to the phenomenon of chromosome imprinting encountered in this genus.

\* In early experiments late 4th-instar larvae or young prepupae were injected with a solution containing 50  $\mu\text{Ci/ml}$   $^3\text{H}$ -thymidine (specific activity 1.88 Ci/mM), and kept until mid-pupal life when spermatogenesis occurred and the testes were dissected out. Because of high mortality following injection, later experiments used gonads given a 30 min exposure in a drop of tissue culture medium containing 10  $\mu\text{Ci/ml}$   $^3\text{H}$ -thymidine (specific activity 5 Ci/mM).

- 16 Ritossa, F. M., Atwood, K. C., Spiegelman, S. A MOLECULAR EXPLANATION OF THE BOBBED MUTANTS OF *Drosophila* AS PARTIAL DEFICIENCIES OF "RIBOSOMAL" DNA. *Genetics* 54 (1966) 819-834.

A high level of labelling was achieved by modifying the standard medium and adding  $^3\text{H}$ -uridine. Eggs were labelled. Final preparations of pure ribosomal RNA had a specific activity ranging from 60 000 - 110 000 cpm/ $\mu\text{g}$ . All counting was done in a liquid scintillation counter on nitrocellulose membrane, permitting assay of  $^{32}\text{P}$  and  $^3\text{H}$  in the same sample. The proportion of DNA complementary to ribosomal RNA was estimated by means of annealing experiments in four bobbed (bb) mutants of independent

origin in D. melanogaster. All were found to be partially deficient in this DNA. These experiments, together with other evidence concerning the localization of this DNA and the characteristic of bb mutants, have led the authors to offer the conjecture that the bb locus is the nucleolus organizer and that the typical genetic basis of bb is partial deletion of the nucleolus organizer.

- 17 Samostrel'skiĭ, A. I. et al. EXPERIMENT ON THE USE OF RADIOACTIVE ISOTOPES TO MARK Onithodoros papillipes TICKS. Trudy Inst. Ėpidem. Pastera 29 (1966) 86-89.  
Ticks can be labelled with radioactive isotopes from feeding on guinea pigs and retain their label for five months.
- 18 Taimr, L., Diabola, J. VYUZITI RADIOFOSFORU PRO OZNACENI KRISU PRI STUDIUM MIGRACE V TERENU. (Application of radiophosphorus for the marking of leafhoppers in the study of their migration.) Ochr. Rost. (No 1) 3 (1965) 75-83. (In Czech, with Russian and English summaries)  
A method was developed for labelling leafhoppers (Javesella pellucida F. (= Calligynona)) with  $^{32}\text{P}$ , by feeding on radioactive plants dipping into a  $\text{Na}_2\text{H}^{32}\text{PO}_4$  solution. The penetration rate of the label into the insect and the feeding rate and sucking intensity were studied. An aqueous solution of  $\text{Na}_2\text{H}^{32}\text{PO}_4$  with an activity of 30-50  $\mu\text{Ci}/\text{ml}$ , containing P up to 4 mg/ml proved useful in labelling for field work. The biological half-life was determined. Labelling had no noticeable effect on lifespan. Larvae labelled in the final stage of development retained their radioactivity. A method for marking and locating leafhoppers under field conditions is suggested.
- 19 Thygesen, T. RADIOAKTIV MAERKNING AF INSEKTER. (Radioactive labelling of insects.) Ugeskr. Land. 92 (1966) 531-537. (In Danish, with English summary)  
Young plants of beet, swede, and winter rape were labelled with  $^{32}\text{P}$ . One method consisted of splitting the stalk or stem, and fastening a small glass container with a  $^{32}\text{P}$ -solution round it. (Other methods consisted in growing the plant in a pot, and watering the soil with a labelled solution, or injecting a labelled solution directly into the plant.) Aphids (Mysus persicae Sulz.) feeding on labelled plants became radioactive themselves. When feeding on small plants with only three to four leaves, a dose of 10  $\mu\text{Ci } ^{32}\text{P}$  was sufficient to effect labelling of the aphid. Older plants need a much larger dose for marking their aphid population. The amounts used had no retarding effect on development and reproduction of the aphids. When a labelled adult is removed from the labelled host plant, offspring are labelled - A scintillation counter was used.
- 20 Trout, W. E., III. THE SO-CALLED RECOVERY PHENOMENON AFTER IRRADIATION IN Drosophila melanogaster. Diss. Abstr. 27, 2 (1966) 646-B.  
Methods are described for investigating the use of sperm by the male, by using  $^{32}\text{P}$  and thymidine as sperm labels. By feeding or injecting males with  $^3\text{H}$ -thymidine, and preparing squashes of the sperm transferred to females, the label transferred can be studied quantitatively by scintillation counting. The samples are also in a form suitable for chemical treatment, staining and autoradiography. Studies with injected adult males delayed in mating suggest that seminal fluid is accumulated to some extent in unmated males, and is used rapidly by mating males. Evidence concerning the time from injection or feeding of the label to the transfer of labelled seminal fluid or sperm to females, suggests that in these experiments the time is the same for  $^{32}\text{P}$  and  $^3\text{H}$ -thymidine labelling. Moreover, the time seems to be the same for males labelled as larvae, and as young adults. There appear to be two labelling peaks, one about 7-8 d after labelling, which may represent the use of labelled seminal fluid; and another peak 12-13 d after labelling, perhaps representing the 1st use of labelled sperm. The x-ray experiments offer strong evidence that the recovery phenomenon is the result of a radiosensitivity gradient in sperm used on different days after irradiation, and is not the result of an actual recovery process. The results from the labelling experiments are not inconsistent with this view. (From DA)
- 21 Trout, W. E., III. SCINTILLATION COUNTING OF TRITIATED THYMIDINE TRANSFERRED TO FEMALES BY LABELLED Drosophila melanogaster MALES. p. 61 of "Biology Division Semiannual Progress Report for Period ending January 31, 1966". ORNL-3922, Oak Ridge National Lab., Tenn. May 1966, 207p.  
Adult males injected with  $^3\text{H}$ -thymidine and mated daily lost 90% of their radioactivity within the first 3 d. About 1% of the label lost on each of these days was taken up by the females. Only 10%,

at most, of the female label was received through mating. At least 50%, and probably all, of the remaining label was received through ingestion of label excreted by the males and as surface contamination of the females. Male larvae which had been fed  $^3\text{H}$ -thymidine for several hours and allowed to mate 4-8 d later as adults also transferred label to females during the first 2-3 d of mating. Although some of this transferred label was taken up by the female through ingestion, there are indications that much of the radioactivity was transferred through mating as labeled sperm and seminal fluid.

- 22 Vargas, L. THE USE OF LABELED MOSQUITOES FOR STUDYING POPULATION PROBLEMS. BoIn. Inst. Estud. méd. biol. 22, 3 (1964) 443-451. (In Spanish)

The labelling of mosquitoes is a useful tool to estimate the size of populations, the daily survival, the dispersion rate or record travelling. It gives indications about the preference for shelters and a more accurate rate of human blood preferences. The results of the incidence of two characters, i.e. human blood index and resistance to DDT are discussed. Detailed consideration is given to infectious doses transmitted by mosquitoes and their chance distribution. All data available are used to estimate the risk of contracting one infectious dose. (NM)

- 23 Weidinger, N., Jahn, E. MARKIERUNG VON INSEKTEN MIT SELTENEN ERDEN ZUM STUDIUM VON FORSTSCHÄDLINGEN. (Insect labelling with rare earths for studying forest pests.) Anz. Schädlingssk. 39 (1966) 77. (In German), Anz. Schädlingssk. 38 (1965) 16, p. 291-294 of "Gemeinsame Tagung der Deutschen Gesellschaft für Biophysik E.V., Österreichische Gesellschaft für Reine und Angewandte Biophysik, Schweizerische Gesellschaft für Strahlenbiologie, Vienna, Austria 14-16 Oct. 1964."

Stable nuclides such europium, dysprosium and thulium were used to study the dispersal of certain pests. Labelling was carried out by spraying (field work), feeding or injection. Labelled insects were subsequently activated in a reactor. Rare earths can be detected with certainty with a gamma spectrometer down to  $10^{-1}\gamma$ , europium even down to  $10^{-2}\gamma$ .

- 24 West, A.S.  $^{32}\text{P}$  TAGGING AND RECOVERY OF BLACK FLIES. Bull. ent. Soc. Am. 12 (1966) 289. Abstr. 271 "Portland Meeting, Portland, Oreg. USA. 28 Nov. - 1 Dec. 1966".

Black fly adults, tagged with  $^{32}\text{P}$  as larvae and pupae in aerated steamside tubs, have been recovered in significant numbers at distances of up to  $3\frac{1}{2}$  miles by use of "sticky" traps and an autoradiographic technique. (Abstr.)

See also:

- 25 The effect of radioactive phosphorus on the growth and development of Culex pipiens molestus Forsk. (Diptera, Culicidae). (Abdel-Malek, A.S., 1961)
- 36 Mutations produced by transmutation of phosphorus-32 to sulfur-32 within Drosophila DNA. (Lee, W.R. et al., 1967)
- 39 Early developments in the use of radioisotopes in agriculture. (Comar, D.L., 1966)
- 41 Atomic energy in life sciences. (Gopal-Ayengar, A.R., 1966)
- 58 Biological half-life of  $\text{Ca-45}$  in fall webworms. (Coleman, D.C. et al., 1967)
- 76 Distribution of  $^{32}\text{P}$  in the male reproductive system of Culex pipiens quinquefasciatus (Say). (Patterson, R.S. et al., 1967)
- 428 Study of the structure and function of the alimentary tract of Megoura viciae Buckt. (Aphididae, Homoptera), with special reference to food uptake and honeydew excretion. (Erhardt, P., 1963)
- 467 Some applications of radioactive isotopes in ecological research. (Noordink, J.P.W., 1965)
- 476 Study of food uptake by Megoura viciae Buckt., a phloem sucking aphid (Homoptera, Rhynchotha). (Erhardt, P., 1961)
- 482 Labelling of aphid saliva with rubidium-86. (Lamb, K.P. et al., 1967)
- 489 Investigation of hibernation of Perillus bioculatus Fabr. tagged with  $^{60}\text{Co}$ . (Wegorek, W. et al., 1965)
- 490 Habitat selection by the queens of two field-dwelling species of ants. (Wilson, O.E. et al., 1966)
- 493 Study of nutritional interchanges in the ant Formica polyctena by means of radioisotopes. (Lecomte, J., 1965)
- 495 Isotopes to estimate colony size of Formica cinerea Mayr (Hymenoptera: Formicidae) (Medler, J.T., 1964)
- 497 Note on the means of dispersion of Rhodnius prolixus Stål. (D'Ascoli, A. et al., 1966)

- 498 Some results obtained with the application of the tracer method in insect migration and dispersion studies. (Diabola, J. et al., 1965)
- 499 Study of the dispersal of the nun moth, Lymantria monacha L., by means of rare earths. (Jahn, E. et al., 1966)
- 502 Flight range, lengths of gonotrophic cycles, and longevity of  $^{32}\text{P}$ -labelled Anopheles stephensi mysorensis. (Quraishi, M. S., 1966)
- 503 Migration and dispersal patterns of  $\text{Fe}^{59}$ -labelled lone star ticks. (Smittle, B.J. et al., 1967)
- 504 Radioisotopes as tracers used for migration studies of the leafhopper species Calligypona pellucida F. (Taimr, L. et al., 1963)
- 554 Evaluation of activity of honeybee colonies moved to a lucerne seed field. (Šedivý, J. et al., 1966)
- 555 Definition of the range of host plants of the fruit fly (Oscinella frit L.) by means of  $^{32}\text{P}$  in the feeds of the insects. (Taimr, L. et al., 1967)
- 558 Radiophosphorus in labelling the desert locust for population estimation. (El-Minawi, S.F. et al., 1964)
- 568 Use of radioisotopes for studies on the ecology of tick vectors of disease. (Sonenshine, D.E., 1966)
- 954 Basic fertilization phenomena and gametic lethality in Drosophila. (Michigan State Univ., East Lansing, 1966)
- 1023 Drosophila cytology and genetics. (Oak Ridge National Lab., Tenn., 1966)
- 1283 Interactions of oxygen at high pressure and radiation in Drosophila. (Thomas, J.J., Jr. et al., 1966)
- 1556 Application of radioactive isotopes to the investigation of methods of biological control of insect pests. I-V. Tests with  $\text{P}-32$  and gamma-rays on Ceratitis capitata. (Arroyo Varela, M. et al., 1965)
- 1609 Study on mass breeding and sterilisation of the Mediterranean fruit fly Ceratitis capitata Wied. (A contribution to the autocidal technique.) (Scherney, F. et al., 1967)
- 1611 Studies on the eradication of Anopheles pharoensis by the sterile-male technique using cobalt-60. IV. Mating behaviour and its frequency in the sterilized mosquitoes. (Tantawy, A.O., 1967)
- 1745 Plastic enclosures for in vivo radioassay of insects. (Lippold, P.C., 1967)
- 1791 "Proceedings of FAO/IAEA Training Course on Use of Radioisotopes in Entomology, Gainesville, Fla., 4 Oct. - 26 Nov. 1965" (FAO/IAEA, 1965)

### 1.1.2. Developmental, physiological, and genetic effects of isotopic labels

- 25 Abdel-Malek, A.S. THE EFFECT OF RADIOACTIVE PHOSPHORUS ON THE GROWTH AND DEVELOPMENT OF Culex pipiens molestus Forsk. (DIPTERA, CULICIDAE). Bull. ent. Res. 52, 4 (1961) 701-708.

In a study on the effect of different concentrations of  $^{32}\text{P}$  in the larval medium on the growth and development of C. pipiens molestus,  $^{32}\text{P}$  was found to have little noticeable effect on the growth of the larvae up to a concentration of  $3.0 \mu\text{Ci } ^{32}\text{P}/\text{ml}$  but above this concentration larval growth was greatly retarded. The period of larval development was increased at concentrations  $> 1.0 \mu\text{Ci } ^{32}\text{P}/\text{ml}$ , and pupation occurred two weeks later than in the controls. In concentrations  $> 5.0 \mu\text{Ci}/\text{ml}$ , pupation was completely inhibited, larvae became sluggish, stopped feeding and finally died. The effect of  $^{32}\text{P}$  in the larval medium on the emergence and radioactivity of the resulting adults was also studied. On the basis of this study, it is recommended that, for efficient utilization of  $^{32}\text{P}$  in large-scale field experiments, a concentration of  $^{32}\text{P}$  of  $1.0 \mu\text{Ci}/\text{ml}$  be employed so that emerging adult mosquitoes may be sufficiently radioactive to be readily detectable. (Auth. summary)

- 26 Anil, Y.D. THE EFFECT OF THYMIDINE- $^3\text{H}$  ON THE MORTALITY OF Drosophila melanogaster LARVAE. Istanbul Univ. Fen. Fak. Mecm. B. 29, 3-4 (1964) 143-147.

Because the effect of radiation on organisms of different ages and on different tissues of the same animal is not the same, D. melanogaster larvae were used, since the number of cells in different tissues seems to be constant and no cell division occurs in postembryonal stages. 6-h-old larvae were transferred to hot nutrient (20 mg sugar, 20 mg yeast, 1 g banana and  $60 \mu\text{Ci}$  thymidine- $^3\text{H}$  (1), maintained in it for 12, 24, or 48 h, and then transferred to normal nutrient. The number of dead and living larvae were

counted at intervals. I had a lethal effect on larvae; the longer the larvae were kept on I-containing nutrient, the greater was the mortality rate. A relation between the age of the larvae and the lethal effect of I was found. The radiation from I had a greater effect on the cells which were at the beginning of differentiation than on the cells with already established differentiation. (CA 64: 1966, 14565g)

- 27 Arroyo, M., Jiménez, A., Meilado, L., Caballero, F. APLICACION DE ISOTOPOS RADIOACTIVOS A LA INVESTIGACION DE METODOS SOBRE LUCHA BIOLOGICA CONTRA LAS PLAGAS. V. EFECTOS DE LA RADIACION GAMMA SOBRE PUPAS DE *Ceratitis capitata* Wied., PREVIAMENTE MARCADAS CON P-32. (Application of radioactive isotopes to the investigation of methods for the biological control of pests. V. The effects of  $\gamma$ -radiation on pupae of *C. capitata* previously labelled with  $^{32}\text{P}$ .)

It was found impracticable to use larvae of *C. capitata* already labelled with  $^{32}\text{P}$  as a source of pupae to be subjected to  $\gamma$ -radiation, since their vitality was so much reduced by the labelling that irradiation resulted in excessive adult mortality. (from RAE-A 55: 1967, ref. 256)

- 28 Bond, V.P., Feinendegen, L.E. INTRANUCLEAR  $^3\text{H}$  THYMIDINE: DOSIMETRIC, RADIOBIOLOGICAL AND RADIATION PROTECTION ASPECTS. *Hith Phys.* 12 (1966) 1007-1020. BNL-9435, Oak Ridge National Lab., Upton, N.Y.

It appears from a study of available data that the degree of early somatic biological effect from intranuclear  $^3\text{H}$  is that anticipated on the basis of calculated average absorbed dose to the cell nucleus, at least down to doses of 5 rad or less. This result indicates that the absorbed dose concept holds down to the order of  $10^{-11}$  g or less, and somatic effects can be predicted on this basis; the distribution of  $^3\text{H}$  atoms incorporated into DNA as  $^3\text{H}$ TdR (and ion pairs from the beta particles) are randomly distributed as far as dose calculations for the purpose of predicting somatic effects are concerned; every part of the cell nucleus lies within one  $^3\text{H}$   $\beta$ -range of some part of a chromatid, and the nucleus contains no sizeable contiguous insensitive volume of a radius exceeding the effective range of  $^3\text{H}$   $\beta$ -rays (1-2  $\mu\text{m}$ ); the origin of  $^3\text{H}$   $\beta$ -tracks in, or their close juxtaposition to the DNA molecule does not appear to enhance the degree of somatic effect. Transmutation effects of the disintegrating  $^3\text{H}$  nuclide in DNA appear to have been demonstrated to produce genetic mutations in micro-organisms, and data suggestive of this mechanism in *Drosophila* are available. The degree to which this factor in addition to absorbed dose must be taken into account in estimating possible genetic effects in the mammal cannot be assessed adequately at this time, but appears to be small. From available data, it appears that the degree of genetic effect in mammalian cells can be approximated closely from the average absorbed dose to the nucleus. The intrinsic RBE of  $^3\text{H}$   $\beta$ -particles appears to be 1.0 (compared to 250 kVp x-rays), rather than the value of 1.7 currently used for radiation protection. (Auth.)

- 29 Caspari, E.W. SOMATIC MUTATIONS IN THE MOTH *Ephestia*. Report on Research, August 1, 1965 - September 15, 1966. p.4 in "NYO-2902-5 (and Suppl.), Rochester Univ., N.Y." 24p.

Injections of  $^3\text{H}$ -thymidine into larvae 8 d after the last moult result in death within 6 h when 2  $\mu\text{Ci}$  are injected. With 1  $\mu\text{Ci}$  some animals survive several days. Their wing buds become reduced in size and finally disappear. No mitosis or incorporation of  $^3\text{H}$ -thymidine was observed. At 0.5  $\mu\text{Ci}$  about 2/3 of the animals survive and pupate. The toxic effect seems to be due to the radioactivity, since equivalent amounts on non-radioactive thymidine are ineffective.

- 30 Dustan, G.G. EFFECTS OF TAGGING AMOUNTS OF RADIOACTIVE PHOSPHORUS ON ADULTS OF THE ORIENTAL FRUIT MOTH, *Grapholitha molesta* (Busck) (LEPIDOPTERA: TORTRICIDAE). *Can. Ent.* 98 (1966) 305-311.

Feeding adults on a tagging solution of  $^{32}\text{P}$  containing 20-50  $\mu\text{Ci}/\text{ml}$  had no apparent effect on their longevity, mating, fecundity, and motility or on the viability of their eggs. The rate of 20-50  $\mu\text{Ci}/\text{ml}$  was in the range used for dispersal studies. Moths thus tagged had radioactivities of about 1500-2500 cpm. Female moths fed on solutions of  $^{32}\text{P}$  at 100  $\mu\text{Ci}/\text{ml}$  showed counting rates of more than 5000 cpm. These females, when mated with normal males, laid approx. 33% fewer eggs than normal females. Also, the fertility of the eggs was reduced by about 25% when only the female parent was radioactive and 15% when only the male parent was radioactive to the extent of over 5000 cpm. (From auth.)

- 31 Ghelelovitch, S. EFFETS TUMORIGENES DE LA LEUCINE, DE L'URIDINE ET DE LA THYMIDINE TRITIÉES CHEZ LES LARVES DE LA DROSOPHILE. *C.R. hebdo. Séanc. Acad. Sci., Paris* 262, 10 D (1966) 1173-1176.

La substitution des composés tritiés à la leucine, l'uridine ou à la thymidine dans la nourriture des larves, permet d'augmenter l'incidence des tumeurs mélaniques chez les mouches d'une souche tumorale de *D. melanogaster*. A cet égard la thymidine tritiée est beaucoup moins active que l'uridine ou la leucine. L'uridine tritiée n'a pas permis d'induire la formation des tumeurs chez les larves d'une souche non tumorale. (Aut.)

- 32 Heslop, J.P. TOPICALLY APPLIED *n*-DECYL ACETATE AS A PRECURSOR FOR METABOLIC INVESTIGATIONS IN INSECTS. *Nature*, Lond. 213 (1967) 291.

The use of *n*-decyl 1-<sup>14</sup>C-acetate was investigated on *Calliphora erythrocephala*, applied topically to 3 to 4-d-old larvae (1 µl). No effects on mortality, pupation or emergence were observed. The lack of time lag in the production of <sup>14</sup>CO<sub>2</sub> (cf. 11/266, on *Musca domestica*) was interpreted as rapid hydrolysis of *n*-decyl acetate, the free acetate produced being further metabolized as soon as it is absorbed by the insect. The use of a fat-soluble precursor therefore permits a water-soluble metabolite to be given to an insect, however small, in a dose smoothly applied over several hours without loss of blood, risk of mechanical damage or unnecessary metabolic disturbance.

- 33 Kaplan, W.D., Gugler, H.D., Kidd, K.K. DISTRIBUTION OF LETHALS INDUCED BY TRITIATED DNA PRECURSORS IN *Drosophila melanogaster*. *Genetics* 53 (1966) 499-511.

Sex-linked recessive lethals have been induced in male *Drosophila* by feeding <sup>3</sup>H-deoxycytidine to 1st-instar larvae. The lethals have been localized and their distribution compared to the one previously obtained with <sup>3</sup>H-thymidine. The two independently produced distributions differ from each other at the 4% level of significance. Two regional differences have been noted, one of high mutability after <sup>3</sup>H-thymidine and one of high mutability after <sup>3</sup>H-deoxycytidine. Data combined from the two treatments closely parallel the distribution of lethals induced by x-rays and γ-rays, suggesting that the combined distribution reflects the regional content of DNA along the X-chromosome. The differences in lethal distributions produced by the two DNA precursors suggest that significant local variations in thymine and cytosine content may exist within chromosomal DNA. (Auth.)

- 34 Lee, W.R., Oden, C.K., Bart, C.A., Debney, C.W., Martin, R.F. STABILITY OF *Drosophila* CHROMOSOMES TO RADIOACTIVE DECAY OF INCORPORATED PHOSPHORUS-32. *Genetics* 53, 5 (1966) 807-822.

Transmutation of <sup>32</sup>P to <sup>32</sup>S and the accompanying energy released by steric changes in the molecule and recoil of the <sup>32</sup>S nucleus have been separated from the mutagenic effect of accompanying β-radiation by storage of <sup>32</sup>P-labelled spermatozoa in unlabelled *Drosophila* females. Because of the high energy of the <sup>32</sup>P β-particle, only a negligible dose is received by sperm from β-radiation from 2-d-old males that had been fed <sup>32</sup>P as larvae. Females recently mated to <sup>32</sup>P-labelled males were divided into two groups. One group was allowed to produce progeny immediately. The other was first stored on sugar agar media at 18°C for three weeks to inhibit oviposition, and then permitted to oviposit freely on cornmeal media. The mutation rates of progeny of the two groups were compared to measure additional genetic damage, if any, caused by <sup>32</sup>P decay in sperm during the storage period. There was no significant change during storage in the rate either of chromosome breakage (as measured by loss of the marker B<sup>8</sup> on the marked B<sup>8</sup> Y-chromosome) or of sex-linked recessive lethals that could be attributed to transmutation and recoil. By combining the min. increase in mutation rate that could have been detected statistically with the minimum estimate of <sup>32</sup>P disintegrations, it was concluded that less than one <sup>32</sup>P disintegration in 2000 in the X-chromosome will produce a "complete" recessive lethal. (From auth. summary)

- 35 Lee, W.R., Sega, G.A., Alford, C.F. THE PRODUCTION OF MOSAICS BY INCORPORATION OF P<sup>32</sup> INTO DNA OF *Drosophila melanogaster* SPERMATOZOA. *Genetics* 56, 3 Pt. 2 (1967) 572. Presented at the "1967 Meetings of the Genetics Society of America. Stanford, Calif., 31 Aug. - 2 Sep. 1967".

The effect of transmutation of <sup>32</sup>P to <sup>32</sup>S in DNA was separated from the mutagenic effect of accompanying β-radiation as in a previous experiment (*Genetics* 53: 1966, 807-822) by storage of <sup>32</sup>P labelled spermatozoa in unlabelled females. The multipurpose breeding scheme used in this experiment was designed to detect: (1) F<sub>1</sub> females with completely mutant germ lines by scoring for sex-linked recessive lethals in the F<sub>2</sub>, and F<sub>1</sub> females with mosaic germ lines by scoring for sex-linked recessive lethals in the F<sub>2</sub>; (2) F<sub>1</sub> males either complete or mosaic for loss of a Y-chromosome marked with γ+. The results confirmed the earlier experiment in that there was no increase in the rate of either complete or partial

loss of the marked Y-chromosome or of sex-linked recessive lethals scored in the  $F_2$  during 23 d of storage. However sex-linked recessive lethals scored in the  $F_3$  generation increased significantly from 0.6% (N = 2247) to 1.5% (N = 2479) during the 23 d the spermatozoa were stored in unlabelled females. The frequency of mutant cleavage nuclei in these mosaics must be less than 50% and is best fit by 12% or less because, as shown in a previous experiment (Genetics 55:1966, 619-634), the  $F_2/F_3$  ratio would be 2.4 if mosaic embryos had averaged 50%, whereas the  $F_2/F_3$  ratio would be reduced to 0.6 if mosaic embryos averaged 12%. (Abstr.)

- 36 Lee, W.R., Sega, G.A., Alford, C.F. MUTATIONS PRODUCED BY TRANSMUTATION OF PHOSPHORUS-32 TO SULPHUR-32 WITHIN *Drosophila* DNA. *Proc. natn. Acad. Sci. USA* 58, 4 (1967) 1472-1479.

To determine the proportion of  $^{32}\text{P}$  incorporated into DNA, sperm cells were double-labelled with  $^{32}\text{P}$  and  $^3\text{H}$ -thymidine. Evidence is presented that transmutation of  $^{32}\text{P}$  to  $^{32}\text{S}$  within the DNA molecule of *Drosophila* spermatozoa produce lethal mutations that are detected only in the  $F_3$ . If the delay in expression of the lethal mutation is due to  $F_1$  mosaicism, the mutant cells must average 25% or less of the  $F_1$  mosaic fly, or otherwise a significant fraction of the lethals will be detected in the  $F_2$ . Mosaics of 25% or less may be the results of multistrandedness or the mechanism of mutation. Regardless of which explanation may be correct, it is clear that the conventional explanation for the origin of mosaics by the alteration of one strand of double-stranded DNA to produce a cell lineage with half mutant and half nonmutant cells cannot be applied to these mutation induced by transmutation of  $^{32}\text{P}$  to  $^{32}\text{S}$ . The implications for estimating genetic damage from radionuclides incorporated into DNA must be re-evaluated in view of these results. Furthermore, these results indicate the inadequacy of conventional  $F_2$  lethal tests for screening potential mutagenic agents.

- 37 Strayer, J.R. EFFECT OF RADIOPHOSPHORUS ( $\text{P}^{32}$ ) ON LIGHT RESPONSE OF *Aedes aegypti* (L.) LARVAE. *Fla Ent.* 48, 2 (1965) 81-84.

The normally negatively phototactic larvae of *A. aegypti* (L.) were exposed to varying levels of  $^{32}\text{P}$  in order to determine whether this would affect their light responses. 3rd-instar larvae were kept for 24 h in beakers containing 250 ml water either alone or with the addition of 1 ml of a 0.5 N solution of orthophosphoric acid, or radioactive material giving 0.1 or 5  $\mu\text{Ci } ^{32}\text{P}/\text{ml}$ . The larvae were then transferred to fresh water and provided with food. For observations on light responses, which were carried out in a darkened room, an 18 in. cylinder was used, a trap funnel being fixed in an upright position about half-way up. The bottom of the funnel was closed or opened by a stopper operated by a string that passed through a drain plug at the base of the cylinder. The cylinder, which was lit initially from below, was filled with water and the funnel was closed. Larvae was poured in through the top, and when they had settled, the funnel was opened and the bottom light was turned out. After a few seconds' pause, a top light was turned on for 20 sec, after which the funnel was immediately closed. The larvae in the top and bottom parts of the cylinder were counted separately, and their radioactivity was determined. No differences in the level of radioactivity were detected between those larvae in the top section and those in the bottom. However, the average percentages of larvae in the top were 29.6 and 26 for those that had been kept in water and in the non-radioactive orthophosphoric acid solution respectively, as compared with 43.6 and 67 for those exposed to 0.1 and 5  $\mu\text{Ci } ^{32}\text{P}/\text{ml}$ , indicating that these concentrations of radioactive phosphorus affected the light response of the *Aedes* larvae. (From RAE-B55: 1967, ref. 171)

See also:

- 3 Application of radioactive isotopes to the investigation of methods for the biological control of pests. II. Tests on marking larvae of *C. capitata* with  $^{32}\text{P}$ . (Arroyo, M., 1965)
- 15 The elimination and differentiation of chromosomes in the germ line of *Sciara*. (Rieffel, S.M. et al., 1966)
- 74 A study of the retention and the mutagenic mode of action of radioactive phosphorus in *Drosophila melanogaster*. (Ofstedal, P., 1959)
- 75 Some aspects of transmutation studies in *Drosophila*. (Ofstedal, P. et al., 1967)
- 292 Genetic and radioautographic evidence for a DNA-containing body in the cytoplasm of the adult testes of *Drosophila melanogaster*. (Kaplan, W.D. et al., 1967)
- 501 Flight range, lengths of gonotrophic cycles, and longevity of  $^{32}\text{P}$ -labelled *Anopheles stephensi mysorensis*. (Quraishi, M.S. et al., 1966)

- 950 Molecular and radiation genetics. Annual Report 1965 (Leiden Rijksuniversiteit, Netherlands, 1966)
- 1429 Effect of tritiated thymidine and irradiation on the mortality of adult *Drosophila melanogaster* larvae. (Bolukbasi, E.K., 1965)

## 1.2. INSECT PHYSIOLOGY AND BIOCHEMISTRY

### 1.2.1. General Articles, Surveys

- 38 Anonymous. RADIOISOTOPES IN TROPICAL MEDICINE. Wld Hlth Orgn. Chron. 17 (1963) 83-86.
- Numerous applications of radioisotopes in biological and medical problems of the tropics are reviewed. In tropical medicine four phenomena appear repeatedly in varied combinations: infection, diarrhea, malnutrition and anemia. Infection and malnutrition interact particularly closely. Isotope techniques have proved invaluable in studies of every aspect of water and electrolyte physiology, the most useful methods so far being based on the dilution principle.  $^{24}\text{Na}$ ,  $^{42}\text{K}$ , and  $^{82}\text{Br}$  (used as a chloride substitute) and deuterium or  $^3\text{H}$  are the isotopes most often employed in such studies. Radioisotopes such as  $^{55}\text{Fe}$ ,  $^{59}\text{Fe}$ ,  $^{51}\text{Cr}$ , and vitamin  $\text{B}_{12}$  labelled with  $^{60}\text{Co}$  have proved useful in the analysis and separation of conditions that exist in South-East Asia. These isotopes facilitate the study of haemolysis by providing estimates of the life span of red cells and the pattern and sites of their destruction. A table shows the various isotopes that have been incorporated into insects. Those most often used for ecological investigations are the  $\beta$  emitters  $^{32}\text{P}$ ,  $^{35}\text{S}$  and  $^{89}\text{Sr}$ . Tracer techniques and insect biochemistry are also discussed, whereby the use of radioactive labelled insecticides enables their metabolic fate in insects to be followed with great sensitivity and specificity, and throwing much light on the mechanism of insect resistance. (NSA 19: 1965, 34094)
- 39 Comar, C.L. EARLY DEVELOPMENTS IN THE USE OF RADIOISOTOPES IN AGRICULTURE. Isotopes Radiat. Technol. 4, 1 (1966) 53-58.
- Very general survey of early developments in the uses of radioisotopes in agriculture. These uses, particularly for research studies in plants, animals, soils, waters, and insects, have expanded enormously in the last 20 yr, and a few are indicated briefly. An ever increasing use of nuclear energy techniques in biological and agricultural research is evident and the trend will continue, in view of the variety of radioisotopes instrumentation and equipment available today.
- 40 Gilbert, L.I., Schneiderman, H.A. SOME BIOCHEMICAL ASPECTS OF INSECT METAMORPHOSIS. Am. Zool. 1 (1961) 11-51.
- Comprehensive review article with extensive bibliography. The article is divided into sections on hormones and metamorphosis (including a chapter on hormone identification dealing with the chemical nature of the brain hormone, the prothoracic gland hormone, the juvenile hormone, the egg-diapause hormone, and queen substance), chemical changes during metamorphosis (amino acid content, proteins, peptides, metabolisms of proteins and amino acids, carbohydrates, glycolysis, lipids, lipid metabolism, nucleic acids, other phosphate compounds, and inorganic ions), and hormone action (mode of action of ecdysone, brain hormone, and juvenile hormone). - Radioisotopes had been utilised in many of the studies cited but this fact scarcely emerges from the text.
- 41 Gopal-Ayengar, A.R. ATOMIC ENERGY IN LIFE SCIENCES. Atom. Energy Rev. 4 (1966) 59-71. Commemorative Issue.
- In surveying the impact of atomic energy on the life sciences, the author discusses particularly the various uses of isotopes in biology and biochemistry, instancing in some detail the effectiveness of tracers as a research tool. He touches on such subjects as the use of isotopes in revealing the processes of plant growth and nutrition, in eradicating insect pests, in improving agriculture and in promoting health and combating disease. (From auth.)
- 42 Haisch, A., Süss, A. DIE VERWENDUNG VON RADIONUKLIDEN UND DEREN STRAHLUNG IN DER SCHÄDLINGSBEKÄMPFUNG. (The use of radionuclides and their radiation in (the) plant pest control.) Kerntechnik 8 (1966) 514-517. (In German)

The use of labelling techniques to determine the relation between plant, plant pest, and environment to ensure a more effective use of pesticides is discussed. The use of ionizing radiation for killing sterilising plant pests is also discussed. Pest sterilisation is considered in some detail with the discussion of the modification of the sterilisation by endo- and exogenic factors, and a review of the results obtained in sterilisation studies is given. The desirability of applying the method to some pests in Germany is pointed out.

- 43 Harvey, W.R., Haskell, J.A. METABOLIC CONTROL MECHANISMS IN INSECTS, p.133-205 of "Advances in Insect Physiology. Vol.3". Beament, J.W., Treherne, J.E., Wigglesworth, V.B., Eds. London, Academic Press, 1966, 382p.

This review article is broken down into sections on phosphate acceptor and substrate control of respiration in isolated mitochondria (dealing with sarcosomes and their isolation, energy requirements of insect flight, regulation of energy trapping pathways in flight muscle, oxidative phosphorylation and respiratory control, endogenous uncoupling or controlling agents,  $\alpha$ -glycerophosphate and respiratory control during flight, and biological factors influencing energetics of mitochondria); the regulation of enzyme levels (constant proportion enzymes, oxidative enzymes in silkworm development, and enzymes of tanning reactions); control at the chromosome level (the biochemistry of insect hormones and of giant chromosomes, chromosomal puffing and its relation to development and to synthetic processes in the cell, ecdysone and DNA synthesis, and chromosomal puffs and transport); and the ionic control of protein synthesis and development (ion control during development, and protein synthesis regulated by ion concentrations). An extensive bibliography is attached. Radioisotopes were used in a large number of studies, particularly those dealing with puffing phenomena).

- 44 House, H.L. INSECT NUTRITION. A. Rev. Biochem. 31 (1962) 653-672.

A review of the understanding of nutritional requirements, e.g. the chemical factors of ingested food essential for normal metabolism and development of the insect and the insight into insect metabolism arising from nutritional research. Tracer techniques are carried out in some of the studies reviewed, using  $^{14}\text{C}$ -labelled compounds. (B1)

- 45 Kansu, A. RADIOISOTOPES IN ENTOMOLOGY. Yük. Zir. Enstit. Derg. (1964) 7-28. (In English)

This paper is mainly based on the author's lecture notes for the International Training Course in Radioisotopes in Agriculture, jointly sponsored by the I.A.E.A. and the Atomic Energy Commission of the Republic of Turkey, Ankara, Oct. 2 - Nov. 25, 1962. (See also II/1575)

- 46 Mitchell, H.K. BIOCHEMICAL ASPECTS OF *Drosophila*. A. Rev. Genet. 1 (1967) 185-200.

A review with 114 references.

#### See also:

- 1538 Role of atomic energy in insect study and control. (Huque, H., 1962)  
1791 "Proceedings of FAO/IAEA Training Course on Use of Radioisotopes in Entomology, Gainesville, Fla., 4 Oct. - 26 Nov. 1965". (FAO/IAEA, 1965)

### 1.2.2. Elements. Ions. Inorganic Salts

- 47 Aidley, D.J. THE EFFECTS OF STRONTIUM AND OTHER DIVALENT CATIONS ON POTASSIUM CONTRACTURE IN A LOCUST LEG MUSCLE. J. Physiol. Lond. 77 (1965) 103-111.

It was previously established that Ca ions are necessary for the production of K-induced contractures in the mesothoracic extensor tibialis muscle of the locust *Schistocerca gregaria*, and present experiments investigated whether other divalent cations would substitute for Ca in this reaction. Ringer's solutions containing these abnormal cations were made by substitution of the cation for Ca, and contractures were produced by perfusion of the muscle with a solution containing 164-mM KCl and 100-mM D-glucose. Addition of salts of Sr, Co, and Ni to Ca-depleted muscle in KCl solution caused a brief submaximal contraction. Salts of Mg, Ba, and Mn did not produce contraction under these conditions. After perfusion

of a Ca-depleted muscle with a Ca-free Ringer's solution containing 4 mM  $\text{SrCl}_2$ , perfusion with KCl solution causes a phasic contracture which may reach 25% of the max. tension produced under normal conditions of Ca ion concentration. Successive responses to depolarization in the presence of Sr ions decline in the absence of Ca ions. It is concluded that Ca is probably uniquely important in excitation-contraction coupling in this muscle, and that Sr and other substitute cations act by displacing Ca from some site at which it is bound in the muscle. The comparative physiology of Sr ion action in excitation-contraction coupling is discussed. In the frog heart, Sr and Ba salts are similar to Ca salts in their effects on contractility, and  $^{86}\text{Sr}$  can be used as a tracer for Ca. There is some competition between Ca and Sr ions, and higher tensions are produced in a Ca-free Ringer containing Sr ions than in normal Ringer solution. At low  $\text{Ca}^{2+}$  concentrations, the presence of Sr ions causes an increase in the contraction of cat papillary muscle which was associated with a lengthening of the R-T interval of the electrocardiogram. Sr and Ba ions can substitute for Ca to some extent in the uterine muscle of the rat, but the muscle appears to distinguish Ca from Sr in that after a brief exposure to Ca ions the ability to produce contracture persists for some time, whereas this does not occur after a similar exposure to Sr ions. There are many differences between the actions of Sr and Ca ions on vertebrate muscles, but these differences are fewer than are seen in locust muscle, where Sr is far from being an adequate substitute for Ca. (NSA 19: 1965, 33725)

- 48 Auerbach, S.I., Crossley, D.A. Jr., Dunaway, P.B. et al. RADIOACTIVE WASTE AREA AND RADIATION EFFECTS STUDIES. p. 81-95 of "Health Physics Division Annual Progress Report for Period Ending June 30, 1968". ORNL-3492, Oak Ridge National Lab., Tenn. 30 Sep. 1968, 245p.

A variety of studies is discussed. The use of biological elimination of radioisotopes in insects as indirect measures of metabolism under field conditions was continued with emphasis on the influence of temperature on elimination rates. In geometric caterpillars the biological half-life of  $^{137}\text{Cs}$  was decreased by one-half for a  $10^\circ\text{C}$  rise in temperature. Similar temperature-related trends were found for leaf beetles (*Chrysomela knabi*) and millipedes (*Dixidesmus erasus*).

- 49 Cavalloro, R., Cirio, U. ACCUMULO ED ELIMINAZIONE DI RADIOISOTOPI IN ALCUNE SPECIE DI INSETTI FITOFAGI OLOMETABOLI. (Accumulation and elimination of radioisotopes in several species of phytophagous holometabolous insects.) *Redia* 49, 2 (1964/5) 239-253. (In Italian, with English summary). Presented at the "VI Congresso Nazionale di Entomologia, Padova, Italia. 11-14 settembre 1965."

The fate of  $^{86}\text{Sr}$ ,  $^{137}\text{Cs}$ , and  $^{131}\text{I}$  was studied in various stages of the life cycle of nine species of phytophagous insects belonging to the families of Noctuidae (*Scotia segetum* Schiff, and others), Thaumetopoeidae (*Thaumetopoea pityocampa* Schiff.) and Scarabaeidae (*Melolontha melolontha* L.). Insects were labelled by feeding on labelled plants or solutions. Iodine was translocated most rapidly, followed by Cs and Sr. Data are given on the accumulation, elimination, and biological half-life of the radionuclides. Uptake was regular in Thaumetopoeidae and Scarabaeidae, although equilibrium failed to be reached, whereas it was very irregular in the Noctuidae.  $^{131}\text{I}$  accumulated more rapidly than  $^{137}\text{Cs}$ , and  $^{137}\text{Cs}$  more rapidly than  $^{86}\text{Sr}$ . Elimination curves were always regular and demonstrated, for all life stages, a more rapid loss for  $^{86}\text{Sr}$ , followed by  $^{137}\text{Cs}$  and then  $^{131}\text{I}$ .  $^{131}\text{I}$  was found in large concentrations in the exuviae which indicates preferential utilization of the element in the cuticle. Biological half-life data varied greatly with species and stage in the life cycle.

- 50 Cavalloro, R., Cirio, U. ACCUMULO ED ELIMINAZIONE DI RADIOISOTOPI IN ALCUNE SPECIE DI INSETTI FITOFAGI OLOMETABOLI. (The accumulation and elimination of radioisotopes in some species of phytophagous holometabolic insects.) p. 78-79 of "6th Italian National Congress of Entomology. Padua, Italy, 11-14 Sep. 1965". Abstract of Paper Presented at the Meeting. Published 1966.

Insects were labelled either by ingestion of a sugar solution to which radioisotopes had been added or by feeding on labelled plants. Translocation in the plant was found to be greatest for I, less so for Cs, and slowest for Sr. A table was presented giving accumulation, elimination rates and the average biological half-life of the various isotopes in the insect species under consideration. The values were compared with those reported for other insect species.  $^{131}\text{I}$  accumulates faster than Cs or Sr. No equilibrium was observed. Elimination was most rapid for  $^{86}\text{Sr}$ , followed by  $^{137}\text{Cs}$  and  $^{131}\text{I}$ . Iodine proved most mobile. It was found in large quantities in the exuvia, and is evidently utilized in the cuticle. 28 biological half-life values were found for the three radioisotopes: 12 each for Sr and Cs and 4 for I, depending on the various stages of the life cycle.

- 51 Cavalloro, R. THE ACCUMULATION, DISTRIBUTION, AND ELIMINATION OF  $^{85}\text{Sr}$ ,  $^{131}\text{I}$ , AND  $^{137}\text{Cs}$  IN VARIOUS INSECT SPECIES. p. 601-608 of "Proceedings of the International Symposium on Radioecology, Concept, Processes". Stockholm 1966. Published 1967.

Several species of Lepidoptera, Coleoptera, Hymenoptera, Orthoptera, and *Homocoryphus* were examined. Included were absorption by exoskeleton, haemolymph, salivary glands, gut, malpighian tubules, fatty tissue, muscle, and nervous system. Also included were body-burden determinations at intervals up to 168 h after ingestion of labelled food. No conclusions were drawn. (CA 68:1968, 19311 c)

- 52 Cavalloro, R., Cirio, U. ACCUMULO ED ELIMINAZIONE DI RADIOISOTOPI IN ALCUNE SPECIE DI INSETTI FITOFAGI OLOMETABOLI. (Accumulation and elimination of radioisotopes in some species of phytophagous insects.) *Euratom Inf.* 4, 10 (1966) 1089.

The movement of  $^{85}\text{Sr}$ ,  $^{137}\text{Cs}$  and  $^{131}\text{I}$  has been studied in various stages of the life-cycle of nine species of phytophagous insects from the families Noctuidae, Thaumetopoeidae and Scarabaeidae. The insects were labelled through feeding on labelled plants or solutions. Labelling the plants indicated that I was translocated most rapidly, then Cs and then Sr. Data are given on the accumulation, elimination and biological half-life of the three radionuclides. Uptake was regular in the Thaumetopoeidae and Scarabaeidae, although equilibrium was not reached, whereas it was very irregular in the Noctuidae.  $^{131}\text{I}$  was accumulated more rapidly than  $^{137}\text{Cs}$  and the latter more so than  $^{85}\text{Sr}$ . Elimination curves were always regular and demonstrated, for all life stages, a more rapid loss for  $^{85}\text{Sr}$ , followed by  $^{137}\text{Cs}$  and then  $^{131}\text{I}$ . The latter isotope, more strongly retained, was found in large concentrations in the exuviae which indicates a preferential utilization of the element in the cuticle. Data on biological half-lives were quite variable as to species and life-cycle. (NM)

- 53 Cavalloro, R., Cirio, U. DISTRIBUZIONE DI STRONZIO, CESIO E IODIO RADIOATTIVI NEL CORPO DI ALCUNI INSETTI. (The distribution of radioactive strontium, caesium and iodine in the body of some insects.) *Redia* 50 (1966/1967) 187-196. (In Italian, with English summary)

The distribution of  $^{85}\text{Sr}$ ,  $^{137}\text{Cs}$ , and  $^{131}\text{I}$  was investigated in larvae of ten species and adults of 11 species of insects belonging to the following groups: Orthoptera (Conocephalidae, Tettigoniidae, Gryllidae, Gryllotalpidae and Acrididae), Lepidoptera (Noctuidae, Thaumetopoeidae, Saturniidae, Lasiocampidae, Papilionidae and Pieridae), Coleoptera (Silphidae), and Hymenoptera (Argidae)\*. The radioisotopes were administered by ingestion of labelled food or solutions. The insects were fasted prior to dissection. Tissues analysed for accumulation were exoskeleton, muscles and nervous system, alimentary canal (gut), salivary glands, haemolymph, gonads, fat body, and Malpighian tubules. The distribution of Sr and Cs was generally similar.  $^{85}\text{Sr}$  and  $^{137}\text{Cs}$  accumulated primarily in the muscles and nervous system and in the haemolymph; some differences related to their respective mobilities were noted. There was a higher accumulation of  $^{137}\text{Cs}$  in the gonads of the Lepidopteran species (14.3%) than in those of the Orthopteran species (3.1%). In the holometabolic species  $^{137}\text{Cs}$  and  $^{85}\text{Sr}$  accumulated mostly in the gut.  $^{131}\text{I}$  accumulated primarily in tissues with high lipid content, i.e. the fat body and exoskeleton.

\* In the adult and larval stages of *Phytometra gamma* L., *Rhyacia c-nigrum* L., *Scotia segetum* Schiff., and *Triphaena pronuba* L., the adult stages of *Ephippiger ephippiger* Fieb., *Gryllotalpa gryllotalpa* L., *Gryllus campestris* L., *Homocoryphus nitidulus* Scop., *Necrophorus* sp., *Oedipoda coerulescens* L., and *Trigonophora meticulosa* L.; and the larval stages of *Arge pagana* Panz., *Endia pavonia* L., *Macrothylacia rubi* L., *Papilio machaon* L., *Pieris brassicae* L., and *Thaumetopoea pifyocampa* Schiff.

- 54 Cavalloro, R., Cirio, U. DISTRIBUZIONE DI STRONZIO, CESIO E IODIO RADIOATTIVI NEL CORPO DI ALCUNI INSETTI. (Body distribution of radioactive strontium caesium and iodine in some insects.) *Redia* 50 (1967) 187-196. (In Italian)

The distribution of  $^{85}\text{Sr}$ ,  $^{137}\text{Cs}$  and  $^{131}\text{I}$  was investigated in larvae of 10 species and adults of 11 species of insects belonging to the following groups: Orthoptera (Conocephalidae, Tettigoniidae, Gryllidae, Gryllotalpidae and Acrididae), Lepidoptera (Noctuidae, Thaumetopoeidae, Saturniidae, Lasiocampidae, Papilionidae and Pieridae), Coleoptera (Silphidae), Hymenoptera (Argidae). The radioisotopes were administered by ingestion of labelled food or solutions. The insects were fasted prior to dissection. The distribution of Sr and Cs were generally similar. They were concentrated preferentially in muscle and in the nervous system, with some differences related to their respective mobility. In the holometabolic species, however, these isotopes were found mostly in the gut. I was associated mostly with the lipids, either in the fat body where important or in the exoskeleton.

- 55 Cavalloro, R. DATA ON THE ACCUMULATION, DISTRIBUTION AND ELIMINATION OF  $^{86}\text{Sr}$ ,  $^{131}\text{I}$  AND  $^{137}\text{Cs}$  IN VARIOUS INSECT SPECIES. p.601-608 of "Radioecological Concentration Processes. Proceedings of an International Symposium. Stockholm, Sweden, 25-29 April 1966". Pergamon, Oxford, 1966.
- Tabulated data are presented on (1) the biological half-life of the above isotopes in larvae and adults of various insect species, belonging to Lepidoptera (Noctuidae and Thaumetopoeidae) and Coleoptera (Scarabaeidae); (2) the distribution of the radioisotopes in last-instar larvae of *Scotia segetum* Schiff and *Thaumetopoea pityocampa* Schiff following ingestion of labelled food; (3) the distribution of  $^{86}\text{Sr}$  in various last-instar larvae after ingestion of labelled food; (4) of  $^{131}\text{I}$ ; and (5) of  $^{137}\text{Cs}$ ; (6) the distribution of  $^{86}\text{Sr}$ ,  $^{131}\text{I}$  and  $^{137}\text{Cs}$  in adults of *Homocoryphus nitidulus* Scop. and *Triphaena pronuba* L. following ingestion of labelled food; (7) distribution of  $^{86}\text{Sr}$  in various adult insects after ingestion of labelled food; (8) of  $^{131}\text{I}$ ; (9) and of  $^{137}\text{Cs}$ . Graphs are given of (1) the body-burden of  $^{86}\text{Sr}$  and  $^{137}\text{Cs}$  in last-instar larvae of *S. segetum* and *Triphaena pronuba*, feeding on labelled plant leaves, as a function of time; (2) daily uptake of  $^{86}\text{Sr}$  and  $^{137}\text{Cs}$  by last-instar larvae feeding on labelled plant leaves. Daily uptake = net increase in body activity + activity of liquid and solid excrements produced over 24 h; (3) specific activity of  $^{86}\text{Sr}$  and  $^{137}\text{Cs}$  in solid excrements in last-instar larvae feeding on labelled food; and (4) loss curves of  $^{86}\text{Sr}$ ,  $^{131}\text{I}$  and  $^{137}\text{Cs}$  in last-instar larvae and adults after ingestion of labelled food.
- 56 Chaplain, R. A. THE EFFECT OF  $\text{Ca}^{++}$  AND OF FIBRE ELONGATION ON THE ACTIVATION OF THE CONTRACTILE MECHANISM OF INSECT FIBRILLAR FLIGHT MUSCLE. *Biochem. biophys. Acta* **131**, 2 (1967) 385-392.
- The relationship between fibre length and the  $\text{Ca}^{++}$ -activated ATPase (adenosine triphosphate phosphohydrolase, EC 3.6.1.3) of insect fibrillar muscle was studied on the dorsal longitudinal muscles of the giant water bugs *Lethocerus cordofanus* and *Hydrocyrius columbiae*, the beetle *Amphimallon solstitialis* and the bumble bee, *Bombus lucorum*. Fibre bundles which had been stretched by the required amount in the relaxing solution were immediately transferred to an activating solution containing  $3.55 \times 10^{-8} \text{ M Ca}^{++}$ , and allowed to equilibrate. Stabilized  $^{45}\text{Ca}$  concentrations were obtained by adding  $^{45}\text{Ca}$ -EGTA, prepared by combining  $^{45}\text{CaCl}_2$  and EGTA in equivalent concentrations. The amount of bound  $^{45}\text{Ca}$  was determined, special care being taken to reduce the contaminating unlabelled  $\text{Ca}^{++}$  present in the reagents used in the  $^{45}\text{Ca}$ -binding studies. Low levels of  $\text{Ca}^{++}$  and small degrees of fibre extension have been found to increase the ATPase activity. Either factor influences the sensitivity of the material to the other factor. In insect fibrillar muscle, where the A filaments are continuous with the Z line, it is possible to strain the A filaments directly in the absence of permanent actin-myosin interactions. The higher ATPase activity as a result of fibre elongation is accompanied by an increase in  $\text{Ca}^{++}$  binding. The relevance of the findings to the mechanism of oscillatory contraction of insect fibrillar muscle is discussed.
- 57 Coleman, D.C., Monk, C.D. THE BIOLOGICAL HALF-LIFE OF  $^{45}\text{-CALCIUM}$  IN THE FALL WEB-WORM, *Hyphantria cunea* (Drury). *Bull. ecol. Soc. Am.* **48**, 3 (1967) 129.
- 58 Coleman, D.C., Monk, C.D. BIOLOGICAL HALF-LIFE OF  $\text{Ca-}^{45}$  IN FALL WEBWORMS. "New York Meeting of the Ecological Society of America. New York, N.Y. Dec. 26-31, 1967". AED-CONF-1967. 396-001. 5p.
- The uptake, assimilation and loss of  $\text{Ca}$  by *Hyphantria cunea* (Drury) was studied after allowing them to feed on persimmon trees which had been labelled by injecting 2 mCi of  $^{45}\text{Ca}$  into holes in the trunk. Values for biological half lives from last-instar larvae were determined under varying experimental conditions, and proved extremely labile. The assimilation rates were determined by micro-bomb calorimetry of leaves and faeces. It proved to be very low, with a fairly rapid flux of assimilated isotope ( $\sim 11\%/d$  for the fed larva).
- 59 Cornwell, P.B. A PRELIMINARY INVESTIGATION INTO THE UPTAKE AND TRANSLOCATION OF PHOSPHORUS IN *Theobroma cacao* L. *Trop. Agric., Trin.* **34**, 2 (1957) 117-132.
- $^{32}\text{P}$  was introduced as carrier free phosphorus in the chemical form of orthophosphoric acid. It was administered by trunk implantation, the immersion of roots and application to the soil. Radioactive assay showed that the distribution of activity in the trunk and branches was influenced by the location of the holes of implantation and of the roots engaged in absorbing the active material. There was

little lateral movement in the conducting vessels of the trunk so that only those branches which originated near the path of translocation took up  $^{32}\text{P}$ . It would appear that the most uniform distribution of activity in the trees is obtained after application to the soil. The distributions and levels of activity in the branches are considered in relation to the suitability of these techniques for labelling the mealybug vector, *Pseudococcus njalensis* Laing, of cacao virus at their feeding sites in the field. No activity could be detected in the immature stages. Considerably higher levels of activity than those employed in the present work are required in the branches before such insects can be used in ecological studies. Ants, *Crematogaster striatula* Emery, attending the mealybugs on these trees gave counts two or three times higher than those obtained from adult *P. njalensis*. The results are of further interest in relation to other fields of cacao research, involving the application of systemic insecticides, the uptake of nutrients and the effect of shade.

- 60 Ehrhardt, P., Lamb, K.P. VERSUCHE ZUR AUFNAHME UND EXKRETION VON JOD-131 BEI *Aphis fabae* Scop. (APHIDAE, HOMOPTERA, INSECTA). (Experiments on the absorption and excretion of  $^{131}\text{I}$  by *Aphis fabae* Scop. (Aphidae, Homoptera, Insecta).) *Int. J. appl. Radiat. Isotopes* **18**, 7 (1967) 543-544. (In German)

The absorption of  $^{131}\text{I}$  (in the form of NaI in thiosulfate and carrier-free aqueous solution, specific activity 2.8 mCi/ml) was studied in larvae and adults. At the start of feeding  $^{131}\text{I}$  accumulates in the insect and is not excreted as rapidly as other tracers; it is excreted 4-5 d later when no further I is stored by the organism.  $^{131}\text{I}$  may also be given via *Vicia faba* leaves, and again it takes 4-5 d to reach maximum activity. The tracer is evenly distributed in the phloem.  $^{131}\text{I}$  is excreted via honeydew, in only small amounts to begin with, and in considerable quantity by adults via larvae. Except for marked losses of radioactivity (from 14.6 - 39.6%) through moulting, presumably by I-incorporation into cuticle proteins, larvae show only slight losses (e.g. from 1304 counts/100 s to 1275 counts/100 s in 48 h). Losses via the saliva are unimportant quantitatively. The incorporation of  $^{131}\text{I}$  appears to be relatively uniform, except for the cuticle.

- 61 Eldefrawi, M.E., O'Brien, R.D. PERMEABILITY OF THE ABDOMINAL NERVE CORD OF THE AMERICAN COCKROACH, *Periplaneta americana* (L.) TO QUATERNARY AMMONIUM SALTS. *J. exp. Biol.* **46**, 1 (1967) 1-12.

The influx and efflux of two series of tritiated quaternary alkylammonium cations in the abdominal nerve cord have been studied. Series I had the general structure  $\text{RN}+(\text{CH}_2)_n\text{I}^+$ , where  $\text{R} = \text{C}_2\text{H}_5$ ,  $\text{C}_4\text{H}_9$ ,  $\text{C}_6\text{H}_{13}$ ,  $\text{C}_8\text{H}_{17}$  and  $\text{C}_{10}\text{H}_{21}$ . Series II had the general structure  $\text{C}_2\text{H}_5\text{N}+(\text{R}')_3\text{I}^+$ , where  $\text{R}' = \text{CH}_3$ ,  $\text{C}_2\text{H}_5$ ,  $\text{C}_3\text{H}_7$ ,  $\text{C}_4\text{H}_9$  and  $\text{C}_6\text{H}_{13}$ . The data are interpreted as showing that increasing liposolubility tends to increase penetration, and increasing size (with respect to the smallest cross-sectional area) decreases it. The influx rates of the quaternary alkylammonium cations into the cockroach central nervous system (CNS) is 2-7 times lower than  $\text{Na}^+$  when equimolar concentrations are compared. Acetylcholine penetrates into the CNS rapidly due to its metabolism, since in the presence of eserine its influx rate becomes similar to that of the analogous alkylammonium cation which is unmetabolized. The alkylammonium cations that penetrate into the CNS appear to be distributed into fast and slow pools, as judged by their relative rates of efflux. These may represent the distribution of cations between extracellular and intracellular spaces or possibly free and bound cations in the extracellular spaces. Although the alkylammonium cations penetrate the CNS, they apparently encounter a regulatory system that discriminates against large size, positive charge and polarity.

OMISSION. Reference should here be made to 464 and 465, erroneously listed in the wrong context.

- 464 Espinola, H.N., Capuñay, R., Silva, J.E., da. EFFECTIVE HALF-LIFE OF  $^{32}\text{P}$  IN THE SANDFLY *Lutzomyia longipalpis* (Lutz AND Neiva, 1912) (DIPTERA PSYCHODIDAE). *Revta bras. Biol.* **26** (1966) 175-179.

- 465 Fairbanks, L.D., Burch, G.E. TURNOVER OF RADIOSODIUM IN *Drosophila melanogaster* ADULTS OF DIFFERENT AGES. *J. Insect Physiol.* **12** (1966) 591-599.

- 62 Getsova, A.B., Volkova, G.A. NEW DATA ON THE ACCUMULATION OF  $^{35}\text{S}$  AND  $^{106}\text{Ru}$  BY LARVAE OF DRAGONFLIES. *Ent. Obozr.* **46**, 1 (1967) 68-69. (In Russian)

The accumulation of  $^{35}\text{S}$  and  $^{106}\text{Ru}$  in larvae of dragonflies (*Aeschna grandis*) decreases with an increase of physiolog. age. (CA 67; 1967, 30098p)

- 63 Grosch, D.S. DISTRIBUTION AND EFFECTIVE HALF-LIFE OF COBALT-58 IN Habrobracon. Nature, Lond. 208 (1965) 906-907.

Young Bracon hebetor females were fed a single meal from a mixture containing 100  $\mu$ Ci of carrier-free  $^{58}\text{CoSO}_4$ /ml of saturated sugar water. The average wasp weighing 1.32 mg ingested  $\sim 0.5 \mu\text{Ci}$  of  $^{58}\text{Co}$ . The radioactivity of each of a sample of 31 wasps was determined daily until death, using a conventional scintillation, well counter connected to an integrating decade scaler. The eggs deposited were collected daily and hatchability determined after 48 h. From other samples, the radioactivity for the whole body, for the anterior and the posterior parts transected at the petiole, the gut, the fat body with urates and the genital system obtained by dissection was determined (usually three counts/specimen). The average life span of wasps fed  $^{58}\text{Co}$  compared well with controls (25.6 $\pm$ 3.1 d against 28.4 $\pm$ 2.0 d). The effective half-life was attained by the 3rd-d following a  $^{58}\text{Co}$ -meal. During this period most of  $^{58}\text{Co}$  was abdominal, falling from 98% in the first 2 h through 94% at the end of the 1st-d to 90% on the 3rd-d. Even after 20 d, 89% of the  $^{58}\text{Co}$ -burden was abdominal. The level of the gut and its contents paralleled that of the abdomen. Neither ovaries nor eggs became appreciably radioactive. Hatchability was low only on the 1st-d (76%), rising to 98% on the 2nd-d. - Investigations of the bioaccumulation of isotopes of the transition elements appear essential for an understanding of potential environmental hazards.

- 64 Harvey, W.R., Nedergaard, S. SODIUM-INDEPENDENT ACTIVE TRANSPORT OF POTASSIUM IN THE ISOLATED MIDGUT OF THE Cecropia SILKWORM. Proc. natn. Acad. Sci. U.S.A. 51 (1964) 757-765.

The lumen of an isolated larval midgut is 84 mV positive to the blood side. K diffusion potentials contribute to but do not account for the trans-midgut potential. The  $^{42}\text{K}$  flux toward the lumen is 32 times greater than predicted from the electrochemical potential. At least 87% of the short-circuit current is carried by K moving towards the lumen. Apparently, Na plays no role in this active K transport since similar potential differences, short-circuit currents, and K fluxes are obtained with or without Na. (Auth.summary)

- 65 Harvey, W.R., Haskell, J.A., Zerahn, K. ACTIVE TRANSPORT OF POTASSIUM AND OXYGEN CONSUMPTION IN THE ISOLATED MIDGUT OF Hyalophora cecropia. J. exp. Biol. 46, 2 (1967) 235-248.

Flux measurements with  $^{42}\text{K}$  reveal that in the isolated midgut of H. cecropia 90 - 100% of the short-circuit current is carried by the active transport of K from the blood-side to the lumen. When K-transport is strongly depressed, either by withholding K from the blood side or by imposing a large positive potential on the lumen, the O-uptake of the isolated gut remains virtually unchanged. If the K-transport were to be energized by the negligible increase in oxygen uptake about 40  $\mu$ -equivalent of K would have to be transported for every  $\mu$ -equivalent of extra oxygen taken up. This ratio of K-transport to O-uptake is thermodynamically impossible. The ratio of K-transported to total O consumed when the midgut is bathed with 32 mM K on both sides is about 1.3 at temperatures of 25 and 15°C. The ratio must be smaller at lower K concentrations and is 2.0 at 73.5 mM-K, which may be approaching the max. value. Although the O-uptake is independent of the K-transport, the reverse is not true. There is a close dependency of K-transport on oxygen consumption. K-transport by the midgut contrasts with Na-transport by the frog skin because Na-transport stimulates oxidative metabolism whereas K-transport does not. Evidently the coupling of transport to energy supply is different in the two systems. (Auth.)

- 66 Haskell, J.A., Clemons, R.D., Harvey, W.R. ACTIVE TRANSPORT BY THE Cecropia MIDGUT. I. INHIBITORS, STIMULANTS, AND POTASSIUM-TRANSPORT. J. cell. comp. Physiol. 65 (1965) 45-56.

Harvey and Nedergaard (see ref. 64) have shown the midguts isolated from mature larvae of the Cecropia silkworm, when perfused in aerated, agitated physiological solution, exhibit a large electrical potential with the lumen-side positive to the blood-side. Isotope studies\* show that potassium carries 83% of the current generated by the midgut when the potential is short-circuited. These and other data demonstrate that potassium is actively transported from blood-side to lumen-side of the midgut epithelium. Neither the potential nor the current requires sodium. The effects of various chemicals on this Na-independent active transport of K were examined. The short-circuit current

\* $^{42}\text{K}$

was rapidly and reversibly inhibited by anoxia and 2,4-dinitrophenol. An irreversible inhibition was effected by iodoacetate. No observable change was produced by cholinesterase inhibitors, adrenalin, pituitary hormones or small changes in pH. Ouabain, a cardiac glycoside which is thought to be a specific inhibitor of sodium transport, was without effect at concentrations as high as  $10^{-4}$  M. Barely affected by 5%  $\text{CO}_2$ , the current was strongly and reversibly depressed by 25%  $\text{CO}_2$ . The carbonic anhydrase inhibitor hygroton at  $10^{-3}$  M was without effect but the related sulfonamide cardrase caused 36% inhibition at this concentration. The sulfonamides are barely soluble in water and perhaps penetrate the midgut cells with difficulty. Another type of carbonic anhydrase inhibitor, sodium sulfide, caused reversible inhibitions of 31% at  $10^{-4}$  M and 87% at  $10^{-3}$  M respectively. Clearly the K transporting system of *Hyalophora cecropia* has important differences from Na systems, and possibly employs a K, H ion-linked pump. (Auth.)

- 67 Hungate, F.P. RESPONSE OF INSECTS TO RADIATION. p.1 of "Pacific Northwest Laboratory Monthly Activities Report, April 1966, on AEC Division of Biology and Medicine Programs". BNWL-257, Battelle-Northwest, Richland, Wash. Pacific Northwest Lab. May 1966, 18p.

In a preliminary study to investigate the pathways of insecticides (distribution, concentration, translocation) in the flour beetle,  $^{131}\text{I}$  was injected orally. After an (?) interval assumed sufficient for distribution ~50% of the injected  $^{131}\text{I}$  was found in the thorax rather than in the abdomen as expected.

- 68 Jones, T.H. THE UPTAKE OF STRONTIUM 90 BY *Chironomus varus*. *Entomologist* 100 (1967) 2.

It was observed that 20 out of 30 dissected snails, *Limnaea pereger* (Mull), contained larval stages of *Chironomus* (*Parachironomus*) *varus* in the mantle cavity. The Ca ion concentration of the Shropshire Union Canal where they were found was 66 ppm  $\text{Ca}^{++}$  and the pH was 7.4. The radioactivity was expressed as an Accumulation Factor (AF), based on the fresh weight of the organism.

$$\text{AF} = \frac{\text{cpm of larva}}{\text{fresh weight in g}} \times \frac{\text{volume of liquid taken}}{\text{cpm of liquid}}$$

The larvae concentrated  $^{90}\text{Sr}$  (AF of 6 at 5 d) and since these larvae form the diet of other aquatic organisms there is a possibility of further contamination in the food chain. Since  $^{90}\text{Sr}$  has a half-life of 28 y it would have a long term effect on other organisms, with possible genetic effects.

- 69 Kallay, N., Tigyi-Sebes, A. LOCALIZATION OF  $^{32}\text{PO}_4^{3-}$  IN ISOLATED MUSCLE FIBRIL. *Acta biochim. biophys.* 2, 2 (1967) 221-224.

Bees were fed labelled  $\text{Na}_2\text{HPO}_4$  and the thorax muscle examined for  $^{32}\text{P}$  uptake. Approximately 75% of the activity was found in the A-band of the fibril, 17% in the Z-line, and the remainder in the I-band. (CA 67: 1967, 106283t)

- 70 Lahiri, S.K., Roy, A., Banerjee, D., Jamdar, S.C., Basu, S.R., Nag, B.D. IONIC DISTRIBUTION STUDIES IN HEMOLYMPH FROM DIFFERENT PARTS OF *Periplaneta americana* BY RADIOACTIVE TRACERS. *Indian J. exp. Biol.* 5, 1 (1967) 27-28.

Adult cockroaches were injected with carrier-free  $^{24}\text{NaCl}$ ,  $\text{H}^{36}\text{Cl}$ ,  $\text{Na}^{131}\text{I}$ , and  $\text{Na}_2\text{H}^{32}\text{PO}_4$  in aqueous solutions and  $^{59}\text{FeCl}_3$  in HCl between the 3rd and 4th-somite of the abdomen. The radioactivity was measured at regular intervals in the haemolymph collected from the antenna and femur of each insect. In general, the femoral haemolymph was richer in inorganic cation and poorer in anion than the antennal haemolymph. The concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{HPO}_4^{2-}$ , and  $\text{I}^-$  differed in the antennal and femoral haemolymphs, while the concentration of  $\text{Fe}^{3+}$  was the same in the 2 haemolymphs. The antennal haemolymph had larger amounts of  $\text{Na}^+$  and  $\text{I}^-$  and lesser amounts of  $\text{Cl}^-$  and  $\text{HPO}_4^{2-}$  than the femoral haemolymph. The internal radiation had no effect on the relative ion distribution in the antennal and femoral haemolymph, even when heavy doses, i.e. up to 100  $\mu\text{Ci}$  of the isotope was administered to each insect. (CA 67: 1967, 30134x)

- 71 Lezzi, M., Kroeger, H. AUFNAHME VON  $^{22}\text{Na}$  IN DIE ZELLKERNE DER SPEICHELDRÜSEN VON *Chironomus thummi*. (The uptake of  $^{22}\text{Na}$  into cell nuclei of salivary glands of *Chironomus thummi*.) *Z. Naturf.* 21b, 3 (1966) 274-8 (In German with English abstract)

The in vivo and in vitro uptake of  $^{22}\text{Na}$  from the haemolymph into cell nuclei of larval salivary glands was measured and compared. The uptake of  $^{22}\text{Na}$  in vivo follows approximately a saturation

curve. The respective in vitro curve has a much steeper slope during the first 8 min and this phase is followed by an interval during which the  $^{22}\text{Na}$  content of the nuclei decreases (8 - 16 min). After the 16th-min it increases again to reach a nuclear  $^{22}\text{Na}$  content approximately twice as high as that of nuclei in vivo at 60 min. The uptake curves are discussed in relation to recent findings on the induction of puffs in polytene chromosomes by inorganic ions. (Auth.)

- 72 Lezzi, M. SPEZIFISCHE AKTIVITÄTSSTEIGERUNG EINES BALBIANIRINGES DURCH  $\text{Mg}^{2+}$  IN ISOLIERTEN ZELLKERNEN VON Chironomus. (Specific activity increase in a Balbiani ring in isolated cell nuclei of Chironomus by means of  $\text{Mg}^{2+}$ .) Chromosoma 21, 1 (1967) 109-122. (In German, with English abstract)

$\text{Mg}^{2+}$  induces in isolated salivary gland nuclei of C. thummi and C. tentans a specific enlargement of one Balbiani ring, whereas  $\text{Na}^+$  or  $\text{K}^+$  do not. Incorporation of  $^3\text{H}$ -uridine suggests that this specific enlargement is a sign of increased RNA synthesis of this chromosome region. Based on recent studies on the structure and function of Balbiani rings a model is proposed to explain the mechanism by which  $\text{Mg}^{2+}$  differentially influences chromosomal RNA synthesis. (Auth.)

- 73 Moulder, B.C. ECOLOGICAL STUDIES OF FOREST FLOOR SPIDER POPULATIONS. Paper presented at the "American Institute of Biological Sciences Meeting. College Station, Tex., Aug. 1967".

Measurements of radiocaesium turnover and oxygen consumption were made on  $^{134}\text{Cs}$  tagged spiders as part of a radiotracer study to measure the energy balance of forest floor spider populations. Various lycosids (Lycosa punctulata, Pardosa sp., and Schizocosa sp.) and gnaphosids (Gnaphosa fontinalis and Drassyllus virginianus) were used in the experiments. A body size relationship occurred with both radiocaesium turnover and oxygen consumption, with larger individuals tending to have longer biological half-lives for  $^{134}\text{Cs}$  and lower  $\text{O}_2$  consumption rates. Measurements made at three temperatures (15, 20, and 25°C) showed an increase in metabolic activity with higher temperatures, reflected by shorter mean biological half-lives and higher mean respiratory rates. Number and biomass estimates, obtained by bi-weekly collections of 0.1 m<sup>2</sup> and 1 m<sup>2</sup> litter samples, indicate the importance of spiders as predators in the forest cryptozoan community. Data on radiocaesium elimination rates and equilibrium values in the field, respiratory rates, number and biomass estimates, and calorific equivalents will be used to calculate the energy budget for this major group of forest floor predators. (Abstr.)

- 74 Oftedal, P. A STUDY OF THE RETENTION AND THE MUTAGENIC MODE OF ACTION OF RADIO-ACTIVE PHOSPHORUS IN Drosophila melanogaster. Hereditas 40 (1959) 245-331.

The genetic effects of  $^{32}\text{P}$  on different stocks of D. melanogaster were examined.  $^{32}\text{P}$  was added to standard medium when raising males. Only few experiments were conducted with  $^{91}\text{Y}$ . Some data were obtained on the part played by  $^{32}\text{P}$  and  $^{91}\text{Y}$  in metabolism, their incorporation into sperm, and their mutagenic effects especially in the rate of induction of lethals.

- 75 Oftedal, P., Kaplan, W.D. SOME ASPECTS OF TRANSMUTATION STUDIES IN Drosophila. "Biological Effects of Decay of Incorporated Radioisotopes, Panel Meeting. Vienna, Austria, 9-13 Oct. 1967".

- 76 Patterson, R.S., Smittle, B.J., Lofgren, C.S. DISTRIBUTION OF  $^{32}\text{P}$  IN THE MALE REPRODUCTIVE SYSTEM OF Culex pipiens quinquefasciatus (Say). Bull. ent. Soc. Am. 13, 3 (1967) 201. Abstr. 317 "New York Meeting of the Entomological Society of America. New York, N.Y., USA. 27-30 Nov. 1967".

4th-instar male larvae of Culex p. quinquefasciatus exposed for 48 h to  $^{32}\text{P}$  at the rate of 0.25  $\mu\text{Ci}/\text{ml}/\text{larva}$  accumulate enough radioactivity so that they can transfer a detectable amount to females during insemination. The radioactivity is found in both the sperm and the accessory gland fluid. (Abstr.)

- 77 Plummer, G.L. BIOACCUMULATION OF RADIONUCLIDES ON GRANITIC OUTCROPS IN THE GEORGIA PIEDMONT. Final Report. NP-16464, Georgia Univ., Athens. Oct. 1966, 68p.

A 5 yr study of the accumulation of radioisotopes by plants and animals inhabiting the granitic outcrop ecosystems of the Georgia Piedmont was conducted beginning in 1961. Natural and fallout radioactivity was investigated by assaying the granites, soils, some plants, insects, and animals for quantities of radioisotopes. Attempts were made to explain actions and interactions between various radioisotopes, soils, plants, water-flow through communities and concentrations within components of the respective

ecosystems.  $^{137}\text{Cs}$ ,  $^{90}\text{Sr}$ , and  $^{54}\text{Mn}$  concentrations were of the greatest interest and data on their uptake by plants, insects, and deer are detailed. (NSA 21; 1967, 19759)

- 78 Quraishi, M.S., Brust, R.A., Lefkovitch, L.P. UPTAKE, TRANSFER, AND LOSS OF  $^{32}\text{P}$  DURING METAMORPHOSIS, MATING, AND OVIPOSITION IN *Aedes vexans*. J. econ. Ent. 59, 6 (1966) 1331-1338.

The uptake of  $^{32}\text{P}$  as a function of time per unit dry weight of *A. vexans* (Meigen) larvae has been studied. There was a significant uptake of  $^{32}\text{P}$  by instar IV larvae up to 72 h, although the rate of uptake was very rapid during the first 24 h. There was little, but measurable, transfer of radioactivity from radioactive males during induced copulation. The radioactive female deposited appreciable radioactivity with the eggs. (Auth.)

- 79 Reichle, D.E. THE BEHAVIOUR OF RADIOCAESIUM DURING INSECT METAMORPHOSIS. ASB Bull. 13, 2 (1966) 44. Abstr.

The evergreen bagworm, *Thyridopteryx ephemeraeformis*, was employed as a model for the uptake and fate of  $^{137}\text{Cs}$  during transformation in insect populations feeding upon contaminated vegetation. Pupating female larvae were placed in artificial cases simulating natural bagworm cases in which normal development occurs (88 d), and reared to adults at mean field temperatures of  $72^\circ\text{F}$  (40 d). Essentially the entire radiocaesium body burden was transferred from the larval to pupal stage, with only 0.3% lost with the cast skin at ecdysis. The activity density in the pupa increased by about 16% over the larval stage. Expressed as a percentage of pupal radioactivity the distribution was: 10% to puparium and miscellaneous residues, 40% to egg clutch, and 50% remaining with the female after oviposition. Slightly less than half the female body burden of radiocaesium (44.5%) was transferred in egg production, which in itself accounted for 82.4% of the total female biomass. (Abstr.)

- 80 Reichle, D.E. MEASUREMENT OF ELEMENTAL ASSIMILATION BY ARTHROPODS. Bull. ecol. Soc. Am. 48, 2 (1967) 64. Abstr.

Measurements of nutrient assimilation by animals from dietary inputs often are based upon analyses of chemical contents of food and faeces. Assimilation measurements based upon faecal concentrations, however, are biased by the organism's turnover of materials through normal excretory and secretory processes. Developments in radioisotope tracer methodology provide means for estimating nutrient absorption from food. Animals fed upon radioisotope tagged food acquire body burdens of isotope (Q) which decrease as  $dQ/dt = -kQ$ . For many organisms, the turnover rate of isotope (k) consists of a two-component system with two interacting rate coefficients ( $k_1$  and  $k_2$ ). The coefficient  $k_1$  represents turnover of non-assimilated isotope in food in the gut, and  $k_2$  the turnover of assimilated isotope in body tissues. Graphical analyses of retention curves and separation of rate coefficients, permit estimation of percent of whole-body radioactivity (Q) lost at each rate. For single feedings of isotope, these percentages may be related to the fraction of Q in body tissue ( $q_1$ ) and that remaining with undigested food ( $q_2$ ). Element assimilation and  $q_2$ , under these conditions, are equivalent. (Abstr.)

- 81 Shyamala, M.B., Bhat, J.V. MINERAL ASSIMILATION IN THE SILKWORM *Bombyx mori* L. Indian J. exp. Biol. 4, 1 (1966) 31-34.

- 82 Stobbs, R.H. THE EFFECT OF SOME ANIONS AND CATIONS UPON THE FLUXES AND NET UPTAKE OF CHLORIDE IN THE LARVA OF *Aedes aegypti* (L.), AND THE NATURE OF THE UPTAKE MECHANISMS FOR SODIUM AND CHLORIDE. J. expl. Biol. 47, 1 (1967) 35-57.

Measurements of fluxes were carried out on chloride influx alone, chloride outflux alone, simultaneous measurement of sodium and chloride influx, and simultaneous outflux of sodium and chloride. Instead of the short-lived  $^{24}\text{Na}$  isotopes  $^{22}\text{Na}$  and  $^{36}\text{Cl}$  were used (see III/42 for details of method). The anal papillae of the aquatic larva of *A. aegypti* are responsible for 90% of the steady-state exchange of chloride. The relationships between chloride flux and external chloride concentration are approximately described by the Michaelis equation. There is net-uptake of chloride, independent of uptake of sodium, from  $\text{KCl}$ ,  $\text{CaCl}_2$  and  $\text{NH}_4\text{Cl}$ , probably in exchange for  $\text{OH}^-$  or  $\text{HCO}_3^-$ , but the rate is much slower than from  $\text{NaCl}$ . The following ions stimulate influx of chloride from 0.1 mM/l.  $\text{KCl}$ :  $\text{H}^+ = \text{Na}^+ > \text{K}^+$ . The following ions inhibit it:  $\text{OH}^- > \text{HCO}_3^- > \text{NO}_3^-$ . Movements of sodium and chloride ions are explicable in terms of an anionic and a cationic carrier located in an osmotic barrier in the papillae, the carriers being functionally coupled to sodium and chloride pumps located at the inner

surface of the barrier. An attempt is made to relate these findings to recent electron microscopical studies of the papillae.

- 83 Timofeeva-Resovskaya, E.A., Getsova, A.B., Timofeev-Resovskii, N.V. EFFECT OF EDTA ON COEFFICIENTS OF ACCUMULATION OF VARIOUS RADIOISOTOPES FROM WATER SOLUTION BY SWEET-WATER HYDROBIONTS. Trudy Inst. Biol., Sverdlovsk (1965) 47-61. (In Russian)  
The effects of EDTA on the coefficients of accumulation of  $^{35}\text{S}$ ,  $^{59}\text{Fe}$ ,  $^{60}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{90}\text{Sr}$ ,  $^{91}\text{Y}$ ,  $^{95}\text{Zr}$ ,  $^{95}\text{Nb}$ ,  $^{106}\text{Ru}$ ,  $^{137}\text{Cs}$ , and  $^{144}\text{Ce}$  by the sweet-water plants, *Elodea canadensis*, *Ceratophyllum demersum*, *Lemna minor*, *Chara fragilis*, and leeches *Herpodeilla* and larvae of *Culex pipiens* were studied in 3-litre glass vessels with lake water, with or without sand bottom. EDTA was added at 400 mg/litre, radioisotopes in concentrations corresponding to 1000 disintegrations/min/litre of water. Samples were taken after 2, 4, 8, and 16 d; water samples were evaporated, plants and leeches weighed, dried to constant weight, and pulverized. The presence of EDTA did not affect coefficients of accumulation of S by plants, leeches and mosquitoes, increased those for Sr and Cs (except for Cs accumulation by mosquitoes), decreased a little those for Zr, Nb, and Ru, and considerably those for Fe, Co, Zn, Y, and Ce. All four kinds of plants, as well as leeches and mosquitoes, gave practically the same results, with negligible differences in accumulation coefficients. (CA 65: 1966, 5826d)
  - 84 Topozada, A., O'Brien, R.D. PERMEABILITY OF THE GANGLIA OF THE WILLOW APHID, *Tubero-lachnus salignus*, TO ORGANIC IONS. J. Insect Physiol. 13 (1967) 941-954.  
A technique was developed that made possible the study of influx of compounds into the ganglia of the willow aphid. Penetration of  $^{14}\text{C}$  fatty acids into the aphid ganglia was biphasic, and increased with increasing liposolubility from acetate to valerate, but influx of hexanoate and heptanoate was similar to that of valerate. The influx rate of acetate into the aphid ganglia was almost twice that into the American cockroach abdominal nerve cord, but influx of valerate was similar in both species. Increasing liposolubility resulted in a greater increase in influx in the case of the cockroach than the aphid. The influx of  $^3\text{H}$ -quaternary ammonium cations into the aphid ganglia was similar but slightly smaller than influx into the cockroach nerve cord. The kinetics were biphasic, differing in this way from the case of the cockroach. Increasing liposolubility tended to increase penetration, and increasing size, with respect to the smallest cross-sectional area, tended to decrease it. Metabolism of the organic ions in the aphid ganglia increased influx rate, as shown by studies on influx of acetylcholine alone and in the presence of eserine, and valerate alone and in the presence of DNP. The rate of penetration into the aphid ganglia was much higher for the fatty acids than the quaternary ammonium compounds. There seems to exist in the aphid ganglia a 'regulatory system' that restricts influx, especially of large, polar, and unmetabolisable cations. (Auth.)
  - 85 Treherne, J.E. THE EFFECT OF OUABAIN ON THE EFFLUX OF SODIUM IONS IN THE NERVE CORDS OF TWO INSECT SPECIES (*Periplaneta americana* AND *Carausius morosus*). J. exp. Biol. 44 (1966) 355-362.  
The intracellular efflux of  $^{22}\text{Na}$  from the nerve cord is reduced by the presence of ouabain in the bathing medium. With *Periplaneta*  $10^{-4}\text{M}$  ouabain caused a reduction in the rate constant for sodium efflux from  $3.94 \times 10^{-3} \text{ sec}^{-1}$  to  $1.80 \times 10^{-3} \text{ sec}^{-1}$  and in *Carausius* from  $1.14 \times 10^{-3} \text{ sec}^{-1}$  to  $6.18 \times 10^{-4} \text{ sec}^{-1}$ . Uncoupling of the energy supply to the sodium pump, by the addition of cyanide, did not further reduce the sodium efflux from ouabain-treated axons, suggesting that the greater part of the active extrusion of this cation is effected by a single ouabain-sensitive carrier mechanism. The relative insensitivity to ouabain of the insect axons, as compared with squid giant axons, is shown to result from the presence of a rather leaky axon membrane in which the carrier-mediated component forms a much smaller part of the total flux. The concentration gradient of sodium across the insect axon membrane is related to the combined effects of the activity of the sodium pump and the passive permeability of the membrane. (Auth. summary)
- See also:
- 205 The effect of different ions on the incorporation of ( $\text{U-}^{14}\text{C}$ ) valine into fat body protein of the larva of the blowfly, *Calliphora erythrocephala*. (Price, G.M.)
  - 314 Incorporation of radioactive uridine into the RNA of the lepidopteran, *Barathra brassicae*. (Morris, O.N., 1966)

- 443 Health in uranium mining. (Anonymous, 1964)
- 496 Pacific northwest laboratory monthly activities report, October 1966, on AEC division of biology and medicine programs. (Battelle-Northwest, Richland, Wash. Pacific Northwest Lab., 1966)
- 507 Radiation ecology. (Auerbach, S.I., 1967)
- 515 <sup>106</sup>Ru elimination by brown crickets (*Acheta domesticus*). (Crossley, D.A., Jr., 1966)
- 516 Comparative movement of <sup>106</sup>Ru, <sup>60</sup>Co, and <sup>137</sup>Cs in arthropod food chains. (Crossley, D.A., Jr., 1967)
- 518 Etude de la contamination externe de l'abeille et de son milieu par un radio-isotope introduit dans la nourriture. (Donault, P., 1966)
- 519 Amélioration d'une technique de recherche de la contamination de l'abeille marquée au moyen d'un radio-isotope. (Donault, P., 1966)
- 521 Review and discussion of barium. (French, N.R., 1963)
- 522 Accumulation of individual radioisotopes by different aquatic insects. (Getsova, A.B. et al., 1964)
- 523 Bacterial transport of radiophosphorus in a stream ecosystem. (Hooper, F.F. et al., 1966)
- 525 A radionuclide tracer study of arthropod food chains in a *Spartina* salt marsh ecosystem. (Marples, T.G., 1965)
- 529 Radioecology of the Colorado range. (Osburn, W.S., Jr., 1964)
- 530 Rates and pattern of radioisotope release from fresh tree litter at three levels of mesic forest development. (Petty, R.O. et al., 1965)
- 531 Investigation of <sup>32</sup>P-uptake and clearance in some brackish organisms of the Agiea and the Eforie Lakes. (Pora, E. et al., 1965)
- 533 Trophic level concentrations of Cs-137, sodium, and potassium in forest arthropods. (Reichle, D.W. et al., 1965)
- 535 Possibility of contamination of insectivorous game birds during ecological investigations using insects labelled with <sup>60</sup>Co. (Wegorek, W. et al., 1963)
- 536 Remarques sur l'incorporation de <sup>35</sup>S par une grégarine intestinale d'insecte orthoptère. (Corbel, J.C., 1965)
- 543 Efficiency of radioisotope transfer in predator-prey systems. (Crossley, D.A., Jr. et al., 1966)
- 544 Radioactive tracer measurements of predation by arthropods. (Crossley, D.A., Jr., 1967)
- 548 Radioisotope measurement of food consumption by a leaf beetle species, *Chrysomela knabi* Brown. (Crossley, D.A., Jr., 1966)
- 553 Rates and pattern of radioisotope release from fresh tree litter at three levels of mesic forest development. (Petty, R.O. et al., 1965)
- 638 Insecticide mode of action. Effect of dieldrin on ion movement in the nervous system of *Periplaneta americana* and *Blattella germanica* cockroaches. (Hayashi, M. et al., 1967)
- 659 Interactions of DDT with components of American cockroach nerve. (Matsumura, F. et al., 1966)

### 1.2.3. Carbohydrates.

- 86 Briceux-Grégoire, S., Jeuniaux, C., Florin, M. BIOCHEMISTRY OF THE SILKWORM. XXX. BIOSYNTHESIS OF TREHALOSE AND GLYCOGEN FROM GLUCOSE 1-PHOSPHATE. Comp. Biochem. Physiol. 16, 4 (1965) 333-340.

Glucose 1-phosphate (I) was injected into 5th-instar silkworm (*Bombyx mori*) larvae, which were either well fed or poorly fed. Adipose tissue and haemolymph analysis 4h later showed that fat body glycogen (II) synthesis proceeded more rapidly in the poorly fed larvae. I was used principally for haemolymph trehalose (III) synthesis; the turnover of haemolymph II was similar during both nutritional states. Adipose tissue II and III were poorly labelled with 0.28-0.62% and 0.03-0.14% incorporation respectively, compared with 12-15% radioactivity incorporation in haemolymph III. III could probably be synthesised from pyruvate in locations other than the fat body, and the bio-synthetic III pathway does not necessarily pass through II. III and II syntheses, and II hydrolysis could be controlled, directly or indirectly, by the variation of the haemolymph III levels. When haemolymph III level was increased, as in well-fed larvae, II synthesis occurred, whereas during malnutrition, when III levels decreased, III was synthesised.

- 87 Chefurka, W. ESTIMATION OF PATHWAYS OF CARBOHYDRATE METABOLISM IN INSECTS. Proc. ent. Soc. Ont. 96 (1965) 17-23. Presented at the "102nd Annual Meeting of the Entomological Society of Ontario, London, England. 8 Sep. 1965.

Two approaches have been used: (1) the radiorespirometric method which monitors the yield of  $^{14}\text{CO}_2$  from variously labelled glucose molecules. The pattern of release of  $^{14}\text{CO}_2$  with time permits certain conclusions as to the nature of the pathways involved, the yields and evaluation of the extent of participation of these pathways. A continuous time-course record of metabolic events is obtained. (2) the Katz and Wood method, based on the fact that the pentose cycle randomizes the  $^{14}\text{C}$  between carbons-1, and -3 of glucose-6-phosphate or its derivative, glycogen, when glycogen-2- $^{14}\text{C}$  is metabolised. The extent of the randomization is obtained from the radioactivity of carbon-1 and -3 relative to that of -2 in glucose residues of hydrolysed glycogen. In intact *Periplaneta americana* and *Oncopeltus fasciatus* 13-17% of the glucose is catabolised by the pentose cycle, the bulk of it being dissimilated by the glycolysis-tricarboxylic acid cycle. Very little difference in activity of this pathway was noted between the sexes of *Oncopeltus* and *Melanoplus bivittatus*, but a striking sexual difference was noted for *Periplaneta* where the pentose cycle was more active in the male. The pentose cycle evidently plays a much more prominent role in glucose metabolism in *Melanoplus* than in the other two species. The occurrence of the pentose cycle is accentuated in the presence of inhibitors of glycolysis and/or the tricarboxylic cycle.

- 88 Chefurka, W. INTERMEDIARY METABOLISM OF CARBOHYDRATES IN INSECTS. p.581-667 of "The Physiology of Insecta, Vol.2". Rockstein, M., Ed. New York, Academic Press Inc. 1965.

This review is broken down into sections on the degradation of polysaccharides and oligosaccharides to monosaccharides (subdivided into carbohydrates, transglycosidases, and phosphorylases); the synthesis of polysaccharides and oligosaccharides from monosaccharides (particularly the synthesis of glycogen and trehalose); the fermentation and oxidation of carbohydrates (subdivided into glycolysis, the citric acid cycle, the electron transport system, oxidative phosphorylation, and the pentose phosphate cycle); the in vivo significance of metabolic routes in insects; and the regulation of metabolisms (in terms of enzymic and hormonal regulation). An extensive bibliography is attached containing numerous references to studies in which radioisotopes had been used.

- 89 Cheldelin, V.H. POSSIBLE METABOLIC CONTROL MECHANISMS IN THE PENTOSE PHOSPHATE OXIDATION PATHWAY. Proc. Robert A. Welch. Found. Conf. Chem. Res. 5 (1961) 281-285.

For *Acetobacter suboxydans*, fructose 1,6-diphosphate is hydrolysed to fructose 6-phosphate in the presence of  $\text{Mg}^{++}$  and diphosphothiamine (I). For chick, hog, or beef embryos, and also for successive developmental forms of blowflies, the ratio of yields of  $^{14}\text{CO}_2$  from glucose-1- $^{14}\text{C}$  and from glucose-6- $^{14}\text{C}$  are initially high but decrease with increasing age. Control of pentose oxidation versus glycolysis may lie in cell concentration of NADP or of I and  $\text{Mg}^{++}$ . (CA)

- 90 Clements, A.N., Grace, T.D.C. THE UTILIZATION OF SUGARS BY INSECT CELLS IN CULTURE. J. Insect Physiol. 13, 9 (1967) 1327-1332.

D-glucose- $^{14}\text{C}$  (3.57 mCi/mM) was used to prepare radioactive trehalose biosynthetically. Cells of *Antheraea eucalypti* Scott (Lepidoptera: Saturniidae) in culture utilize trehalose at approximately the same rate as glucose. Sucrose (sucrose- $^{14}\text{C}$  was used, at 22.8 mCi/mM) is not utilized during the first days of a subculture, and its subsequent utilization coincides with a fall in glucose concentration.

- 91 Courtois, J.E., Percheron, F., Guillaux, E. PURIFICATION ET ETUDE DE LA SPECIFICITE DE LA TREHALOSE DU HANNETON (*Melanothia vulgaris*). C.r. hebd. Séanc. Acad. Sci. 265, 25D (1967) 2111-2114.

Les auteurs décrivent la purification de la tréhalase extraite du Hanneton, et indiquent les propriétés de cette osidase. En effectuant une hydrolyse du tréhalose en présence de glucose marqué au  $^{14}\text{C}$ , les auteurs n'ont pu mettre en évidence aucune incorporation de glucose radioactif dans le tréhalose. L'absence d'hydrolyse de dérivés substitués non symétriques du tréhalose montre que les hydroxyles en position 3 et 6 des deux unités glucosyle du tréhalose doivent être libres pour que l'action hydrolisante de l'enzyme se manifeste. De nombreux composés intermédiaires du métabolisme glucidique sont dépourvus de pouvoir inhibiteur, et cette tréhalase est dépourvue d'activité transférante.

- 92 Engels, W., Bter, K. GLYKÖGENSPEICHERUNG WÄHREND DER OÖGENESE UND IHRER VORZEITIGEN AUSLÖSUNG DURCH BLOCKIERUNG DER RNS-VERSORGUNG (UNTERSUCHUNGEN AN Musca domestica L.) (Glycogen accumulation during oogenesis and its premature release by blocking of the RNA supply. (Study on Musca domestica L.)) Wilhelm Roux Arch. EntwMech. 158, 1 (1967) 84-88. (In German, with English summary)

$^3\text{H}$ -uridine, -histidine, -leucine, -lysine, and  $^3\text{H}$ -valine were used. In the oocyte of the house fly, M. domestica L., glycogen is stored for the time at an advanced developmental stage (stage 5 of the classification used). Initial glycogen deposition is marked by a follicle epithelium serrated towards the ooplasm and by the degeneration of the trophocytes. During glycogen synthesis, the oocyte is no longer supplied with RNA, and the incorporation of amino acids declines. At the same time, with the formation of the chorion most of the glycogen grana are deposited into the reticuloplasm of the oocyte excluding the periplasm.  $^3\text{H}$ -glucose is incorporated only during glycogen synthesis. Already after 1 min of incubation, the pattern of labelling corresponds to that of the glycogen granules. Glycogen molecules are therefore concluded to remain at the site of synthesis. In normal development, no glycogen is found in nurse or follicle cells.

- 93-a Florkin, M., Jeuniaux, C. METABOLISME DU TREHALOSE ET DU GLYCOGENE CHEZ LE VER A SOIE, EN RELATION AVEC LA MUE, LE FILAGE ET LES METAMORPHOSES. Bull. Acad. r. Belg. Cl. Sci. 51, 5 (1965) 541-552. (With English summary)

The principal circulating form of saccharidic cellular food in the silkworm is trehalose. The enzyme trehalase, in the haemolymph, is normally inhibited. Inhibition is only suppressed during moulting, causing a decrease in trehalose concentration and an increase in the amount of free glucose. The muscles and most other tissues, such as the digestive tract, are able to use blood trehalose, thanks to an intracellular trehalase. The epidermis and the silk glands are devoid of trehalase: they use the free glucose liberated by the hydrolysis of the haemolymph trehalase during the periods of moulting and spinning. The problem of the origin of the trehalose is discussed, in the light of recent experiments, in which the incorporation of radioactivity from  $^3\text{H}$ -labelled pyruvate and glucose-1-phosphate into a fat body glycogen and haemolymph trehalose has been followed. The chitin of the cuticle is synthesised at every moulting process, partly at the expense of the glucose liberated by the hydrolysis of the trehalose in the haemolymph. On the other hand, the old cuticle is destroyed by the proteolytic and chitinolytic enzymes of the exuvial fluid. The hydrolytic products, especially N-acetylglucosamine, are resorbed by the epidermis and can be used for the biosynthesis of the chitin of the new cuticle. (Essentially auth. summary)

$^3\text{H}$   $^{14}\text{C}$

- 93-b Fristrom, J.W. DEVELOPMENTAL AND BIOCHEMICAL STUDIES ON THE MORPHOLOGICAL MUTANT CRYPTOCEPHAL OF Drosophila melanogaster. Diss. Abstr. 27, 10 (1967) 3402-B - 3403-B.

Studies have been conducted on the morphological mutant cryptocephal of D. melanogaster in an attempt to elucidate the mechanism of action of the mutant gene and also to uncover possible mechanisms of suppression of the cryptocephal gene by other genes in the stock. The main developmental abnormality of the cryptocephal mutant is that its head does not evert at the time of pupation. It has been possible to demonstrate that the mutant has greater mechanical resistance to head emergence than the wild type. Experimental evidence indicates that the increased resistance to head emergence is produced by an increase in rigidity of the integument. It has been possible to show that the cryptocephal mutant has more glucosamine (presumably chitin) in its integument at the time of pupation than the wild type. The increased content of glucosamine is presumed to be responsible for the increased rigidity of the integument. Biochemical evidence obtained from studies with glucose- $^{14}\text{C}$  indicates that the rate of chitin synthesis is higher in the mutant than in the wild type. The phenocopy of the cryptocephal mutant produced by glucosamine was shown to have also an increased content of glucosamine in its integument at pupation and also has increased resistance to head emergence. The common features found between the glucosamine-phenocopy and the cryptocephal mutant offer additional verification to the developmental model presented above for the action of the cryptocephal gene. The modifiers in the cryptocephal stock act by decreasing mechanical resistance to head emergence (and thus allow it) and by decreasing the excess content of glucosamine found in the cryptocephal integument. Cryptocephal stock

could be shown to resist the incorporation of free glucosamine. It is proposed that this resistance is one means by which the modifying genes decrease the content of glucosamine in the integument, and lower the mechanical resistance to head emergence. The relevance of the results to normal genetic activity during development and to the phenocopy concept is discussed. (From DA)

- 94 Gelperin, A. CONTROL OF CROP EMPTYING IN THE BLOWFLY. J. Insect Physiol. **12**, 3 (1966) 331-345.

The control of crop emptying in the blowfly, Phormia regina, was investigated by the technique of x-ray photography. Increasing the osmotic pressure of the solution in the crop slows crop emptying. This effect is independent of the nutritive value, stimulating power, and viscosity of the solution. Increasing the osmotic pressure of the blood also greatly slows crop emptying. Blood osmotic pressure is the controlling factor. The effect of increased blood osmotic pressure in slowing crop emptying is independent of nervous or endocrine elements. The rate of absorption of sugar across the midgut wall was studied using  $^{14}\text{C}$ -labelled carbohydrates. Two in vitro midgut preparations were employed. No evidence for active transport was found.

- 95 Gilby, A.R., Wyatt, S.S., Wyatt, G.R. TREHALASES FROM THE COCKROACH, Blaberus discoidalis: ACTIVATION, SOLUBILIZATION AND PROPERTIES. Acta biochim. polon. **14**, 1 (1967) 89-100.

The thoracic muscle of various insects contains an enzyme capable of hydrolysing trehalose. In several cockroaches and a grasshopper the enzyme activity of muscle homogenates is elevated several-fold repeated freezing and thawing, as previously shown for the Cecropia silkworm. With muscle of several flies, however, such treatment had no effect on trehalase activity. [ $1, 1^{14}\text{C}$ ] Trehalose was prepared by injecting 50  $\mu\text{Ci}$  of [ $1^{14}\text{C}$ ] glucose (0.65 mCi/mM) into each of two mature larvae of Hyalophora cecropia and permitting incorporation for 6 h at 25°C. In the cockroach, B. discoidalis, trehalase activity is concentrated in a microsomal fraction prepared by centrifugation at 105 000 g. Glycosidase activity is then specific for trehalose; it is inhibited strongly by sucrose and weakly by glucose and D-2-glucosamine. In Blaberus muscle microsomal preparations, trehalase activity is enhanced as a result of treatment with several anionic, cationic, and non-ionic detergents, and by incubation with snake venom, as well as by freezing and thawing. Snake venom, either whole or briefly boiled, gave the greatest activation (up to 10-fold). The activated enzyme exhibits maximal activity at pH 6.0; with untreated enzyme, this could not be determined precisely, but appears to be the same. The  $K_m$  for trehalose is 3.3 mM for untreated enzyme and 1.7 mM for activated enzyme. Of the detergents, only deoxycholate solubilizes the microsomal trehalase, and aggregation occurs on removal of the detergent. Snake venom yields trehalase in soluble form which gives single peaks on Sephadex G-200 columns and in sucrose gradient centrifugation. The apparent mol. wt from these methods are 80 000 and 63 000, respectively. It is suggested that this discrepancy may be due to bound lipid. B. discoidalis midgut tissue yields a distinct trehalase, which is entirely in the soluble fraction of homogenates and has pH optimum about 5.0 and  $K_m$  for trehalose 0.5 mM.

- 96 Gussin, A.E.S., Wyatt, G.R. SOLUBLE AND MEMBRANE-BOUND TREHALASES IN INSECTS. Proc. natn. Acad. Sci. India **74** (1965) 350.

- 97 Horie, Y. PATHWAYS OF CARBOHYDRATE METABOLISM IN INSECTS AND ITS REGULATION. Seibitsu Kagaku **17**, 2 (1965) 55-68.

A review. Radioisotopes were used in some of the studies cited. (\*)

\* Original article not available.

- 98 Kobayashi, M., Kimura, S. ACTION OF ECDYSONE ON THE CONVERSION OF  $^{14}\text{C}$ -GLUCOSE IN DAUER PUPA OF THE SILKWORM, Bombyx mori. J. Insect Physiol. **13** (1967) 545-552.

The effect of ecdysone on the incorporation of  $^{14}\text{C}$ -glucose in dauer pupa (brain removed) of B. mori was examined. Incorporation was compared in pupae 18 h after injection of ecdysone or water. Cumulative recovery of  $^{14}\text{CO}_2$  from  $^{14}\text{C}$ -glucose was much the same in the ecdysone-injected pupa as in the control, and in both the recovery of  $^{14}\text{CO}_2$  from  $1^{14}\text{C}$ -glucose was more than that from  $6^{14}\text{C}$ -glucose. Labelled glucose was converted to blood trehalose after ecdysone

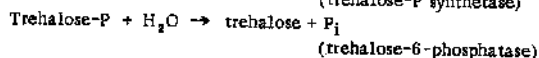
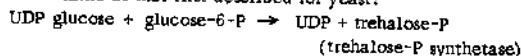
injection but to glycogen in the control. This therefore, suggests the possibility that ecdysone affects the relative activities of enzymes at the branching point of the pathways of trehalose and glycogen syntheses in the fat body, so that the pathway of trehalose synthesis is activated. The blood-sugar content of the 60-d-old dauer pupa with or without the injection of ecdysone was about 1.14 mg/ml, which is much lower than in a normal pupa. (Auth.)

- 99 Kobayashi, M., Kimura, S., Yamazaki, M. ACTION OF INSECT HORMONES ON THE FATE OF  $^{14}\text{C}$ -GLUCOSE IN THE DIAPAUSING BRAINLESS PUPA OF *Samia cynthia pryeri* (LEPIDOPTERA: SATURNIIDAE). *Appl. Ent. Zool.* 2, 2 (1987) 79-84.

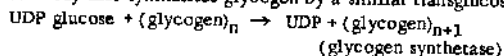
The mode of action of insect hormone and its related substance upon the metabolism of diapausing pupa was studied on the pupa of *S. cynthia pryeri*. The brain was extirpated from each pupa at the dormant stage. The brainless pupae were kept at 25°C for five weeks prior to use.  $^{14}\text{C}$ -glucose (0.1  $\mu\text{Ci}/\mu\text{g}$ ) was injected into the pupae with ecdysone, proteinic brain hormone or cholesterol having brain hormone activity, respectively. 24h following injection, the incorporation of radioactive glucose into carbon dioxide, trehalose, glycogen, crude lipid, and crude protein was measured by several methods, obtaining the following results. All of the substances acted on carbohydrate metabolism in the brainless pupa and promoted the biosynthesis of trehalose. In control insect without hormone or cholesterol injection, however, radioactive glucose was incorporated into glycogen in the fat body and scarcely incorporated into blood trehalose. On the other hand, incorporation of  $^{14}\text{C}$ -glucose in crude lipid and crude protein showed a low value, whether insect hormone or cholesterol was injected. It is concluded that insect hormones, ecdysone and brain hormone, are concerned with the carbohydrate metabolism of *S. cynthia pryeri*. (Essentially auth.)

- 100 Murphy, T.A., Wyatt, G.R. ENZYMATIC REGULATION OF TREHALOSE AND GLYCOGEN SYNTHESIS IN THE FAT BODY. *Nature*, Lond. 202 (1964) 1112-1113.

The chief site of trehalose synthesis in insects is the fat body. There is evidence that the pathway is the same as that first described for yeast:



Fat body also synthesises glycogen by a similar transglucosylation:



These pathways have been verified in homogenates of fat body of *Hyalophora cecropia*. Trehalose-P synthetase was detected by measuring incorporation from both glucose- $^{14}\text{C}$ -6-P and UDP glucose- $^{14}\text{C}$  into trehalose, and by measuring the release of UDP from UDP glucose. The glycogen synthetase is activated by glucose-6-P, but not by glucose-1-P. The trehalose-P synthetase is activated by  $\text{Mg}^{++}$  and inhibited by trehalose. - The physiological significance of trehalose inhibition was tested in experiments with intact larval fat body in a haemolymph-like medium. In medium without trehalose, the fat body incorporated glucose- $^{14}\text{C}$  into trehalose ten times faster than into glycogen. Addition of trehalose at levels approximating those in larval haemolymph (~50 mM) resulted in decreased incorporation into trehalose and increased incorporation into glycogen. Inhibition of trehalose-P synthetase by trehalose would allow the level of UDP glucose to rise, thus increasing glycogen synthesis. Such a mechanism could explain the apparent regulation of blood trehalose-level in insects. The conversion of carbohydrates which follows injury in saturniid pupae may also be explained by these results together with the activation of glycogen phosphorylase.

- 101 Murphy, T.A., Wyatt, G.R. THE ENZYMES OF GLYCOGEN AND TREHALOSE SYNTHESIS IN SILK MOTH FAT BODY. *J. biol. Chem.* 240 (1965) 1500-1508.

Enzymes which synthesise trehalose and glycogen from uridine diphosphate glucose are present in homogenates of fat body from larvae and pupae of the silkworm, *Hyalophora cecropia*. Glycogen synthetase sediments in the 37 000 x g particulate glycogen fraction. It is activated by glucose 6-phosphate and has kinetic properties similar to those of mammalian glycogen synthetase. Trehalose phosphate synthetase, which remains soluble at 137 000 x g, requires glucose 6-phosphate as a substrate and is inhibited by trehalose. Kinetic evidence indicates that trehalose binds to a site separate from the catalytic site, which can be functionally eliminated by treatments which cause mild protein denaturation. With intact fat body, trehalose in the medium inhibits incorpora-

tion of glucose into trehalose and stimulates incorporation into glycogen. This is interpreted on the basis of feedback inhibition of trehalose phosphate synthetase, which is believed to play a role in regulation of blood trehalose in this species. Glucose-1-<sup>14</sup>C was used. (Essentially auth. summary)

- 102 Puro, J. MUTATIONAL RESPONSE OF THE PREMEIOTIC GERM-CELL STAGES OF ADULT *Drosophila melanogaster* MALES TO x-IRRADIATION. *Annls zool. fenn.* 3, 2 (1966) 99-126.

The mutagenic effect of 3000 R acute x-irradiation on the spermatogenesis of adult *D. melanogaster* was studied by means of the brood pattern technique. The irradiation brought about a period of low fertility on the 8th-9th day. almost complete sterility occurred on the 8th day. Restoration of fertility took place in two steps, with a secondary decline during the 11th-12th d. Control level was reached on the 13th- or 14th-day. - Recessive 3rd-chromosome lethal mutations, Y, three translocations and male sterility mutations (probably arising from damage to the Y-chromosome) showed the familiar brood patterns with gradually increasing mutation rates in the first three successive 2 or 3 d broods, corresponding to progressively earlier postmeiotic germ-cell stages at the time of irradiation. (Auth.)

- 103 Takahashi, S.Y., Ohnishi, E. ENZYMIC SYNTHESIS OF THE GLUCOSIDE OF PROTOCATECHUIC ACID IN THE COCKROACH, *Periplaneta americana*. (*Seikagaku Zasshi* 60, 4 (1966) 473-475. (J. Biochem., Tokyo). (In English))

Labelled  $\beta$ -glucosylprotocatechuic acid was formed during incubation of homogenates of fat bodies from the female cockroach, *P. americana*, with <sup>14</sup>C-labelled UDP-glucose and protocatechuic acid. (CA 66 : 1967, 687 v)

- 104 Villar, E. del. Mosnaim, D. EFFECT OF DDT ON INCORPORATION OF <sup>14</sup>C- LABELED GLUCOSE INTO PROTEIN AND SOLUBLE INTERMEDIATES OF NYMPHAL *Triatoma infestans*. *Expl Parasit.* 21, 2 (1967) 186-194.

The mechanism for the greater resistance of *T. infestans* 4th-instar larvae to DDT in comparison with 3rd-instar larvae was investigated. Both developmental stages rapidly incorporated <sup>14</sup>C-labelled glucose into protein and several soluble intermediates, but the rate of incorporation was higher in 4th-instar larvae. DDT increased glucose incorporation into total protein, and this increase was paralleled by the amount of protein in 4th-instar larvae. However, this effect was less apparent in 3rd-instar larvae. DDT also significantly increased free proline in 4th-instar larvae. Chromatographic profiles of larval protein indicated the presence of an active Krebs cycle in these insects, which was apparently inhibited by DDT only in third instar larvae. (CA 68 : 1968, 28728 w)

- 105 Wiens, A.W. ASPECTS OF HORMONAL AND METABOLIC REGULATION OF CARBOHYDRATE METABOLISM IN THE INSECTS *Hyalophora cecropia* AND *Leucophaea maderae*. *Diss. Abstr.* 27 (1967) 2503-B.

Glycogen phosphorylase in cockroach and *Cecropia* fat body occurs in forms having differential sensitivity to 5'-AMP, is bound to glycogen and is soluble. Phosphorylase activity in cockroach fat body is 4 and 70-fold greater, respectively, than adult and pupal fat body of *Cecropia*. Phosphorylase phosphatase in adult moth fat body is responsible for the decrease in concentration of active phosphorylase, without a decrease in total phosphorylase. In vitro, the rate of decrease varies with the ionic nature of the medium. Phosphorylase kinase, requiring ATP and Mg<sup>++</sup> or Mn<sup>++</sup>, mediates the conversion of active from inactive phosphorylase, and is highly active in adult cockroach fat body and only slightly active in adult flight muscle. Glucose,  $\alpha$ -acetylglucosamine and trehalose inhibit the active phosphorylase of adult male moth fat body in a cell-free system, while sucrose, ribose and mannose do not. Fat body active phosphorylase per animal increases 3-fold during adult development without a significant change in total phosphorylase. In vitro incubation of cockroach corpus cardiacum or the retrocerebral complex of *Cecropia* does not activate *Cecropia* fat body phosphorylase. Under identical conditions cockroach fat body is always activated by roach corpus cardiacum. The inability of *Cecropia* fat body to respond to hormones by activation of phosphorylase is not due to the lack of enzyme systems capable of interconverting the inactive and active forms. Activation of phosphorylase in fat body of male *L. maderae* in vitro is maximal 10 min after the addition of corpus cardiacum extract. As little as  $5 \times 10^{-5}$  pair of corpora cardiaca per ml of incubation medium elicit an increase in active phosphorylase.

Female fat body, ovaries and the developing embryos of L. maderae show marked changes in glycogen related to events in the reproductive cycle. The activation of phosphorylase by extracts of corpora cardiaca from animals at various stages suggests that the hormone content of the gland is greatest at times when the glycogen level in the fat body is decreasing and the converse. Trehalose release by the fat body of male L. maderae in vitro is stimulated by the hormone. The percent stimulation reaches a maximum after 60 min of incubation. Incubation of fat body with extracts of corpus cardiacum stimulates  $O_2$  consumption and decreases the respiratory quotient. The rate of oxidation of glucose- $U-^{14}C$  to  $C^{14}O_2$  and its incorporation into glycogen is also reduced. The hormone stimulates the oxidation of acetate- $1-^{14}C$  and palmitate- $1-^{14}C$  to the degree that the metabolic rate is increased, possibly compensating for the decrease in carbohydrate oxidation. Trehalose is synthesised from glucose-6-P ( $U-^{14}C$ ) and UDPG more rapidly in a cell-free extract of tissue that had previously been incubated with corpus cardiacum extracts, suggesting an activation of trehalose-6-P synthetase. The data indicate that a corpus cardiacum hormone(s) regulates more than one site in the metabolism of glycogen. The hormone increases glycogen degradation by activation of phosphorylase and the phosphorylated hexoses are shunted into trehalose, rather than entering glycolysis at an accelerated rate. Consequently endogenous fat body metabolism shifts toward the increased utilization of lipid. Mechanisms that may be responsible for these effects are discussed. (From DA)

- 106 Wiens, A.W., Gilbert, L.I. VARIATIONS IN THE GLYCOGEN CONTENT OF FAT BODY, OVARY, AND EMBRYO DURING THE REPRODUCTIVE CYCLE OF Leucophaea maderae. J. Insect Physiol. **13** (1967) 587-594.

Changes in the fat body glycogen reserves of female L. maderae during the reproductive cycle follow a biphasic curve during oogenesis and a large single phase of synthesis and degradation during embryogenesis. Glucose- $U-^{14}C$  and palmitate- $1-^{14}C$  were used. Glycogen in the ovary and developing embryos increases slowly except for a short-term rapid build-up in the last half of embryogenesis, which is rapidly depleted just prior to and following parturition. These changes are related to several other physiological events in the reproductive cycle and suggest a basis for further studies of the control of carbohydrate metabolism by hormonal or metabolic mechanisms.

- 107 Wiens, A.W., Gilbert, L.I. REGULATION OF CARBOHYDRATE MOBILIZATION AND UTILIZATION IN Leucophaea maderae. J. Insect Physiol. **13** (1967) 779-794.

Hormonal control of the phosphorylase system, metabolism of radioactive substrates, and trehalose synthesis were examined in the fat body. Fat body phosphorylase is maximally activated within 10 min after being exposed to corpus cardiacum extracts and is sensitive to very low concentrations of the extract. The concentration of phosphorylase-activating factor in the gland appears to be related to changes in glycogen content during oogenesis. The hormone elicits an accelerated rate of trehalose release, an increase in  $O_2$  consumption, and a decrease in the respiratory quotient. The rate of oxidation of glucose to  $CO_2$  is decreased, but that of acetate and palmitate are both increased in fat body incubated with corpus cardiacum extracts. A hypothesis considering the new metabolic effects of the hormone suggests that its regulatory action is effective at a number of different sites. - The following radioactive compounds were used in the study: glucose-6-phosphate, glucose- $U-^{14}C$ , acetate- $1-^{14}C$ , palmitate- $1-^{14}C$  and trehalose- $^{14}C$ .

- 108 Williams, M.W., Benson, N.R. TRANSFER OF  $C^{14}$  COMPONENTS FROM Psylla pyricola (Foer.) TO PEAR SEEDLINGS. J. Insect Physiol. **12**, 2 (1966) 251-254.

Sorbitol was found to be the major  $^{14}C$ -containing compound transferred from the insect P. pyricola (Foer.) to pear seedlings. Other  $^{14}C$  components were also present in the seedlings but the amounts were too small to be identified. The psylla larvae transferred considerably more  $^{14}C$  to the seedlings than did the adults. Analysis of psylla honeydew revealed that it contained 65% sorbitol, 22% sucrose, and 12% reducing sugars on a dry weight basis. (Auth.)

- 109 Wyatt, G.R. THE BIOCHEMISTRY OF SUGARS AND POLYSACCHARIDES IN INSECTS. p. 287-360 of "Advances in Insect Physiology, Vol.4". London, Academic Press, 1967, 415p. Comprehensive review. The article is divided into sections on the occurrence of sugars in insects (glucose and reducing substances, trehalose, sugar content of insect haemolymph and of whole insects and insect tissues); intestinal absorption and the physiology of haemolymph sugar levels

(absorption from the gut, regulation of blood sugar); biosynthesis and utilization of sugars (glucose, use of monosaccharides other than glucose, biosynthesis, cleavage and use of trehalose, physiological roles of trehalose and trehalase, dormancy and the properties of trehalose); glycogen (in insects, accumulation and conversion during growth and metamorphosis, in insect flight, metabolism of glycogen); hormonal effects on carbohydrate metabolism; glycoproteins and chitin (glycoproteins in insects, metabolism of chitin); and on glycerol and sorbitol. - Extensive reference to radioisotope applications (author's own among others) are made throughout the text. A comprehensive bibliography is appended.

See also:

- 40 Some biochemical aspects of insect metamorphosis. (Gilbert, L.I. et al., 1961)
- 146 The course of protein and carbohydrate synthesis during oogenesis in Apis mellifica L. (Engels, W., 1965)
- 147 The time sequence in protein and carbohydrate synthesis in Apis mellifica. (Engels, W., 1966)
- 176 Action of ecdysone on some metabolism during larval-pupal transformation of the housefly, Musca domestica L. (Diptera: Muscidae) (Kobayashi, M. et al., 1967)
- 188 Studies of the mode of action of royal jelly in honeybee development: the utilization of sugar uniformly labeled with  $^{14}\text{C}$  and of aspartic- $1\text{-}^{14}\text{C}$  acid. (Lue, P.F. et al., 1967)
- 192 Studies on the salivary physiology of plant-bugs: transport from haemolymph to saliva. (Miles, P.W., 1967)
- 268 Radioisotope incorporation and nucleic acid synthesis in dipteran embryos. (Eudy, W.W. et al., 1967)
- 419 The deposition of endocuticle in an insect, Calpodus ethlius Stoll (Lepidoptera, Hesperidae). (Condoulis, W.V. et al., 1968)

#### 1.2.4. Amino Acids. Pteridines, Pyrimidines, Purines. Proteins. Enzymes. Hormones.

- 110 Agosin, M., Aravena, L., Neghme, A. ENHANCED PROTEIN SYNTHESIS IN Triatoma infestans TREATED WITH DDT. Exptl Parasit. **16**, 3 (1965) 318-324.

The effect of DDT and DDE, a DDT-non toxic analogue, on protein biosynthesis in T. infestans nymphs and adult specimens has been studied. DDT increases the in vivo rate of incorporation of DL-leucine- $1\text{-}^{14}\text{C}$  into total proteins, while a slight inhibitory effect is observed in adult males. DDE does not appreciably change the rate of protein biosynthesis in nymphs, although it appears to be inhibitory in males. Nymphal microsomes and sarcosomes show the highest specific activity after DL-leucine- $^{14}\text{C}$  injection. This activity is markedly increased by DDT, while DDE again is inhibitory. A cell-free system obtained from nymphal specimens incorporates DL-leucine- $1\text{-}^{14}\text{C}$  into proteins in the presence of ATP, an ATP-generating system, GTP, and Mg ions. In vivo pretreatment with DDT at low concentrations increases the rate of DL-leucine- $1\text{-}^{14}\text{C}$  incorporation into protein by this system. In vitro added DDT also is stimulatory at very low concentrations, while higher ones are inhibitory. The results obtained indicate that protein biosynthesis is stimulated by DDT, but not DDE; an effect that is only evident in nymphal T. infestans. This appears to be consistent with the inductive effect of DDT previously shown with NAD-kinase in this organism (Ilevicky et al., 1964). (Auth.)

- 111 Agosin, M., Fine, B.C., Scaramelli, N., Ilevicky, J., Aravena, L. THE EFFECT OF DDT ON THE INCORPORATION OF GLUCOSE AND GLYCINE INTO VARIOUS INTERMEDIATES IN DDT-RESISTANT STRAINS OF Musca domestica L. Comp. Biochem. Physiol. **19** (1966) 339-349.

House fly strains showing various patterns of cross-resistance to insecticides have remarkable metabolic differences as to levels of NADP, relative participation of glycolysis and the pentose phosphate pathway in glucose utilization, rates of protein synthesis and glutathione turnover. Labelled glucose (uniformly  $^{14}\text{C}$ -labelled, glucose- $1\text{-}^{14}\text{C}$ , glucose- $2\text{-}^{14}\text{C}$ , and glucose- $6\text{-}^{14}\text{C}$ ) was used. DDT treatment stimulates the pentose phosphate pathway, increasing the availability of NADPH\*, and

\* NADP, oxidized nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate.

enhances the rates of protein synthesis and glutathione turnover in a pyrethrin-resistant strain, which is also cross-resistant to DDT. On the other hand, a susceptible strain did not evidence any of the above DDT effects. It is suggested that the metabolic changes observed are closely linked to the activity of detoxifying enzymes and constitute an expression of insecticide resistance.

- 112 Agosin, M., Ilivicky, J., Litvak, S. THE INDUCTION OF NAD KINASE BY DDT IN Triatoma infestans. Can.J. Biochem. 45, 5 (1967) 619-626.
- The NAD kinase (EC 2.7.1.23) from T. infestans was purified and a specific antiserum against it prepared. Immunochemical techniques showed that the increase in the levels of NAD kinase in nymphs of T. infestans was accompanied by an increase in the amount of enzyme protein. The enzyme is labelled after injection of  $^{14}\text{C}$ -labelled leucine in both induced and non-induced insects, but labelling is greater in the former, which further supports the concept that a de novo synthesis of enzyme protein occurs during induction by DDT. The enzyme is heterogeneous by DEAE-cellulose and DEAE-Sephadex column chromatography but the antiserum does not distinguish this heterogeneity. NAD kinase induction may correspond to a protective mechanism of the insects by increasing the availability of coenzymes required for DDT detoxication. (CA 67: 1967, 10648q)

- 113 Allen, R.R., Newburgh, R.W. AMINO ACID-ACTIVATING ENZYMES IN Sarcophaga bullata. Comp Biochem. Physiol. 17, 1 (1966) 309-317.

Amino acids were studied in the supernatant from fat bodies from the larval and pupal stages of the fly. The enzymes were examined by the pyrophosphate-ATP exchange method. The optimal pH for pyrophosphate- $^{32}\text{P}$ -ATP exchange was 7.6 in the presence of 10 mM Mg. The exchange rate varied with the amino acid substrate, but the total activity decreased with age for the 1st 6 d of pupal life then increased to a max. 1-2 d before adulthood. Free amino acid concentration in the pupae decreased during the period of amino acid-activating activity. (CA 64: 1966, 13141f)

- 114 Atallah, Y.H., Killebrew, R. ECOLOGICAL AND NUTRITIONAL STUDIES ON Coleomegilla maculata: AMINO ACID REQUIREMENTS OF THE ADULTS DETERMINED BY THE USE OF  $\text{C}^{14}$  LABELED ACETATE. Ann. ent. Soc. Am. 60 (1967) 186-188.

Fifty adult Coleomegilla maculata (De Geer) were fed on 250 ml of an aqueous solution of sodium acetate- $^{14}\text{C}$  which contained 600  $\mu\text{g}$  of the salt; total  $^{14}\text{C}$  was 10  $\mu\text{Ci}$ . After 4 h, the beetles were homogenized, the amino acids were extracted and separated by thin-layer chromatography, and the activity was measured, using a scintillation counter. Glycine, serine, aspartic acid, glutamic acid, proline, and lysine showed high activity, and are considered to have been synthesised in vivo. They are apparently nonessential, in contrast to threonine, phenylalanine, isoleucine, and valine, which are essential or derived exclusively from essential dietary constituents. The low activity shown by alanine, leucine, arginine, and histidine indicated a low level of  $^{14}\text{C}$  incorporation. Three unknown ninhydrin-positive compounds were isolated, in addition to the 19 amino acids that were identified. (Auth.)

- 115 Berendes, H.D. THE HORMONE ECDYSONE AS EFFECTOR OF SPECIFIC CHANGES IN THE PATTERN OF GENE ACTIVITIES OF Drosophila hydei. Chromosoma 22, 3 (1967) 274-293.

The hormone ecdysone induces a large number of changes in the puffing pattern of mid 3rd instar larvae of D. hydei. The pattern of changes occurring after experimental administration of the hormone are identical with those observed in normal development during a 6-h period before puparium formation. After administration of the hormone a considerable number of puffs react with a change in activity within 15-20 min. During this period 3 puffs arise newly, 12 puffs show a strong increase in activity, 6 puffs show a less pronounced increase in activity and 12 puffs show a decrease in activity. At a period of 4-6 h after administration of the hormone another 5 puffs arise newly. The effect of the hormone was identical in both in vivo and in vitro experiments. - Diameter measurements on several puffs reacting within 30 min with an increase in diameter showed that these puffs reacted simultaneously. Most of the puffs that showed a decrease in activity reacted with some delay. - A study of the effect of different hormone concentrations revealed that the kinetics of 4 puffs with respect to the relationship between concentration and puff size was identical over a range of concentrations from  $33 \cdot 10^{-5}$  to  $33 \text{ CU}/\mu\text{l}$ . Three of these puffs showed a reaction with even lower concentrations. Maximum puff size is attained by all puffs at a concentration of  $33 \cdot 10^{-4} \text{ CU}/\mu\text{l}$ .

Among the puffs studied no difference in their reaction threshold was found. — A study of the behaviour of 5 puffs of the group reacting within 15-20 min and one of the group reacting after 4-6 h in mid-intestine and Malpighian tubules revealed that these puffs showed the same reaction after injection of the hormone as observed in the salivary glands. — Chromosomal RNA synthesis was studied by injecting the larvae with 1  $\mu$ l of an ecdysone solution (33 CU/ $\mu$ l) containing 1  $\mu$ Ci of uridine- $^3$ H (specific activity 16,800 mCi/mM), the salivary glands being prepared 30 min after injection. Specific labelling of the activated puffs was improved by injecting uridine- $^3$ H into larvae that were injected with an ecdysone solution 30 min earlier. The glands were prepared at 3 min after injection of the uridine. All puffs activated by administration of the hormone showed particularly strong uptake of  $^3$ H uridine and accumulation of acidic protein. — Ecdysone is concluded to induce a pattern of changes in gene activity that is far more complex in *D. hydei* than in *Chironomus tentans*.

- 116 Berendes, H.D. AMINO ACID INCORPORATION INTO GIANT CHROMOSOMES OF *D. hydei*. *Drosoph. Inf. Serv.* 42 (1967) 102-103.

Staining salivary gland chromosomes with Fast green at pH 2.4 revealed that normally occurring puffs as well as those produced by a temperature shock contain a high amount of proteins which are absent in the non-puffed condition of these regions. Various  $^3$ H amino acids were injected into larvae to trace their incorporation into the chromosomes and the possible relation of protein synthesis to the origin of puffs. No preferential labelling, after the injection of 140-h-old larvae with 1  $\mu$ l of  $^3$ H-tryptophan (specific activity 3,02 Ci/mM), of the induced puffs was ever observed. In many cells the number of grains over the puffed region was even lower than over the neighbouring part of the chromosome. In many cases, however, certain unpuffed bands showed a reproducible preferential labelling. None of the puffs specific for puparium formation which arise during the period of incubation showed a preferential uptake of the labelled amino acid. Similar results were obtained with proline, histidine, leucine and arginine. On account of these data it is clear that the regional increase in protein content during puffing is not caused by a synthesis in the puff itself, but is presumably due to an accumulation of pre-existent proteins from some place in the cell. Larvae of 136 h showed amino acid labelling in 100% of the salivary gland nuclei after 5 h of incubation. After the same period of incubation with  $^3$ H-thymidine only 37-46% of the nuclei of similar glands were labelled. It therefore might be suggested that there is no apparent correlation between chromosomal protein synthesis or accumulation and chromosomal replication. (From DIS)

- 117 Biryukova, N.V., Kullyev, P., Mamedniyazov, O.N. INCORPORATION OF LABELED AMINO ACIDS INTO THE FIBROIN IN THE SILK-SECRETION GLAND OF *Bombyx mori*. *Izv. Akad. Nauk turkmen. SSR, Ser. Biol.* 1 (1966) 58-63. (In Russian).

*B. mori* caterpillars were given glycine- $^{14}$ C, DL-alanine- $^{14}$ C, DL-lysine- $^{14}$ C, DL-methionine- $^{35}$ S, or 0.1 ml 0.14M NaCl in the fall. After 3 h, their silk-secretion glands were removed, homogenized, and radioactivity measured. Fibroin synthesis reached its max. some days after completed RNA formation in glandular cells and increased incorporation of labelled amino acids into total protein as well as accumulation of the secretion occurred in the 2nd half of the 5th growth phase of the caterpillars. (CA 65: 1966, 14159b)

- 118 Boyd, J.B. PART I: TURNOVER OF THE HEMOLYMPH PROTEINS OF *Drosophila melanogaster*. PART II: A NEW METHOD FOR THE DETECTION OF DEOXYRIBONUCLEASES AND ITS APPLICATION TO STUDIES OF *Drosophila melanogaster*. *Diss. Abstr.* 26, S (1966) 4203.

In part I, a method is described developed for measuring the radioactivity of proteins labelled with  $^{14}$ C or  $^3$ H following their separation by disc electrophoresis. The radioactivity is measured directly in acrylamide gel with scintillation techniques after the water in the gel has been replaced with a toluene-based scintillator solution. 74 Gel slices can be prepared with a minimum of handling in 9-18 h depending upon the size of the slices. This technique has been used in combination with densitometry measurements of separately stained gels to study the turnover of the haemolymph proteins of *D. melanogaster*. By injecting labelled homologous proteins into unlabelled animals, active turnover of most of the haemolymph proteins has been demonstrated in both larvae and pupae. One particular group of proteins begins to turn over rapidly after puparium formation with a half life of 13 h. Another group of proteins has been shown to be relatively inert in pupae. A postulated mechanism for protein turnover in metamorphosing insects is discussed. No radioisotopes were used in part II.

- 119 Boyd, J.B., Mitchell, H.K. TURNOVER OF THE HEMOLYMPH PROTEINS OF Drosophila melanogaster. Archs Biochem. Biophys. 117 (1966) 310-319.

Turnover of the haemolymph proteins of D. melanogaster was studied with the aid of disc electrophoresis. By injecting labelled homologous haemolymph proteins into unlabelled animals, active turnover of the major haemolymph proteins was demonstrated in both larvae and pupae. Parallel experiments with a heterologous protein demonstrated that the observed turnover was specific for homologous proteins. One group of haemolymph proteins turned over rapidly after puparium formation, with an average half-life of 13 h. Another group of proteins was relatively inert in pupae. (CA 66: 1967, 679e).

- 120 Briceux-Grégoire, S., Verly, W.G., Florkin, M. PROTEIN SYNTHESIS IN Sphinx ligustri PUPAE. Nature, Lond. 179 (1957) 678-679.

The study was aimed at determining whether the larval proteins are hydrolysed to free amino acids before being used for adult protein synthesis, or whether adult proteins are built up from larger units that might be carried by phagocytes which are known to destroy the larval tissues. The mean specific rates of protein synthesis (rate of synthesis/amount of proteins) from one free amino acid, glycine, in Sphinx ligustri pupae, either in diapause or at the moment of the development of the adult organs were compared.  $112 \mu\text{g}$  of glycine- $1-^{14}\text{C}$  (433 000 Ci/min) was injected into the body cavity of each of five pupae. The mean specific rate of non-blood protein synthesis from free glycine proved much higher in the transforming pupa than in the diapausing animal. This seems to support the view of adult protein synthesis occurring directly from the free amino acid pool.

- 121 Brookes, V.J. THYMIDINE KINASE AND THYMIDYLATE KINASE IN THE SILKWORM, Antheraea pernyi. Biochim. biophys. Acta 119, 2 (1966) 268-275.

Thymidine and thymidylate kinases were assayed by a chromatographic method (cf. J. biol. Chem. 238:1963, 1467) in terms of the disappearance of [2- $^{14}\text{C}$ ]thymidine and the formation of phosphorylated products, deTMP, deTDP, and deTTP. Thymidine kinase (ATP: thymidine-5'-phosphotransferase, EC 2.7.1.21) and thymidylate kinase were extracted from the wing epidermis of developing adults of Antheraea pernyi and partially characterized. Activity of both enzymes was low in dialysed extracts unless  $\text{Mg}^{2+}$  was added. Non-dialysed extracts contained sufficient  $\text{Mg}^{2+}$  for the full activation of thymidine kinase but not for full activation of thymidylate kinase. High concentrations of  $\text{Mg}^{2+}$  or of ATP inhibited thymidine kinase but this inhibition disappeared when both cofactors were added in equimolar concentrations. High concentrations of thymidine also inhibited thymidine kinase; this inhibition was diminished by lowering the concentration of ATP. Thymidine kinase was inhibited about 45% when thymidine and deTTP were equimolar, deCTP also inhibited thymidine kinase but to a lesser degree; i.e., by about 50% when the deCTP concentration was 10 times that of thymidine. deGTP and deATP caused no detectable inhibition. However, to a certain extent, these factors were able to satisfy the requirements for ATP.

- 122 Brunel, C. ANALYSE DE QUELQUES DERIVES PURIQUES ET PYRIMIDIQUES ACIDO-SOLUBLE DANS LES GLANDES SERICIGENES CHEZ Bombyx mori. Thesis, Lyon Univ. (France). 1965.

The thesis consists of two parts. The first concerns the development of a column-chromatographic method\* for fractionating acid-soluble nucleotides of the silk gland of Bombyx mori. The second part is devoted to the study of these nucleotides. With the exception of CTP (cytidine triphosphate) and UTP (uridine triphosphate) all the simple ribonucleotides have been identified: AMP, ADP, ATP (adenosine mono-, di- and triphosphate); GMP, GDP, GTP (guanosine mono-, di- and triphosphate); UMP (uridine monophosphate) and several UDP; CMP and CDP (cytidine mono- and diphosphate). These results are compatible with those obtained for other animal tissues, and the order of elution of the nucleotides corresponds to that given by Hurlbert et al. Possible explanations for the absence of CTP and UTP are put forward. Orotic acid, a precursor of the pyrimidine bases, was used in radio-active (orotic-6- $^{14}\text{C}$ ) form but did not yield further information.

\* To some extent a modification of Hurlbert's method (J. biol. Chem. 209: 1954, 23).

- 123 Burdette, W.C., Coda, R.L. EFFECT OF ECDYSONE ON INCORPORATION OF C- $^{14}$ -LEUCINE INTO HEPATIC PROTEIN IN VITRO. Proc. Soc. exp. Biol. Med. 112 (1963) 216-217.

As much as a 10-fold increase in incorporation of leucine occurred when ecdysone was present. DL-leucine was labelled in position 1 with  $^{14}\text{C}$ . The level of incorporation was increased significantly

(t test) when ecdysone was present in concentrations of  $\geq 250$  *Calliphora* units (c.u.)/ml and the activity of leucine adjusted to 10 mCi/mM. The effect was more pronounced with higher doses, and a threshold was present at concentrations  $> 100$  c.u./ml. It would appear that extracts of *Bombyx* containing ecdysone activity (*Calliphora* test) enhance the rate of synthesis of mammalian protein in vitro.

- 124 Bursell, E. ASPECTS OF THE FLIGHT METABOLISM OF TSETSE FLIES (*Glossina*). *Comp. Biochem. Physiol.* 19, 4 (1966) 809-818.

Injection of  $^{14}\text{C}$ -labelled amino acids and the tracing of the radioactivity in extracts of thoracic muscles of the tsetse fly after flight show that proline is the substrate for flight metabolism. During flight proline is converted to glutamic acid and subsequently to alanine. (CA 65: 1967, 26873z)

- 125 Bursell, E. THE EXCRETION OF NITROGEN IN INSECTS. p.33-67 of "Advances in Insect Physiology", Vol. 4". London, Academic Press. 1967, 415p.

This review article is divided into two main parts: the formation and the excretion of nitrogenous end products. The first deals with the uricolytic and the uricotelic pathways, the formation of urea and ammonia, and with amino acids and some other N-containing substances. The excretion of nitrogenous end products is considered in Collembola, Orthoptera, Odonata, Dermoptera, Hemiptera, Coleoptera, Neuroptera, Hymenoptera, Diptera, and Lepidoptera. The tremendous progress made in the last 15 years is stressed, as are the remaining complexities of insect excretory metabolism. - In some unpublished work Bursell has shown that arginine becomes rapidly labelled following injections of  $^{14}\text{C}$ -glutamate; this substance may therefore play a more active role in excretion than had originally been envisaged. - A number of studies are cited in which radioisotopes had been used but no mention of this is made in the text.

- 126 Bursell, E. THE CONVERSION OF GLUTAMATE TO ALANINE IN THE TSETSE FLY (*Glossina morsitans*). *Comp. Biochem. Physiol.* 23, 3 (1967) 825-829.

A series of experiments was carried out to determine whether the pattern of incorporation of radioactivity from glutamate labelled in the 1, the 5, or the 3,4 position into alanine molecules would conform to expectations for a previously proposed pathway for this conversion in flight metabolism in *G. morsitans*. The level of incorporation from glutamate-3,4  $^{14}\text{C}$  was significantly higher than that from glutamate-5- $^{14}\text{C}$ , which was greater than that from glutamate-1- $^{14}\text{C}$ . Results were the same when resting flies or flies flown for 1.5 min were used. These results support the view that the previously proposed metabolic pathway is operative in the tsetse fly. (CA 68: 1968, 47363f)

- 127 Casidy, J.D. BASE ANALOGUE INCORPORATION IN THE FEMALE GERM PLASM OF *Microbracon hebetor* Say. *J. Insect Physiol.* 13 (1967) 487-493.

The biochemical fate of an ingested purine analogue was traced in adult female braconid wasps and their ova. Synchronous oögenesis facilitated day-to-day radiometric analysis of germinal cell lines treated in vivo. The data showed differential incorporation of  $^{14}\text{C}$ -8-azaguanine into various cell types. Maximum isotopic incorporation occurred in the most differentiated oöcytes. The same meiotic cells expressed maximum analogue susceptibility as measured by the proportion of aberrant embryos. Homogenates of embryonic wasps derived from treated cells were used for extraction of five metabolic fractions. Incorporation occurred in DNA and RNA, and analogue transmission was identified to the 3rd generation. Biochemical, genetical, and cytological results suggest an alteration in the transcription of genetic information for cellular specialization and early braconid development. (Auth.)

- 128 Chan, S.K., Margoliash, E. BIOSYNTHESIS OF CYTOCHROME c IN DEVELOPING PUPAE OF *Samia cynthia*. *J. Biol. Chem.* 241 (1966) 2252-2255.

The content of cytochrome c in metamorphosing pupae of the saturniid moth, *Samia cynthia*, rises rapidly from an undetectable level during the final 2-3 d of development. To test whether this increase in cytochrome c during the later stages of development is a consequence of protein synthesis de novo,  $^{14}\text{C}$ -L-arginine (5.8 mμM, uniformly labelled, 174 mCi/mM) was injected into the middorsal line of the 3rd abdominal segment on the 17th day immediately before the rapid rise in the concentration of the protein. A total of 1.50 μM of cytochrome c was recovered from the 200 animals 2 d after adult emergence. The incorporation of  $^{14}\text{C}$ -L-arginine into tryptic peptides from *S. cynthia*

cytochrome c and their characterization was studied and the results are tabulated. The increase in cytochrome c could be shown to result from de novo synthesis of the entire polypeptide chain.

- 129 Chaudhary, K.D., Srivastava, U., Lemonde, A. MONOAMINE OXIDASE IN *Tribolium confusum* Duval. *Biochim. biophys. Acta* 132, 2 (1967) 290-299.

Certain biochemical characteristics of monoamine oxidase (monoamine: O<sub>2</sub> oxidoreductase (deaminating), EC 1.4.3.4) in *T. confusum* homogenate have been determined by using a radioisotope method (<sup>14</sup>C-tryptamine). Like the mammalian enzyme, the insect monoamine oxidase shows maximum activity at pH 7.4. Besides, this enzyme possesses a very high affinity for tryptamine used as substrate ( $K = 8.7 \times 10^{-5}$  M) and has an activation energy ( $\Delta E$ ) of the order of 10 440 cal/mol within the limits of optimum temperature (37° C). At a final concentration of 0.5 M, EDTA causes about 70% loss in the activity. Addition of Zn<sup>2+</sup> and Co<sup>2+</sup> (final concentration 10<sup>-3</sup> M of each) in the presence of EDTA not only recovers the lost activity but also activates the enzyme considerably. Both parnate sulfate and harmine inhibit monoamine oxidase reversibly, their respective inhibition constants ( $K_i$ ) being  $0.26 \times 10^{-3}$  M and  $1.4 \times 10^{-3}$  M. Monoamine oxidase activity is lodged in the particulate fraction of which the mitochondria, in terms of specific activity, contains about 5.7 times more activity than nuclei. The enzyme is tightly bound to mitochondria. During the life cycle of the insect, monoamine oxidase titer follows a more or less inverse trend to that of acetylcholinesterase (acetylcholine acetyl-hydrolase, EC 3.1.1.7).

- 130 Chefurka, W. INTERMEDIARY METABOLISM OF NITROGENOUS AND LIPID COMPOUNDS IN INSECTS. p. 669-768 of "The Physiology of Insecta. Vol.2". Rockstein, M., Ed. New York, Academic Press Inc. 1965.

This review is divided into sections dealing with the breakdown of proteins (subdivided into endopeptidases and exopeptidases); the transamination, decarboxylation, and oxidation of amino acids (D- and L-amino acid oxidases), and the metabolism of amino acids (glutamic acid, glycine, serine, alanine, lysine, arginine, sulphur amino acids, tryptophan, and aromatic amino acids); end products of N metabolism: metabolism of pterins; the synthesis of proteins (in whole cells and cell-free systems, and in relation to amino acid incorporation and to nucleic acid metabolism); and finally lipid metabolism (oxidation and synthesis of fatty acids). The extensive bibliography contains numerous references to work utilizing radioisotopes.

- 131 Chen, P.S. AMINO ACID AND PROTEIN METABOLISM IN INSECT DEVELOPMENT. p.53-132 of "Advances in Insect Physiology. Vol.3". Beament, J.W., Treherne, J.E., Wigglesworth, V.B., Eds. London, Academic Press. 1966, 382p.

Comprehensive review article, dealing with embryonic development (changes in free amino acid pools, and enzyme patterns); larval development (amino acids peptides and other amino acid derivatives, and haemolymph proteins); pupal development (metabolism of amino acids and proteins, and changes in enzyme activities); and the adult, in terms of sex-specific differences in amino acids, peptides and proteins, and protein metabolism in relation to reproduction. A section is devoted to some genetic aspects of protein metabolism in insects, and deals with patterns of protein metabolism in lethal mutants, synthesis of enzymes and other specific proteins, and the regulation of gene activity. It is clear that profound changes in protein metabolism take place at various periods during insect development. The major morphogenetic events, such as the determination of the organ (anlagen) during embryogenesis, growth and moulting during larval life, as well as transformation from larva to pupa at metamorphosis are accompanied by characteristic variations in the patterns of amino acids, peptides and proteins. A reasonable picture of the causal relationship of hormone action, protein synthesis, growth and differentiation has been provided by physiological and biochemical studies, though the precise mechanism involved is still far from clear. The genetic aspect of protein metabolism has been reviewed to a very limited extent on hand of a few selected examples. - A very extensive bibliography is appended. In a large number of studies radioisotopes were used, a fact which is not always brought out in the text.

- 132 Chen, P.S., Levenbook, L. STUDIES ON THE HAEMOLYMPH PROTEINS OF THE BLOWFLY *Phormia regina* - CHANGES IN ONTOGENETIC PATTERNS. *J. Insect Physiol.* 12 (1966) 1595-1609.

In order to examine the breakdown process more critically experiments were carried out entailing the injection of <sup>14</sup>C-labelled larval haemolymph into various developmental stages of the black blowfly *P. regina*. By following the formation of <sup>14</sup>CO<sub>2</sub> and the distribution of radioactivity in both the free

amino acid pool and protein fractions following the injection of labelled haemolymph proteins, the extent of protein degradation was estimated. Detailed analyses of the haemolymph during development of blowfly *P. regina* have demonstrated a 12-fold increase in haemolymph volume during the 2nd to 5th day of larval life. Within the same period the total concentration of proteins increases about 24-fold, and reaches a max. value of about 20% (w/v) shortly before pupation. During metamorphosis both haemolymph volume and haemolymph protein concentration fall distinctly, the most rapid decline occurring during the time of transformation from larva to white pupa and at adult emergence. In the newly emerged flies the total protein concentration amounts to 3.5% (w/v) which is only about one-sixth of that in the mature larva. Acrylamide gel electrophoresis indicates the presence of at least 19 protein bands in haemolymph. Characteristic changes in the ontogenetic patterns have been observed during development, including the appearance of new protein fractions and the disappearance of others in both pupal and adult stages. Furthermore, the distinct difference in the electrophoretic patterns between haemolymph and moulting fluid is indicative of the different origin of the respective protein components.

- 133 Chen, P.S., Levenbook, L. STUDIES ON THE HAEMOLYMPH PROTEINS OF THE BLOWFLY *Phormia regina* - II. SYNTHESIS AND BREAKDOWN AS REVEALED BY ISOTOPIC LABELLING. *J. Insect Physiol.* 12 (1966) 1611-1627.

The breakdown of proteins during metamorphosis of the blowfly *P. regina* was investigated by injecting  $^{14}\text{C}$ -labelled haemolymph protein into larvae, pupae, and developing adults. Measurements of  $^{14}\text{CO}_2$  production and radioactivity in free amino acids indicate that the breakdown process is phase-specific; it begins in the mature larva shortly before puparium formation and reaches a max. in the white pupa. However, the overall rate of protein degradation is very low, and amounts to only about 5-15% of the injected dose 10 h after injection. Analyses of adult flies which received labelled haemolymph protein before pupation showed that about three-quarters of the total body radioactivity was in the tissues. On the basis of this finding and the fact that the total free amino acid concentration remains essentially unchanged during metamorphosis, the possibility of a direct utilization of larval haemolymph proteins by adult tissues is suggested. Injection of  $^{14}\text{C}$ -labelled *Chlorella* protein hydrolysate showed that incorporation of the radioactive amino acids into haemolymph proteins is most rapid during the growth period of larval life. The corresponding values for pupae are very low, and progressively decline during the course of metamorphosis. (Auth.).

- 134 Chitra, C., Shyamala, M.B. ACCUMULATION OF GLYCINE IN THE FAT BODY OF THE SILKWORM, *Bombyx mori* L. *Nature*, Lond. 216 (1967) 386-387.

When glycine-2- $^{14}\text{C}$  was administered in vivo to 5th-instar larvae, 96% of the radioactivity was incorporated into various tissues within 1 h whereas in vitro only 19% of the activity was transported by the mid-gut. This suggests that continued absorption of glycine by the intestine could be aided by a facilitated diffusion mechanism in which amino acids are rapidly removed from the site of absorption either by accumulation into other tissues or by degradation. The experiments described show that the silkworm fat body possesses an efficient mechanism for accumulating glycine and that both the accumulation and the release of glycine are metabolically controlled. Glycine uptake was studied by suspending 50 mg of fat body in 0.5 ml of Ringer solution containing 5  $\mu\text{l}$  of glycine-2- $^{14}\text{C}$  (specific activity 0.1 mCi/9.8 mg dissolved in 2 ml) and 7.5 mg of carrier glycine (200 mM/l Ringer solution). Comparison between middle 5th instar and spinning larvae shows a statistically significant reduction in uptake even at the early spinning stage (see typical double-layered plasma membrane for the fat body cell discovered presumably corresponding to discrete structures in fat body cells discovered electron microscopically in *Philosamia cynthia* by other workers).

- 135 Chmuryńska, W., Zielińska, Z. METABOLIZM FRAGMENTÓW JEDNOWĘGLOWYCH U OWADÓW. 1. INKORPORACJA WĘGLA Z  $^{14}\text{C}$  MROWCZANU DO NIEKTÓRYCH WOLNYCH AMINOKWASÓW HEMOLIMFY *Acantholyda nemoralis* (Thoms). (Metabolism of monocarbon fragments in insects. I. Incorporation of carbon from  $^{14}\text{C}$  formate into certain free amino acids of the haemolymph of *Acantholyda nemoralis* (Thoms)) p. 67 of "Biochemistry of Lipids". 4th Symposium of the Polish Biochemical Society. 11-12 Jun. 1965. Abstr.

A solution of  $^{14}\text{C}$  formate (0.08  $\mu\text{Ci}/100$  mg insect weight) was injected into larvae (q) of *Acantholyda* at diapause. Free amino acids were isolated from haemolymph taken a certain time after injection and were separated by paper chromatography; after elution the radioactivity of the individual amino acids was measured by means of a Micromil (Nuclear Chicago Co.) end-window counter. The

incorporation of  $^{14}\text{C}$  into alanine, glycine, methionine and serine was established. The specific activity of the alanine and glycine was about 700 and 150 cpm/ $\mu\text{M}$  in these experiments, in which the similarly determined specific activity of serine and methionine reached 20 000 cpm/ $\mu\text{M}$  or even more. The high activity of the serine, also the high activity of the enzymatic systems bringing about synthesis and interconversion of the active monocarbon fragments (Grzelakowska, Zielifiska) give grounds for believing that the incorporation of  $^{14}\text{C}$  formate into serine occurs with the participation of the enzymatic systems in question. The high radioactivity of the methionine may indicate a considerable metabolic activity of this amino acid. (From translated abstr.)

- 136 Clarke, J.M., Maynard-Smith, J. INCREASE IN THE RATE OF PROTEIN SYNTHESIS WITH AGE IN *Drosophila subobscura*. *Nature*, Lond. **209** (1966) 627-629.

Flies were fed a solution of  $5 \times 10^{-4}\text{ M}$   $^3\text{H}$ -leucine in 10% sucrose, with a specific activity of 0.5 mCi/ml. Label can be detected from within 5-10 min of the start of feeding. Flies were allowed to feed for 1 h. The mean survival time of even the most rapidly turning-over species of protein molecule must be at least 100 h, and may be much greater. The rate of protein synthesis is about twice as great in old as in young flies. Possible interpretations are discussed.

- 137 Clarke, K.U., Gillott, C. STUDIES ON THE EFFECT OF THE REMOVAL OF THE FRONTAL GANGLION IN *Locusta migratoria* L. - I. THE EFFECT ON PROTEIN METABOLISM. *J. exp. Biol.* **46**, 1 (1967) 13-25.

Studies were made on the 3rd-, 4th-, and 5th-instar nymphs of *L. migratoria* L. from which the frontal ganglion had been removed, on control-operated, and on starved animals. The effects of this operation on protein metabolism were observed by study of: electrophoresis of haemolymph proteins, chromatography of haemolymph amino acids, production of protease in the midgut, and the incorporation of  $^{14}\text{C}$ -glycine into protein by the body cells. Third-instar locusts were injected with  $^{14}\text{C}$ -glycine (specific activity 8.0 mCi/mM, 106.7  $\mu\text{Ci}/\text{mg}$ ) in distilled water (concentration 0.1 mCi/ml) either as a fixed volume (5  $\mu\text{l}$ ) or as a proportion of the body weight (1  $\mu\text{l}/20\text{ mg}$ ). The total protein concentration in the haemolymph of operated locusts did not increase with time as did that of controls, in which the increase was almost entirely due to changes in the second of the three bands which normally separate out. The concentration of the free amino acids in the haemolymph fell to about 70% of that in operated controls. In operated locusts the proportions of the amino acids relative to one another changed. The incorporation of  $^{14}\text{C}$ -glycine into protein was slower and the equilibrium concentration less in operated than in control-operated animals. The time taken to reach equilibrium was the same in both cases. In both operated and control-operated animals the protease activity of the mid-gut wall was very low; no difference could be detected between them. The protease activity of the mid-gut contents expressed per mid-gut was lower in operated than in control-operated animals. The protease activity expressed per mg was found to be the same in operated, control-operated and starved animals. The hypothesis that the effects of the removal of the frontal ganglion were mediated through changes in the secretion of hormones from the corpus cardiacum was tested by giving daily injections of freshly prepared corpus cardiacum extract to locusts from which the frontal ganglion had been removed and observing the growth in weight of these animals. A permanent increase in weight amounting to 100% of their initial weight was found. Animals injected with distilled water showed a temporary increase amounting to 30%. Uninjected animals maintained approximately constant weight. (Essentially auth. summary)

- 138 Cline, R.E., Pearce, G.W. UNIQUE EFFECTS OF DDT AND OTHER CHLORINATED HYDROCARBONS ON THE METABOLISM OF FORMATE AND PROLINE IN THE HOUSEFLY. *Biochemistry* **2** (1963) 657-662.

Insecticide-treated and control flies were injected with  $^{14}\text{C}$ -labelled biochemicals, and 3 h later the soluble radiometabolites were extracted for identification and assay by paper chromatography and radiometric techniques. Of the injected compounds, DDT was found to interfere most with the metabolism of formate, glycine and proline. Thus after injection of  $^{14}\text{C}$ -formate, more uric acid and allantoin and less proline were recovered as radiometabolites from flies treated with DDT and related hydrocarbon insecticides than from flies untreated or treated with nontoxic analogues. However, insecticides of other types such as pyrethrum, organic phosphates, and phosphonates and a carbamate, interestingly, failed to show a significant effect on formate metabolism, providing additional evidence for a different mode of action. (Auth.)

- 139 Coles, G.C. STUDIES ON RESILIN BIOSYNTHESIS. *J. Insect Physiol.* **12** (1966) 679-691.

The cells that deposit resilin in *Schistocerca gregaria* have been studied to test the hypothesis that this protein is formed by the cross-linking of proresilin by a peroxidase enzyme. A peroxidase, resembling that from horse-radish, is present in these cells, and also in the gut, flight muscle, and cuticle with attached epidermal cells. There are proteins in the cells that deposit resilin that can be cross-linked by the peroxidase enzyme to form di- and trityrosine residues. However, it cannot be concluded that the natural function of the peroxidase enzyme is to cross-link proresilin and similar cuticular proteins because it has not been possible to demonstrate its presence in the cuticle or at the surface of the epidermal cells. The difficulties of providing conclusive evidence for the existence of proresilin are discussed. Both the *in vivo* and *in vitro* incorporation of  $^{14}\text{C}$ -tyrosine show that only a short time is required for incorporation of free tyrosine into resilin cuticle, indicating a rapid turnover of proresilin and suggesting that only a small pool is present. Some observations on chitin synthesis by these cells are reported. The incorporation of  $^{14}\text{C}$ -glucose and -glucosamine was used to investigate the synthesis of chitin by "resilin cells".

- 140 Collins, J.V., Locke, M. THE SEQUESTRATION OF PROTEIN BY FATBODY CELLS IN AN INSECT. *J. Cell Biol.* **31**, 2 (1966) 23A. Abstracts of Papers presented at the "6th Annual Meeting of the American Society for Cell Biology, Houston, Tex., USA. 17-19 Nov. 1966". Abstr. 42.

The fat body of metamorphosing holometabolous insects stores protein in the form of granules. In the larva of *Calpodethiulus* Stoll (Lepidoptera: Hesperidae), the granules begin to form about 30 h before pupation. Most of the protein in the granules is sequestered over a period of 16 h. The source of the protein sequestered has been demonstrated in two ways. The incorporation of  $^3\text{H}$ -tyrosine and -leucine into the protein of the granules was observed radioautographically. The level of incorporation into the fat body is no higher at the time of granule formation than at any other time during the stadium. In contrast, there is considerable incorporation into the epidermis. It is unlikely that the fatbody synthesises much of the protein in the granules at this time. An alternative hypothesis is that the protein is sequestered from the blood. The concentration of blood protein increases steadily throughout the stadium and decreases during the period of granule formation. Females have a higher concentration of blood protein than males, and a higher proportion of protein in the fat body cells. Horse-radish peroxidase injected into the haemolymph during the early phase of granule formation is sequestered mostly into the granules. It is concluded that the fat body sequesters blood protein to make the protein granules. The high concentration of blood protein is not a sufficient stimulus for sequestration. The cells fail to form protein granules if the source of the moulting hormone is removed, although the blood protein concentration may be max. at that time. Electron micrographs of fat body cells during granule formation show that blood protein is concentrated in intercellular and intracellular channels, from which vesicles bud off into the cytoplasm. This mechanism differs from that involved in yolk protein uptake in oocytes. (Abstr.)

- 141 Collins, J.V. THE FORMATION OF PROTEIN GRANULES IN THE FAT BODY OF AN INSECT, *Calpodethiulus* Stoll, LEPIDOPTERA, HESPERIIDAE. *Diss. Abstr.* **28**, 6 (1967) 2269-B - 2270-B.

The fat body of metamorphosing holometabolous insects stores protein in the form of granules. In the larva of *C. ethiulus* Stoll (Lepidoptera, Hesperidae), the granules are formed over a period of 18 h beginning 30 h before pupation. Grain counts of autoradiographs show that although the fat body synthesises protein during the intermolt, the rate of incorporation of  $^3\text{H}$ -amino acids is lowest at the time of granule formation. The granules contain little newly synthesised protein and are formed from protein taken up from the blood. This conclusion is supported by the changes in amount of protein reserves in the blood throughout the stadium. The concentration of protein in the blood is constant from the 4th-5th moult until the critical period for the action of the brain on the prothoracic glands 66 h later. It then increases until the end of the intermolt at the time of the activation of the tissues by the prothoracic glands. This correlation between blood protein synthesis and the critical period for the action of the brain hormone has not been made in other insects. Following this critical period there is a sudden decrease in the concentration which exactly coincides with the formation of the protein granules. There is a sex difference in the amount of protein in the blood and in the amount stored in the fat body granules. Females have more protein in their blood and also store more protein in the granules of the fat body at pupation. The intermolt fat body also sequesters horse radish peroxidase from the haemolymph into granules. These granules are much smaller than those formed at pupation. This sequestration occurs during the period when the blood protein is increasing. The protein sequestered

at this stage is not stored in large granules as at pupation and must therefore be degraded by the cells. This suggests that the fat body is engaged in the turnover of blood proteins. The uptake of the blood proteins during the intermolt is not dependent on the presence of hormones from the head and thorax. "Dauer" larvae, produced by thoracic ligation to prevent moulting, continue to sequester peroxidase from the blood. The cells thus have an intrinsically controlled ability to take up proteins. In contrast, ligation experiments show that the formation of the large granules at pupation occurs only after the action of the moulting hormone. The moulting hormone may influence both the rate of sequestration and the rate of destruction of the protein. Protein uptake is a two stage process. The first step is the concentration of the blood proteins in the intercellular spaces between the fat body cells. These spaces are continuous with intracellular channels formed by the extensive invagination of the plasma membrane. The concentrated protein is then ingested in vacuoles which pinch off from the tips of the intracellular channels. The vacuoles fuse to form granules in which the protein is either lysed or stored, depending on the stage in the moulting cycle. Not all the granules are formed from sequestered blood protein. There is a phase of autolysis before protein sequestration during which mitochondria and a small amount of the endoplasmic reticulum are isolated and lysed. After the sequestration of blood proteins to form the major portion of the granules, a second phase of autolysis occurs. This involves the isolation and partial autolysis of the endoplasmic reticulum. The residual bodies from the two autolytic phases are stored along with the granules of sequestered protein and are probably used by the pupa to provide precursors for adult development. (From DA)

- 142 Dinamarca, M.L., Levenbook, L. OXIDATION, UTILIZATION, AND INCORPORATION INTO PROTEIN OF ALANINE AND LYSINE DURING METAMORPHOSIS OF THE BLOWFLY *Phormia regina* (Meigen). *Archs Biochem. Biophys.* 117, 1 (1966) 110-119.

L-alanine-U-<sup>14</sup>C (123 mCi/mM) and L-lysine-U-<sup>14</sup>C (223 mCi/mM) were used. Blowflies at the desired experimental stage were injected quantitatively with 1.0  $\mu$ l of isotope, the adults into the thorax, the pupae after piercing of the external puparium in the abdominal region lateral to the heart. The extent of alanine and lysine oxidation by various developmental stages of the blowfly is stage specific. Both amino acids are most slowly oxidized at about half way along adult development, but alanine is most rapidly oxidized by the adult fly, and lysine by the white pupa at the commencement of pupation. The rate of utilization ( $K_a$ ) for free pool alanine is at all corresponding stages higher than for lysine. Variations in  $K_a$ -alanine during metamorphosis are far more pronounced than for  $K_a$ -lysine,  $K_a$ -alanine being particularly high in the white pupa. Alanine and lysine were incorporated into total protein at identical rates ( $K_p$ ) during metamorphosis except in the mature fly, where  $K_p$ -lysine was higher than  $K_p$ -alanine. The  $K_p$  values, at all times low, decreased markedly during adult development. It is suggested that protein synthesis during blowfly metamorphosis is less extensive than had previously been assumed.

- 143 Doe, F., Schurin, M., Federbush, M. SYNTHESIS OF RIBOSOMES AND PROTEINS IN THE LARVAE OF *Drosophila virilis*. *J. Cell Biol.* 23, 2 (1964) 24A-25A. Abstr. 45. "4th Annual Meeting of the American Society for Cell Biology, Cleveland, Ohio, USA, 11-13 Nov. 1964".

Preparations of ribosomes from *D. virilis* yield populations of mainly 20S or 80S particles depending on the composition of the isolating media. It has been suggested that the largely protein 20S particles are the structural subunits of 80S ribosomes with which they are antigenically identical. It has now been possible to quantitatively convert 20S particles to 80S ones in vitro (but not vice versa). The in vivo relationship has been pursued by studies of the formation of proteins in the RNP fraction, using sucrose density gradient analyses of <sup>14</sup>C-leucine incorporation with an isolating buffer that produces some polysomes, some 20S, and mainly 80S. Long term incorporation (2-4 d) results in an almost equal excess of label in both the 20S and polysome regions when the radioactive peak is normalized to the 80S peak. Chase experiments on cold media after the long term incorporation remove the excess label of the polysomes but not that of the 20S, indicating that the incorporation at the polysome area was transient while that at the 20S was not. Populations were also sampled at various times after exposure to radioactive food. After 2 h, both 20S and polysomes were hot but the 80S area was cold. After 6 h, an 80S radioactive peak commences. At 10 h, the 80S peak can be normalized to the 80S OD peak; the polysomes still have a moderate excess of label and the 20S a large excess. This suggests that synthesis is initiated on 20S and on polysomes, with the 80S labelled secondarily. The constancy of the polysome excess is consistent with a flow of material such as is expected in the site of general protein synthesis. To follow 80S synthesis, a 2 h label was followed by an 8 h chase. The radioactivity showed equal amounts of label in the 20S and 80S regions, approximating the straight

10 h labelling result. This suggests that the label of the 80S ribosomes comes from the hot 20S particles, as if 20S units were the precursors of 80S ribosomes. (Abstr.)

- 144 Duffy, J.P. BETA ALANINE INCORPORATION INTO INSECT PROTEINS.\* *Bull. ent. Soc. Am.* 13 3 (1967) 190. Abstr. 57. "New York Meeting of the Entomological Society of America, New York, N. Y., 27-30 Nov. 1967".

Beta alanine incorporation into TCA precipitated proteins of whole body homogenates of T. molitor and Drosophila melanogaster. (Abstr.)

\* Following request (MB) for further information the author sent the abstract below, on  $\beta$ -alanine metabolism in Tenebrio molitor.

"Beta alanine 1- $C^{14}$  metabolism in Tenebrio molitor pupae.

One to four  $\mu$ l of (1- $C^{14}$ ) beta alanine (0.5  $\mu$ Ci) was injected into the thoracic dorsum of T. molitor pupae. The radioactive carbon dioxide which resulted from 0-5 h went from 0-3.5% radiation, while the 95% ethanol, 5% TCA whole body precipitate ranged from 3.5-5.0% radiation for the same time intervals. The supernatant that resulted was the most active fraction and ranged from 26-36% radiation with a slight increase in the 5th hour sample. A 24-h sample showed no outstanding increase in radioactive  $CO_2$ , or the 95% ethanol, 5% TCA precipitate or supernatant. Paper chromatography of the supernatant established radioactivity entered glutamic acid (92 cpm) and proline (18 cpm) with some residual radioactivity in beta alanine (117 cpm). This supernatant sample was after 24 h. Starch gel electrophoresis of the haemolymph of the T. molitor pupae did not indicate any activity in the four soluble proteins demonstrated by this method."

- 145 Ellgaard, E.G. GENE ACTIVATION WITHOUT HISTONE ACETYLATION IN Drosophila melanogaster. *Science*, N. Y. 157 (1967) 1070-1072.

Chromosomal puffing, generally believed to represent gene activation in Drosophila, was induced in the presence of sodium acetate- $^3H$  (25  $\mu$ Ci/ml of saline). There is no preferential uptake of these labelled molecules in regions of gene activation. Uridine- $^3H$  (25  $\mu$ Ci/ml of saline) was also used. It is concluded from the results of autoradiography that the acetylation of histones does not play a general role in the regulation of RNA synthesis in D. melanogaster.

- 146 Engels, W. DER ZEITLICHE ABLAUF VON PROTEIN- UND KOHLENHYDRATSNTHESEN IN DER OOGENSE BEI Apis mellifica L. (The course of protein and carbohydrate synthesis during oogenesis in Apis mellifica L.) p.243-251 of "Verhandlungen der Deutschen Zoologischen Gesellschaft, Jena, 1965". (In German).

The sequence and mutual interaction of protein and carbohydrate syntheses during oogenesis in the honey bee was investigated autoradiographically. A mixture of  $^3H$ -D-glucose and  $^3H$ -L-histidine was used. By using enzymes to break down polysaccharides and checking against controls it was possible to assess the role played by proteins alone. Protein and glycogen syntheses could therefore be followed in one ovary. Laying 1-yr-old queens were used, and the labelled substances injected abdominally (involving a mixture of equal parts of an aqueous solution of  $^3H$ -D-glucose (specific activity 0.45 Ci/mM and  $^3H$ -L-histidine (specific activity 1.7 Ci/mM), with 1  $\mu$ Ci/ml total activity). Incubation periods of 5, 10, 30, and 60 min were allowed. Five functional phases could be distinguished, with some overlap in their sequence. They are described in some detail. RNA is transported into the ovum, whereas older oocytes no longer receive newly synthesised RNA. The reduction in RNA supply to the cytoplasm, followed by marked protein synthesis appears to be a first step in certain cytoplasmic maturation processes. Maximal protein synthesis and carbohydrate deposition appear to be mutually exclusive. Reserves of carbohydrates are stored during the final phase.

- 147 Engels, W. DER ZEITLICHE ABLAUF VON PROTEIN- UND KOHLENHYDRATSNTHESEN IN DER OOGENSE BEI Apis mellifica L. (The time sequence in protein and carbohydrate synthesis in Apis mellifica.) *Zool. Anz.*, Suppl. 29 (1966) 243-251. (In German)

An aqueous mixture of  $^3H$ -D-glucose (specific activity 0.45 Ci/mM) and  $^3H$ -L-histidine (specific activity 1.7 Ci/mM), with a total activity each of 1 mCi/ml was injected abdominally into the haemocoel of ovipositing 1-yr old queens. Brief mention is also made of results obtained from using

<sup>3</sup>H-uridine and <sup>3</sup>H-thymidine. Details of the autoradiographic techniques are given. Five functional phases may be distinguished. The 1st, during which there is very limited protein synthesis, is followed by the euplasmatic growth phase which involves prolonged, slowly increasing protein synthesis; the deutoplasmatic growth phase; the 4th phase of oogenesis when carbohydrates are stored in the egg plasma in the form of glycogen, and the 5th and last phase in which the egg is surrounded by the chorion. The hypothesis that maximal protein synthesis and glycogen storage are mutually exclusive appears to fit the Apis-oogenesis data. The results and their significance are discussed.

- 148 Florkin, M. APPROCHE ORGANISMIQUE ET APPROCHE MOLECULAIRE DANS L'ETUDE DES ADAPTATIONS. SOURCES METABOLIQUES D'UNE SYNTHÈSE PROTEIQUE ADAPTIVE (COCON DU VER A SOIE). Bull. Acad. r. Belg. Cl. Sci. 51, 5 (1985) 441-463.

Review article drawing on data obtained from work on insects and spiders. A very detailed study has been made of the molecular aspects of the differentiation involved in cocoon spinning in the silkworm. The physical properties of fibroin on which the protective function of the cocoon is based depend on the characteristics of the protein synthesis in the posterior part of the silk gland. The structure of fibroin seems to be explained by the special nature of the messenger RNA liberated at the cellular level in the posterior part of the gland. Detailed analysis has shown, however, that if the nature of the cocoon depends on a specific gene, the formation of the cocoon is the result of several other factors: the type of nutrition available to the silkworm; the behaviour pattern which attracts the silkworm to a specific nutrient, making it chew and finally swallow it, and which leads to the outstanding appetite characterizing the middle of the 5th stage; the way in which amino acids are removed from the haemolymph by the gland; pupal weight control, etc. Differentiation evidently has a clearly polygenic basis which can only be elucidated by a molecular approach. - Radioisotopes were used in the majority of the studies cited.

- 149 Forrest, H.S., Menaker, M., Alexander, J. STUDIES ON THE PTERIDINES IN THE MILKWEED BUG, Oncopeltus fasciatus (Dallas). J. Insect Physiol. 12 (1966) 1411-1421.

A quantitative re-examination of the pteridines in Oncopeltus has given results at variance with those published previously. The precursors of the red pteridine, erythropterin, are xanthopterin and, probably, oxalacetic acid. The conversion of xanthopterin-8a-<sup>14</sup>C into erythropterin was studied in 3-5 d old eggs. Xanthopterin is not present as such in Oncopeltus eggs but exists as 7,8-dihydro-xanthopterin. Pyruvic acid-2-<sup>14</sup>C, on the other hand, proved not to be a precursor of erythropterin. Chrysopterin and bioppterin have also been isolated and identified from later growth stages of the bug.

- 150 Fox, A.S., Kan, J., Kang, S.H., Wallis, B. PROTEIN SYNTHESIS IN CELL-FREE PREPARATIONS FROM Drosophila melanogaster. J. Biol. Chem. 240, 5 (1965) 2059-2065.

Uniformly labelled L-leucine-<sup>14</sup>C, and in some cases L-leucine-<sup>14</sup>C or L-leucine-<sup>3</sup>H were used. A cell-free system was developed capable of incorporating <sup>14</sup>C-labelled amino acids into protein. The system includes microsomes or ribosomes, soluble RNS (sRNA) or pH 5 fraction, the 20 standard amino acids, ATP and an ATP-generating system, and GTP. Mg ion concentration is optimal at 10<sup>-2</sup>M. Microsomes and ribosomes exhibit a significant level of endogenous incorporation in the absence of sRNA and pH 5 fraction. sRNA stimulates incorporation more effectively than pH 5 fraction. The requirements for amino acids, ATP, and GTP are not absolute. In the presence of sRNA, the incorporation of 3.20 μM of L-leucine/mg of microsomal protein/h/μM of L-leucine in an incubation mixture has been observed. This yields an estimate of 8 μg of protein synthesised/mg of microsomal protein. For ribosomes, the corresponding value is 19.83, or an estimate of 52 μg of protein synthesised/mg of ribosomal protein. Ribosomes in 10<sup>-2</sup>M MgCl<sub>2</sub> exhibit a single peak in the ultracentrifuge with a sedimentation coefficient of 72 S. In 10<sup>-3</sup> M MgCl<sub>2</sub> two peaks are observed at 57 S and 44 S, respectively. Amino acid-activation enzymes are bound to microsomes and ribosomes: no activity has been observed in the soluble fraction, the pH 5 fraction, or the pH 5 supernatant. It is found that tobacco mosaic virus RNA is capable of stimulating incorporation by Drosophila microsomes, apparently functioning as messenger.

- 151 Frisrom, J.W., Knowles, B.B. STUDIES ON PROTEIN SYNTHESIS IN IMAGINAL DISCS OF Drosophila melanogaster. Expl Cell Res. 47, 1-2 (1967) 97-107.

<sup>14</sup>C-l-L-leucine (34.1 mCi/mM), <sup>3</sup>H-4,5-L-leucine (5.0 Ci/mM), <sup>14</sup>C-l-glycine (5.69 mCi/mM), <sup>3</sup>H-2-glycine (200 mCi/mM), and <sup>3</sup>H-uridine (11.9 Ci/mM) were used in protein determinations. Comparisons were made of protein synthesis in imaginal discs of different developmental stages using

acrylamide gel electrophoresis of  $^3\text{H}$ - and  $^{14}\text{C}$ -labelled protein. No qualitative differences were found between middle 3rd-instar discs and late 3rd-instar discs, and small differences between late 3rd-instar discs and early prepupal discs. Using actinomycin D the functional half-life of mRNA of imaginal discs has been minimally estimated to be  $\sim 2\frac{1}{2}$  h long. It is proposed, on the basis of the life of mRNA, that no large differences in the pattern of protein synthesis in late larval and prepupal discs should be expected because the existing mRNA would buffer the system against changes.

- 152 Fuzeau-Braesch, S. ETUDE DU NOIRCISSEMENT CUTICULAIRE NON-EXUVIAL CHEZ Locusta migratoria. J. Insect Physiol. **12** (1966) 1363-1368.

Secondary of 'homochromic' pigmentation can be induced in adult acridids by rearing the adults on a black background; this occurs in the field when locusts and grasshoppers live on black, burnt land. The secondary pigmentation is achieved in two ways: an epidermal omochrome appears in certain localized areas within 5-10 d, partially covered by a cuticular pigment. Experiments using  $^{14}\text{C}$ -labelled tyrosine show that tyrosine incorporated into the new cuticle before the moult is not a pigment precursor, whereas the tyrosine taken up after ecdysis and during the sclerotization of the cuticle is used for primary pigmentation; the pigment is referred to as 'primary melanin'. The pigment associated with secondary pigmentation appears to have the same nature and origin as the primary pigment, but since it is deposited after the cuticle is sclerotized, it is referred to as 'secondary melanin'. The significance of the secondary melanization is discussed, as well as the possibility of the hormonal control. (Auth.)

- 153 Ghosh, D., Forrest, H. S. ENZYMATIC STUDIES ON THE HYDROXYLATION OF KYNURENINE IN Drosophila melanogaster. Genetics **55** (1967) 423-431.

$^3\text{H}$ -labelled reduced nicotinamide adenine dinucleotide phosphate was used. Kynurenine hydroxylase activity has been detected in wild-type D. melanogaster for the first time. The cinnabar mutant, as expected, lacks this activity, and the white mutant has about one half the wild-type activity. During the life cycle, of the fly, there is a peak of enzyme activity at about the time the brown pigments are being laid down. As in the mammalian system, the enzyme seems to be associated with the "mitochondrial fraction". A theory as to the nature of the biochemical lesion in white mutants is proposed, involving the supposition that kynurenine hydroxylase has a pteridine cofactor.

- 154 Gruzova, M. N. AUTORADIOGRAPHICAL STUDIES OF PROTEIN METABOLISM IN THE NUCLEI OF OOCYTES OF Panorpa communis. Tsitologiya **9**, 1 (1967) 90-92. (In Russian)

Autoradiographic experiments showed that 15 and 60 min after an intravenous injection of leucine- $^3\text{H}$  and phenylalanine- $^{14}\text{C}$ , these amino acids are incorporated mainly into the cytoplasm of P. communis oocytes (polytrophic type) and only slightly into the nucleus. After 24 h the incorporation into the nucleus was twice as high as the incorporation into the cytoplasm. The karyosphere does not demonstrate a specific capacity for preferably binding amino acids, the degree of incorporation is the same as in the rest of the karyoplasm. However, it can be assumed that the karyosphere plays a definite role in the protein metabolism of the nucleus. (CA 66:1967, 102820x)

- 155 Gumilevskaya, N. A., Kuvaeva, E. B., Chumikina, L. V.  $\text{C}^{14}$ -GLYCINE AND  $\text{C}^{14}$ -LEUCINE INCLUSION BY AN ACELLULAR SYSTEM FROM THE SILK GLAND OF Bombyx mori L. Dokl. Akad. Nauk SSSR **174**, 2 (1967) 472-476. (In Russian)

The specific activity of 1- $^{14}\text{C}$ -glycine was 88-100 mCi/g and that of the labelled  $^{14}\text{C}$ -L-leucine was 200 mCi/g. There were no impurities of other  $^{14}\text{C}$ -amino acids. The fractions of both free and bound ribosomes of the silk gland were shown to be able to include  $^{14}\text{C}$ -leucine in the proteins of the acellular system, whereas glycine can be included only by the bound ribosome fraction (15 000 g - precipitate). In each case the ribosome acellular system from the silk gland of Bombyx mori L. did not differ from the classic systems of animals and bacteria.

- 156 Hackman, R. H. MELANIN IN AN INSECT, Lucilia cuprina (Wied.). Nature, Lond. **216** (1967) 163.

Melanins are dark pigments usually bound to protein. A culture of the blowfly was used which is homozygous for three recessive mutants carrying yellow eyes, rusty body and a black puparium (in contrast to the brown puparium of the normal wild strain). When empty puparia, from which pupal cuticles, other residues and lipids have been removed, are hydrolysed thin insoluble membranes

remain, "ghosts" of the original pupae, which are colourless in the normal wild strain but intensely black and thicker in the mutant. The black pigment in the puparia showed the physical properties of a melanin, with degradation products characteristic of melanins of animal origin. The pigment was therefore classified as an indole melanin. Experiments with radioactive tyrosine show it to be formed from tyrosine, confirmed by the nature of the degradation products.

- 157 Harris, S.E., Forrest, H.S. INHIBITION BY CERTAIN PTERIDINES OF RIBOSOMAL RNA AND DNA SYNTHESIS IN DEVELOPING *Oncopeltus* EGGS. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1 (1967) 89-94.

In developing *O. fasciatus* eggs, in which ribosomal RNA is the major type of RNA synthesised, the rate of this synthesis, when it is at its max., can be affected by incubating partially dechlorinated eggs with either of two naturally occurring pteridines, isoxanthopterin, or xanthopterin. Isoxanthopterin and xanthopterin at  $10^{-3}$  M inhibited by 50 or 25%, resp., the incorporation of uridine- $^3$ H into ribosomal RNA. DNA synthesis was also affected by the same two compounds. At later stages of development these inhibitory effects disappeared, and indeed, with respect to ribosomal RNA synthesis, pteridines stimulated incorporation of precursor. (CA 67:1967, 71415 h)

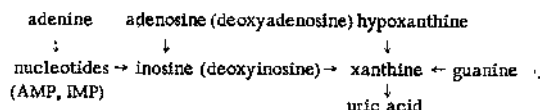
- 158 Heslop, J.P. EFFECTS OF AGE ON [ $^{14}$ C] VALINE TURNOVER INTO LOCUST WING PROTEINS. *Biochem. J.* **104**, 1 (1967) 5P-6P.

The posterior wings of *Schistocerca gregaria* age visibly as the animal gets older (see also ref. 136), becoming brittle and breaking away at the ends, although an active circulation of blood is maintained. The turnover of [ $^{14}$ C]valine into wing protein 2 h after injection of 0.66  $\mu$ Ci/g live wt. (6.9 mCi/mM; 66  $\mu$ μCi/ $\mu$ l) has been measured. At adult emergence the wings weigh 70 mg/g live wt. falling quickly to 30 mg/g at 30 h as the wings dry out and tan, and then more slowly to 16 mg/g at 14 weeks old. The wing protein is 10 mg/g at emergence, 6.3 mg/g at 30 h and 3.6 mg/g at 14 weeks so there is no net synthesis of protein by the wings during adult life. 2 h after injection the wings of newly-emerged adults contained 120  $\mu$ Ci/g of wing falling linearly to 53  $\mu$ Ci/g in wings of 14-week-old insects. The rate of incorporation of valine into wing protein was almost linear over the first 4 h after injection. The specific activity of wing protein 2 h after injection was measured in locusts of different ages. The age given is half way through the incubation period: 0.75 h, 0.17  $\mu$ Ci/g; 3.25, 0.28; 6.3, 0.35; 7.5, 0.69; 9.7, 0.61; 15.4, 0.82; 15.2, 1.0; 26.5, 0.95; 31.2, 1.23; 53.4, 1.27; 73, 0.69; 170, 0.67; 338, 0.61; 530, 0.28; 2120, 0.36; 2340 h, 0.29  $\mu$ Ci/g. There was no indication of an increase of protein synthesis in the wings of older insects.

- 159 Hodge, L.D. PART I. PURINE CATABOLISM IN *Drosophila melanogaster*. (PART II. URINARY EXCRETION OF UNUSUAL METABOLITES IN IDIOPATHIC ANEMICS.) *Diss. Abstr.* **27**, 8 (1967) 2623-B.

In part I, xanthine dehydrogenase, one of the enzymes of purine catabolism in *D. melanogaster*, has been extensively studied and is known to be affected by three separate loci. To date little evidence has been presented concerning the remainder of the pathway. In vitro studies were made of a Pacific strain and other strains carrying these mutant genes: *w* (1-1.5 $\pm$ ), *bw* (2-104 $\pm$ ), *st* (3-44 $\pm$ ), *lxd* (3-33 $\pm$ ), *ry* (3-52 $\pm$ ) and *ma-1* (1-64 $\pm$ ).

These conversions of  $^{14}$ C-purines were observed:



Two mutants of XDH, *ma-1* and *ry*, converted some  $^{14}$ C-xanthine to uric acid. No evidence for adenine deaminase activity or urate oxidase activity was noted. There was no significant difference in enzymic levels of adenosine deaminase, inosine phosphorylase or guanine deaminase in any of the strains. The possibility of pathway control by the genes involved in xanthine dehydrogenase activity: *ma-1* (1-64 $\pm$ ), *ry* (3-52 $\pm$ ) and *lxd* (3-33 $\pm$ ) was ruled out.

- 160 Hodge, L.D., Glassman, E. PURINE CATABOLISM IN *Drosophila melanogaster*. I. REACTIONS LEADING TO XANTHINE DEHYDROGENASE. *Biochim. biophys. Acta* **149** (1967) 335-343.

The pathway of purine catabolism in larval extracts of *D. melanogaster* was investigated. The enzymes prior to xanthine dehydrogenase in the pathway appear to be adenosine deaminase, inosine phosphorylase.

and guanine deaminase. Adenine deaminase and urate oxidase are not present in extracts of larvae. A Canton-S wild-type stock strain did not metabolise guanine- $^{14}\text{C}$ , suggesting a possible mutation affecting guanine deaminase. Some conversion of xanthine- $^{14}\text{C}$  to uric acid was noted in extracts of the xanthine dehydrogenase mutants, ma-1 and ry. (CA 68: 1968, 37150d)

- 161 Howells, A.J., Birt, L.M. AMINO ACID-DEPENDENT PYROPHOSPHATE EXCHANGE DURING THE LIFE CYCLE OF THE BLOWFLY Lucilia cuprina. Comp. Biochem. Physiol. **11** (1964) 61-83.

Some biochemical changes during the metamorphosis of the fly L. cuprina were studied and the amounts of free amino acids, soluble protein and DNA per organism have been estimated at different stages of the life cycle. The effect of variations in the concentrations of ATP, pyrophosphate and  $\text{Mg}^{2+}$  on the total rate of amino acid-dependent ATP-pyrophosphate exchange by soluble fractions were investigated and a suitably sensitive method of assay has been derived. Radioactive phosphate,  $\text{H}_2^{32}\text{PO}_4$  in HCl, was converted into pyrophosphate by pyrolysis at  $400^\circ\text{C}$  for 1 h. Amino-acid-dependent ATP-pyrophosphate exchange was determined by two methods, in both of which  $\text{Na}_4^{32}\text{P}_2\text{O}_7$  was used.

- 162 Howells, A.J., Birt, L.M., Finch, L.R. FURTHER STUDIES OF AMINO ACID ACTIVATION DURING THE LIFE CYCLE OF THE BLOWFLY Lucilia cuprina. J. Insect Physiol. **13**, 8 (1967) 1221-1236.

A technique has been developed for preparing from the soluble fraction of L. cuprina an enzyme system containing about 80% of the RNA and 25% of the total ATP-pyrophosphate exchange activity of the soluble fraction, 16 different amino acids were able to stimulate exchange with this system. Using this technique, enzymes were prepared from tissues at different stages of the life cycle and the variations during the life cycle in the total ATP-pyrophosphate exchange and in the exchanges with 21 single amino acids were measured. The variations were similar to those obtained with the original soluble fraction. The enzyme system incorporated [ $^{14}\text{C}$ ]-amino acids into a form insoluble in perchloric acid, in a reaction which was dependent on ATP and RNA. Evidence suggested that this reaction included an exchange between amino acids attached to sRNA and [ $^{14}\text{C}$ ]-amino acids in the incubation medium. The incorporations of aspartic acid, glutamic acid, and leucine were highly specific; amino acids with similar chemical structures did not compete effectively. Variations during the life cycle in the extent of incorporation both with a mixture and with single amino acids were investigated. The variations with the mixture of amino acids whether expressed per organism, per mg protein, or RNA were similar to those observed in the total rate of amino acid activation. These results are discussed in relation to likely changes in the rate of protein synthesis during the life cycle. Variations in the extents of incorporation for single amino acids, expressed relative to that obtained with the mixture, occur during the life cycle. Most pronounced were the decreases for glutamic acid and serine and the increases for alanine, aspartic, and tyrosine associated with the transition from larval to pupal life and the changes for leucine, proline, and threonine during the development of the pupa and pharate adult. (Auth.)

- 163 Ilivicky, J., Agosin, M. GLUTATHIONE TURNOVER IN Triatoma infestans TREATED WITH, 2,2-bis(p-CHLOROPHENYL)-1,1,1-TRICHLOROETHANE. Expl Parasit. **20**, 3 (1967) 345-356.

The effect of 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT) and 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (DDE) on  $^{14}\text{C}$ -labelled glycine (I) incorporation into glutathione (II) was studied in T. infestans. The levels of glutathione (oxidized) and glutathione (reduced) were similar in nymph and male specimens. DDT increased total II levels in nymphs, but in males this effect was negligible. DDE was without significant effect in nymphs, but markedly decreased total II levels in males. II turnover was increased by DDT in nymphs, whereas the opposite effect occurred in males. DDE markedly increased I incorporation into II in nymphs within the 1st hour of intoxication, but the turnover time was unaltered. These results were attributed to the increased level of enzymes involved in the synthesis and degradation of II. (CA 67: 1967, 116120x)

- 164 Ivie, G.W., Green, L.R., Dorrough, H.W. THE USE OF SODIUM BICARBONATE  $\text{C}^{14}$  TO DETERMINE CHOLINESTERASE ACTIVITY. Bull. envir. Contam. Toxicol. **2**, 1 (1967) 34-40.

Simple and inexpensive methods of determining cholinesterase activity are useful in many phases of research dealing with organophosphorus and carbamate insecticides. Such a method, based on the same principle utilized in manometric techniques has been developed successfully. Sodium bicarbonate- $^{14}\text{C}$  is used to react with acetic acid liberated from the enzymatic hydrolysis of acetylcholine. The resulting  $^{14}\text{CO}_2$  is trapped and quantitatively measured by liquid scintillation counting. The

method is rapid and inexpensive; although so far limited to gross determinations it could be made into a useful tool for more precise studies by additional refinement.

- 165 Jacobs, M. E. DEPOSITION OF LABELLED BETA-ALANINE IN EBONY AND NON-EBONY Drosophila melanogaster WITH NOTES ON OTHER AMINO ACIDS. Genetics 53 (1966) 777-784.

18 different  $^{14}\text{C}$ -labelled amino acids (400 cpm in  $10^{-5}$  ml water) were injected into the haemocoel of early pupae and early adults of the genotypes  $\underline{e}$ ,  $\underline{e}^+$ , and  $\underline{e}^+/e$  females. Following  $\beta$ -alanine injection, pupal cases incorporated more  $^{14}\text{C}$  in  $\underline{e}^+$  than in  $\underline{e}$ . The reverse was true for precipitates from centrifuged homogenized adults, and for excreted  $\text{CO}_2$  -  $\underline{e}$  showed greater incorporation than  $\underline{e}^+$ ,  $\underline{e}^+/e$  was intermediate. With other amino acids,  $\text{C}$  in pupal cases was near background; however, with  $\gamma$ -aminobutyric, aspartic, and glutamic acids and alanine more  $\text{C}$  was incorporated in  $\underline{e}^+$  than in  $\underline{e}$ . Rates of  $^{14}\text{CO}_2$  excretion were the reverse of the  $\text{C}$ -count of pupal cases and of precipitates of bodies of emerging adults. The  $\beta$ -alanine 2nd carbon was excreted as  $^{14}\text{CO}_2$  more slowly than was the 1st carbon. With carboxyl labelled  $\beta$ -alanine, phenylalanine, and valine,  $^{14}\text{CO}_2$  excretion was later in  $\underline{e}$  than in  $\underline{e}^+$ . Centrifuged precipitates from adults homogenized 6 h after injection showed great variation in  $\text{C}$ . With  $\beta$ -alanine, precipitates had lower counts for  $\underline{e}$  than  $\underline{e}^+$ , while  $\underline{e}^+/e$  was intermediate. Rates of  $^{14}\text{CO}_2$  excretion of these teneral adults were in reverse order of  $\text{C}$  in body precipitates.  $\underline{e}^+$  adults aged 9 d before injection showed accelerated  $^{14}\text{CO}_2$  excretion and no- $\text{C}$ -incorporation in body precipitates.  $\beta$ -alanine was injected into teners of other stocks. Oregon-R and two "black" mutants incorporated  $\text{C}$  in body precipitates at high rates, ebony<sup>11</sup> did not, and ebony-sooty was intermediate. (Essentially auth. summary)

- 166 Jenny, E., Hicklin, J. E., Leuthardt, F. IN-VITRO-EINBAU RADIOACTIVER AMINOSÄUREN IN DIE PROTEINE VON Drosophila -PUPPEN. (In vitro incorporation of radioactive amino acids into proteins of Drosophila pupae.) Helv. chim. Acta 45 (1962) 2014-2020. (In German, with English summary)

The incorporation of labelled amino acids (among them  $^{14}\text{C}$ -leucine) into microsomes from D. melanogaster pupae is described. Enzymatic incorporation can only be demonstrated after inhibition of a very active tyrosinase which is present in the microsomal fraction, by phenylthiourea. The system has the following properties: (1) It requires ATP,  $\text{Mg}^{++}$ , an ATP regenerating system, but not GTP. (2) Sucrose-washed microsomes with and without addition of pH 5 enzymes incorporate to the same extent. (3) Microsomes treated with deoxycholate or 0.05 M KCl incorporate less than the untreated ones, the former being nevertheless stimulated by addition of pH 5 enzymes or the pH 5 precipitate of the KCl extract. (4) Rat liver microsomes or pH 5 enzymes can be exchanged with the corresponding fraction from Drosophila.

ATP = adenosine-5'-triphosphoric acid.

GTP = guanosine-5'-triphosphoric acid.

- 167 Deleted.

- 168 Kapitza, W. SOME PROPERTIES OF THE NEUROHORMONES C AND D. Věst. čsl. Spol. zool. 31, 4 (1967) 355-359.

Four active substances have been isolated from the central nervous system of insects and crustaceans; acetylcholine, serotonin, and neurohormones C and D. Studies on S metabolism using  $^{35}\text{S}$  compounds in Periplaneta americana indicated that neurohormones C and D were of a peptide nature with S-containing amino acids. The neurohormones increased the heart beat frequency in Periplaneta until the heart stopped either in systole (with neurohormone C) or diastole (neurohormone D). The heart inhibition could be removed by diluting the neurohormones with Insect Ringer solution. Neurohormone D increased uptake of neutral red dye by Malpighian tubules and intestine of Dixippus morosus, whereas neurohormone C had an inhibitory action. (From CA 68: 1968, 76054v)

- 169 Karlson, P. BIOCHEMISCHE WIRKUNGSWEISE DER HORMONE. (Biochemical mechanisms of hormone action.) Dt. med. Wschr. 86 (1961) 668-674. (In German)

The effect of hormones on certain enzyme systems in terms of inhibition and activation, the effect on membranes by affecting permeability, and the effect on gene substance by activation or inactivation are discussed critically on hand of various examples. The effect of ecdysone is discussed in

connection with the induction of the puffing phenomenon, investigated autoradiographically in Chironomus tentans.

- 170 Karlson, P. BIOCHEMISTRY AND MODE OF ACTION OF ECDYSONE. p.416-419 of "2nd International Congress of Endocrinology. Proceedings, 1964".

Cholesterol-<sup>14</sup>C had been shown by the author to be a precursor of ecdysone. Ecdysone has been demonstrated to induce moulting and processes preparatory to moulting, and to induce the synthesis of mRNA carrying the information for dopa decarboxylase. The induction of puffs in salivary gland chromosomes and the biochemical events leading to the sclerotization of the larval cuticle are discussed.

- 171 Karlson, P. ECDYSON, DAS HÄUTUNGSHORMON DER INSEKTEN. (Ecdysone, the moulting hormone of insects.) Naturwissenschaften 53, 18 (1966) 445-453. (In German)

Review article. After a historical survey, the author discusses the concentration and isolation of ecdysone, the elucidation of its structure, its biological and biochemical effects, and its mechanism of action. Radioisotopes, although used in numerous studies cited are not mentioned specifically in the text.

- 172 Karlson, P., Sekeris, C.E. ECDYSONE, AN INSECT STEROID HORMONE, AND ITS MODE OF ACTION. Recent Prog. Horm. Res. 22 (1966) 473-483.

The chemical, physiological and chemical effects and the mechanism of action of ecdysone are reviewed. It was the first insect hormone to be isolated in pure, crystalline form, and the first to have its structure elucidated. Its mechanism of action, influence on genetic material and induction of messenger RNA synthesis are well documented. The use of radioisotopes formed an essential part of many studies cited (e.g. tracer studies with labelled compounds showed that tyrosine, dopa, and tyramine are incorporated into the cuticle; the stimulation of RNA synthesis, etc.). There are many indications that the same mechanism of action may also be valid for other steroid hormones in mammalian target tissues. (An active discussion on the paper is reported fully: see p. 494-502.).

- 173 Karlson, P. THE EFFECTS OF ECDYSONE ON GIANT CHROMOSOMES, RNA METABOLISM, AND ENZYME INDUCTION. Mem. Soc. Endocr. No.15 (1967) 67-74; discussion 74-76.

A review with conclusions. Ecdysone, the moulting hormone of insects, exerts its action at the level of the chromosome and induces messenger-RNA and in turn enzyme synthesis. In this respect hormones appear as general inducers and may be, in higher organisms, of far greater importance than substrates, which are of primary importance to bacteria. (CA 67:1967, 1059w)

- 174 Kasting, R., McGinnis, A.J. AMINO ACID REQUIREMENTS OF Hypoderma bovis, DETERMINED WITH GLUCOSE-U-<sup>14</sup>C. Exptl Parasit. 19, 3 (1966) 249-253.

Labelled glucose was injected into 3rd-instar larvae of H. bovis, the northern cattle grub, and the concentrations of some of the amino acids in the tissues of the grubs were determined. Alanine, proline, aspartic acid, and glutamic acid were readily synthesised and appeared to be nutritionally non-essential. The absence of radioactivity in phenylalanine, tyrosine, leucine, histidine, lysine, isoleucine, valine, threonine, and methionine showed that they were not synthesised by the cattle grub and were probably essential. Limited synthesis of glycine, serine, and arginine by this insect suggested that it would benefit from a dietary supply of these compounds. (CA 66: 1967, 83577e)

- 175 Kasting, R., McGinnis, A.J. RADIOISOTOPES AND THE DETERMINATION OF NUTRIENT REQUIREMENTS. Ann. N.Y. Acad. Sci. 139 (1966) 98-107.

An indirect radioactivity method for determining nutrient requirements is described. It depends on the presence of a readily metabolized compound normally available in food and labelled with <sup>14</sup>C. Nutrients of interest such as amino acids are subsequently isolated from the organism; after purification, the radioactivity is determined. Substances that contain <sup>14</sup>C are considered to be nutritionally non-essential since they were synthesised by the organism. In contrast, those substances that contain no <sup>14</sup>C are considered nutritionally essential since they were not synthesised. The validity of the method was established by comparing results with those of the classical deletion procedure. Apparent discrepancies can be explained. The radioactivity method has already been applied to several organisms that cannot be reared on chemically-defined diets. In particular, it has been widely used for determining the amino

acid needs of phytophagous and other insects. Conditions used in determining amino acid requirements of different organisms by the indirect radioactivity method are tabulated and include the black blowfly, *Phormia regina*, the pine sawfly, *Neodiprion pratti* Dyar, the wheat stem sawfly, *Cephus cinctus*, the wireworm *Arenicera destructor*, the cutworm *Agrotis orthogonia* Morr., the warble grub *Hypoderma bovis*, and the green peach aphid *Myzus persicae*. The dietary need of the hide beetle, *Dermestes vulpinus*, for cholesterol (and the lack of a dietary requirement for mesoinositol by the rat) were confirmed by the radioactivity method. The amino acid requirements have been examined for a number of other species including *Periplaneta americana* and *Blattella germanica*. Factors that must be considered in the interpretation of results from the radioactivity method are (a) purity of isolated compound, (b) pool size and source of isolated compound, (c) radioactive substrate, (d) metabolism period, and (e) administration of radioactive substrate. Radioisotopic procedures have also been used to demonstrate utilization of protein and cellulose by insects. In addition, radioisotope may be used to measure the amounts of food consumed and digestibility of dietary components by small organisms.

- 176 Kobayashi, M., Akaki, H. ACTION OF ECDYSONE ON SOME METABOLISM DURING LARVAL-PUPAL TRANSFORMATION OF THE HOUSEFLY, *Musca domestica* L. (DIPTERA: MUSCIDAE). *Appl. Ent. Zool.* **2**, 4 (1967) 223-224.

The effect of ecdysone on DNA and RNA synthesis, and on leucine and glucose metabolism was investigated. 5  $\mu$ l water ( $\pm$  2  $\mu$ g ecdysone) were injected into the posterior half of house fly larva, together with 0.2  $\mu$ Ci of  $^3$ H-thymidine, -uridine, -leucine- or -glucose. Most DNA synthesis appears to have been completed 24 h prior to pupation, whereas ecdysone appeared to accelerate RNA synthesis in the tissues examined (epidermis, fat body, muscle) by stimulating RNA-polymerase. Although ecdysone-injected larvae showed some slight incorporation of leucine into the epidermis, muscle, and intestine there was no increase with time, and the incorporation of leucine is not considered to be closely correlated with the presence of the hormone. It appears that injected glucose is converted to polysaccharides or related compounds and stored in the tissue. Ecdysone probably acts on glucose degradation in *M. domestica* larvae in the way it acts on *Samia* pupae. (See 99)

- 177 Kristensen, B.L. INCORPORATION OF TYROSINE INTO THE RUBBER-LIKE CUTICLE OF LOCUSTS STUDIED BY AUTORADIOGRAPHY. *J. Insect Physiol.* **12**, 2 (1966) 173-177.

Incorporation of L-tyrosine, generally labelled with  $^3$ H and given as a single injection into the haemolymph of the desert locust, *Schistocerca gregaria* Forskål, is shown to take less than 6 h, and the lag-phase is less than 1-2 h. The brightly fluorescent bands in resilin are synthesised during the day periods, whereas the faintly fluorescent bands are synthesised during the night periods. It is shown that the part of the prealar arm where two chitin lamellae are missing is synthesised soon after emergence. (Essentially auth.)

- 178 Kuvaeva, E.B., Gumilevskaya, N.A., Chumikina, L.V., Sisakyan, N.M. INCORPORATION OF AMINO ACIDS INTO PROTEINS OF NONCELLULAR SYSTEM OF *Bombyx mori* PUPAE. *Dokl. Akad. Nauk SSSR* **167**, 3 (1966) 695-698. (In Russian)

The cell free centrifugates prepared from crushed pupae 1-7 d of age were examined as to their ability to take up  $^{14}$ C-labelled amino acids into their proteins, with tagged alanine, glutamic acid, L-leucine, and threonine being used. Such incorporation was indeed found. Ribosomes isolated during max. level of histolysis had low activity; ribosomes from 7-d pupae had high activity. Amino acid incorporation was impossible in the presence of active tyrosinase. Amino acid incorporation in the cell-free system requires the presence of ATP and is sensitive to RNase and puromycin. (CA 64:1966, 20036g)

- 179 Deleted.

- 180 Laufer, H., Nakase, Y. CHROMOSOMAL PUFFING AS AN EXPRESSION OF PROTEIN TRANSPORT BY THE DIPTERAN SALIVARY GLAND. *J. Cell Biol.* **23**, 2 (1964) 52A. Abstr. 103, at "4th Annual Meeting of the American Society for Cell Biology, Cleveland, Ohio, USA, 11-13 Nov, 1964".

The primary function of the salivary gland in *Chironomus thummi* and *C. tentans* is to produce a secretion. Puffing of the Balbiani rings on salivary gland chromosomes, which represents sites of heightened RNA synthesis, has often been correlated with the production of secretion (Beermann, Panitz, Laufer). The pattern of Balbiani ring puffing is tissue and stage specific, as is the production of the secretion. Quantitative biochemical and immunochemical analyses of the salivary secretion revealed the presence

of a number of enzymes (Laufer) and antigens. Recently we found that all the major secretions antigens and enzymes occurred in other tissues as well as in the blood. Thus an apparent paradox arose: tissue- and stage-specific puffs appeared to be related to the secretion of proteins (representing more than 50% of the secretory mass), yet these proteins were not tissue specific. The following experiments appear to resolve this paradox. They indicate that salivary glands function predominantly to select, concentrate, and particularly to transport the proteins of the secretion. Furthermore, present evidence indicates that the gland does not synthesise any of the major secretory proteins.  $^{14}\text{C}$ -labelled *Chironomus* blood proteins were injected into larvae. Radioactive proteins promptly appeared in the secretion. Radioautographs of tissue sections showed that the radioactivity was located in cytoplasmic granules; these granules seemed to be formed at the ends of canals connected to the basal surface of the gland. Uptake of proteins therefore appears to occur from the haemolymph by a process akin to pinocytosis. After the injection of radioactive human serum albumin into larvae, this heterologous protein also was recovered in the secretion, retaining its antigenic properties. We concluded that the major proteins and antigens of the secretion normally are derived from the blood. Since the presence of the Balbiani rings in salivary chromosomes is correlated with the secretory process, we suggest that the Balbiani rings reflect the functioning of genes concerned with the synthesis of special "transport" proteins, or "permeases," important in the secretory process. These substances would not appear to be major constituents of the secretion itself. Thus the non-specific secretion even of heterologous protein can be, and indeed seems to be, mediated by tissue-specific puffs. (Abstr.)

- 181 Laufer, H., Doyle, D. SOURCES OF SALIVARY GLAND SECRETION IN *Chironomus tentans*. *J. Cell Biol.* 31, 2 (1966) 66A. Abstracts of Papers Presented at the "6th Annual Meeting of the American Society for Cell Biology, Houston, Tex., USA. 17-19 Nov. 1966". Abstr. 131.

Most proteins found in extracts of isolated salivary glands were also present in the haemolymph, according to results with disc electrophoresis in acrylamide gels. Certain blood proteins were labelled in the absence of the salivary glands when  $^{14}\text{C}$ -amino acids were injected into the body cavity. These blood constituents are identical with proteins of the salivary secretion, since the same number of counts were precipitated from labelled blood by antibodies to either secretion or blood. The transport of protein from the haemolymph to secretion can be demonstrated by  $^{125}\text{I}$ -serum albumin injection into the haemocoel. Labelled protein appeared in the secretion as demonstrated by the recovery of substantial quantities of albumin from the secretion and by high resolution radioautography of sectioned glands. Albumin transfer was not diminished by concomitant injections of iodine or tyrosine. These observations are consistent with a salivary gland transport function and suggest that blood components are synthesised at sites other than the gland, being subsequently transferred by the gland to the secretion. In addition to those components of the secretion which are concentrated by the gland, some fraction of the secretion is also synthesised in situ by the salivary gland. This was revealed by radioautographic analysis of glands cultured in vitro with a pulse of  $^3\text{H}$ -lysine followed by a chase with the unlabelled amino acid. Labelled protein studies indicate that phosphoproteins have a function in the nucleus. The fact that purified nuclear phosphoprotein forms complexes with histones which are less inhibitory to DNA-dependent RNA synthesis than free histones (Langan, in Symposium on "Regulatory Mechanisms in Nucleic Acid and Protein Biosynthesis", *Biochim. et Biophysica Acta*, Library Ser., 1966, in press) suggests the possibility that phosphoproteins may be involved in the regulation of RNA synthesis in eukaryotic chromosomes. (From abstr.)

- 182 Laufer, W. BEITRÄGE ZUR STRUKTUR DES SKLEROTINS UND ZUM CHEMISMUS DER SKLEROTISIERUNG. (Contributions on the structure of sclerotin and the chemistry of sclerotization.) Thesis. Munich Univ. (West Germany). Naturwissenschaftliche Fakultät. 1964, 70p. (In German)

The structural composition and histological differentiation of Diptera cuticles before and after pupation, followed by hormonal control, and chitin tanning under the action of phenol oxydase systems are discussed. The particular aspects studied were the changes in the chemical structure of cuticula by sclerotization, attention being paid to the fate, distribution and compound formation of tyrosine metabolites in the cuticle during different developmental stages; and the incorporation of various labelled precursors of chitin into the cuticula. Labelled amino acids (generally labelled L-tyrosine,  $\alpha$ - $^{14}\text{C}$ -DL-tyrosine,  $\alpha$ - $^{14}\text{C}$ -DL-DOPA, 2,3,5,6- $^{14}\text{C}$ -hydrochiquone, 1- $^{14}\text{C}$ -leucine,  $\alpha$ - $^{14}\text{C}$ -dopamine, acetyl dopamine, acetyl dopamine- $\alpha$ - $^{14}\text{C}$ , acetyl- $^{14}\text{C}$  dopamine, and generally labelled acetyl tyramine) were injected into larvae 1d before pupation, and the distribution of radioactivity between cocoon and pupa determined. Tyrosine incorporation does not involve protein synthesis. Phenolic rather than chinoid substances appear responsible for cuticle tanning. Tyrosine is

incorporated primarily during the first 24 h of pupation, at a percentage considerably above that of dopamine. - During pupation the water content of the cuticula drops from 68% to 7%. The increase in dry weight by 31% is caused by the sclerotizing substance acetyl dopamine, by an unidentified carbohydrate, and a further protein. The chitin content remains unchanged. Experiments with  $^{14}\text{C}$ -tyrosine have shown that tyrosine metabolites are firmly incorporated into the cuticula in the course of sclerotization. Apart from tyrosine, N-acetyl-dopamine, a direct precursor of sclerotizing chinone, was found in the cuticula of prepupae. In 12-h-old, just coloured puparia no N-acetyl-dopamine or radioactive tyrosine can be traced even after extraction and hydrolysis. The presence of phenols and phenol carbonic acids has, in part, been confirmed in Calliphora. They are not, however, formed from tyrosine immediately before pupation. Para-diphenols do not play any part in sclerotization. Fractionated chemical extraction of puparia gave fractions particularly enriched in labelled tyrosine metabolites, the fractions containing only 1.5% protein. The major component consists of what is presumably a carbohydrate with markedly high O-content. The protein which lacks arginine, serine, and threonine can be separated completely by hydrolysis. Radioactivity remains in the carbohydrate fraction. When extracts are hydroxylated before hydrolysis, 4 ninhydrin-negative degradation products are obtained. The radioactive  $\text{C}_4$ -chain proves that a chinoid metabolite is involved in sclerotization. From the various data obtained the author concludes that the chinoid tyrosine metabolite is responsible for the reddish brown colour, the incorporation of the cuticle components as a network within the complex sclerotin structure, and probably also for linking them to the chitin skeleton.

- 183 Liaci, L. SYNTHESIS OF SEPIAPTERINE FROM LABELLED ADENINE IN THE EYES OF Drosophila melanogaster. Riv. Biol., Suppl. 58 (1965) 53-55.

The title synthesis in the mutant se was determined by chromatography and autoradiography. A purine base was utilised, in this case, adenine. (CA 64: 1966, 14647g)

- 184 Linzen, B., Ishiguro, I. 3-HYDROXYKYNURENINE IN Bombyx mori. NEW TRYPTOPHAN METABOLITE, 3-HYDROXYKYNURENINE GLUCOSIDE. Z. Naturf. 21b, 2 (1966) 182-187.

Using a specific quantitative method, 3-hydroxykynurenine was determined through a number of larval, pupal and imaginal stages of the B. mori mutant rb. There is a steady increase in the amount of hydroxykynurenine from the spinning larva to the middle of the pupal stage, reaching 1 mg/animal. During the same period the pattern of fluorescent compounds shows significant changes. After injection of  $^3\text{H}$ -hydroxykynurenine several fluorescent compounds are labelled. The O- $\beta$ -glucoside of 3-hydroxykynurenine appeared after cocoon spinning. Its structure was confirmed by synthesis. (CA 64: 1966, 18077a)

- 185 Linzen, B. ÜBER NEUE OMMOCHROME BEI HEUSCHRECKEN. (The existence of new ommochromes in grasshopper.) Naturw. Rdsch., Stuttg. 12 (1959) 432. (In German). Also presented at "17. Internationaler Kongress für Reine und Angewandte Chemie 30 Aug. - 6 Sep. 1959."

The existence of ommochromes in grasshopper was investigated. The brick-red pigment was isolated from the eyes. Compared with the usual ommochromes the isolated pigment showed different characteristics on analysis by paper chromatography and spectroscopy. It could, nevertheless, be identified as an ommochrome by means of experiments involving the injection of  $^{14}\text{C}$ -tryptophan into larvae.

- 186 Linzen, B. ZUR BIOCHEMIE DER OMMOCHROME. UNTERTEILUNG, VORKOMMEN, BIOSYNTHESE UND PHYSIOLOGISCHE ZUSAMMENHÄNGE. (The biochemistry of ommochromes. Their subdivision, occurrence, biosynthesis, and physiological significance.) Naturwissenschaften 54, 11 (1967) 259-267. (In German)

Work on the dark eye pigments of insects is reviewed. Some radiolabel studies are cited. Thus the red pigmentation in the eye of certain grasshoppers (variously called acridoxanthine, acridioerythrine and insectorubine) could be proved, by means of its incorporation of radioactive 3-hydroxykynurenine, to be of ommochrome, rather than of omnine nature. The term ommidine was subsequently used for it. - The incorporation of labelled precursors into ommines was demonstrated by means of methionine- $^{35}\text{S}$  and 3-hydroxykynurenine- $^3\text{H}$  into Gryllus bimaculatus. It could thus be shown that the ommines are not synthesised continuously but only for a short period at the end of each moult.

- 187 Locke, M., Collins, J.V. SEQUESTRATION OF PROTEIN BY THE FAT BODY OF AN INSECT. Nature, Lond. 210 (1966) 552-553.

Two hours after the injection of  $^3\text{H}$ -tyrosine ( $20 \mu\text{Ci/g}$ ) into 28 *Calpodex* larvae in the 5th and pupal stadia, autoradiographs demonstrated that amino acid incorporation into the fat body was no higher at the time of protein granule formation than at other times in the stadium. In contrast, there was considerable incorporation into the epidermis when the protein granules were forming and the endocuticle was being resorbed, but the protein granules did not have a higher labelled amino acid incorporation than did the rest of the cell. The incorporation of  $^3\text{H}$ -leucine was also examined. Treatment of the larvae with a foreign recognizable protein (horseradish peroxidase) when the protein granules were forming demonstrated, in frozen sections treated with alcoholic benzidine-HCl, that the protein granules may have resulted from the fusion of smaller granules, suggesting that the fat body sequesters proteins from the blood to form the protein storage granules.

- 188 Lue, P.F., Dixon, S.E. STUDIES OF THE MODE OF ACTION OF ROYAL JELLY IN HONEYBEE DEVELOPMENT: THE UTILIZATION OF SUGAR UNIFORMLY LABELLED WITH  $^{14}\text{C}$  AND OF ASPARTIC- $^{14}\text{C}$  ACID. *Can. J. Zool.*, 45 (1967) 595-599.

The amino acid requirements of developing honey bee larvae were determined by the indirect method using glucose- $^{14}\text{C}$  and sucrose- $^{14}\text{C}$ . The amino acids proline, hydroxyproline, alanine, glutamine, glutamic acid,  $\alpha$ -amino- $\eta$ -butyric acid,  $\gamma$ -amino- $\eta$ -butyric acid, glycine, serine, and  $\beta$ -alanine were classified as non-essential. Cystine, aspartic acid, asparagine, isoleucine, leucine, phenylalanine, methionine, tryptophan, valine, threonine, tyrosine, lysine, histidine, and arginine were considered to be essential. The metabolism of sugar and aspartic acid are discussed in relation to caste development. (Auth.)

- 189 Mansingh, A. METABOLISM OF LABELED GLUTAMIC ACID IN ADULT GERMAN COCKROACHES. *J. econ. Ent.*, 59, 1 (1966) 234-235.

Some aspects of the fate and metabolic pathways of the carbon skeleton of glutamic acid are considered. ( $^{14}\text{C}$ )-glutamic acid in distilled water was injected into the body cavity of *Blattella germanica* (L.), the radioactivity of the solution being 0.01 mCi or  $\sim 2500$  cpm. The final values were corrected for self absorption and back counts. The recovery of radioactivity from  $^{14}\text{CO}_2$ , sugars, lipids, and some of the amino acids suggests that the bulk of the administered amino acid was metabolized via Krebs' TCA cycle. The labelled carbon of glutamic acid was found to be incorporated into seven amino acids, proline, glutamine and glycine showing much higher radioactivity. The incorporation of ( $^{14}\text{C}$ )-glutamic acid into various compounds is tabulated and the significance of the results discussed.

- 190 Meinwald, J., Koch, K.F., Rogers, J.E., Jr., Eisner, T. BIOSYNTHESIS OF ARTHROPOD SECRETIONS III. SYNTHESIS OF SIMPLE *p*-BENZOQUINONES IN A BEETLE (*Eleodes longicollis*). *J. Am. chem. Soc.*, 88, 7 (1966) 1590-1592.

Two pathways of synthesis of the *p*-benzoquinones found in arthropod defence secretions were demonstrated in *E. longicollis*. Paper chromatography of 2,4-dinitrophenylhydrazine-treated secretions from L-tyrosine- $^{14}\text{C}$  or DL-phenylalanine-ring- $^{14}\text{C}$ -fed insects showed the synthesis of *p*-benzoquinone to involve the utilization of the aromatic ring of tyrosine or phenylalanine. Direct silicic acid column chromatography of secretions from insects injected with Na acetate- $^{14}\text{C}$  and Na propionate- $^{14}\text{C}$  gave component benzoquinones which were oxidized to acetic and propionic acid by  $\text{KMnO}_4$ . The aliphatic acids were degraded by the Schmidt reaction and the products purified to a constant specific activity. About 30% of the total activity of methyl-*p*-benzoquinone derived from Na acetate- $^{14}\text{C}$  appeared at C-2. Since  $<0.1\%$  of the activity occurred in the Me group of this compound, the remaining 70% must be distributed among the other 5 ring-C atoms. Similarly, with ethyl-*p*-benzoquinone derived from Na acetate- $^{14}\text{C}$ , about 90% of the total activity was found in the analogous 5 ring-C atoms. Thus, the synthesis of methyl-*p*-benzoquinone and ethyl-*p*-benzoquinone involved the utilization of acetate units to form the quinone ring. After injection of Na propionate- $^{14}\text{C}$ , 95% of the activity found in ethyl-*p*-benzoquinone was recovered in the derived propionic acid and was almost entirely localized at C-2 of the ring. The low level of incorporation of the precursor into the remaining ring C of methyl-*p*-benzoquinone and ethyl-*p*-benzoquinone may be due to the slight conversion of the precursor into  $\text{C}_2$  units. (CA 65: 1966, 2693b)

- 191 Melius, M.E., Jr. AN AUTORADIOGRAPHIC ANALYSIS OF BLOOD PROTEIN UPTAKE AND PROTEIN YOLK SPHERE FORMATION BY *Cecropia* MOTH OOCYTES. *Diss. Abstr.*, 27, 5 (1966) 1659-B.

Cecropia moth oocytes selectively remove proteins from the blood during the period of yolk formation; the blood proteins reach the surface of the oocyte by an intercellular route, enter the oocyte by pinocytosis, and become a component of the protein yolk spheres. The extent to which a particular protein enters the oocyte may be determined by its relative affinity for the surface of the oocyte; three basement lamellae that form continuous boundaries between the oocytes and the haemocoel are also candidates for the site of a selective mechanism. The primary objective of the work described in this dissertation was to obtain experimental evidence concerning the relative contributions of the basement lamellae and the oocyte itself to the selectivity of blood protein uptake. An autoradiographic analysis of the uptake of  $^3\text{H}$ -blood proteins injected into the haemocoel indicated that selective transmission through the basement lamellae and selective adsorption onto the oocyte surface both contribute to the selectivity of blood protein uptake. Evidence is presented, too, from studies of the incorporation of  $^3\text{H}$ -leucine into blood proteins and yolk, that the ovary itself synthesises some of the proteins deposited in the yolk spheres. Information concerning the relative rates of yolk formation by various regions of the oocyte cortex was also obtained; the rate of yolk formation by a particular area of the oocyte surface appears to be correlated with the ease with which materials derived from the blood can reach it. (DA)

- 192 Miles, P.W. STUDIES ON THE SALIVARY PHYSIOLOGY OF PLANT-BUGS: TRANSPORT FROM HAEMOLYMPH TO SALIVA. J. Insect Physiol. 13, 12 (1967) 1787-1801.

The insects used in Australia were 5th-instar larvae of Eumecopus punctiventris Stål (Pentatomidae) collected from under the bark of sugar gums (Eucalyptus cladocalyx F. Muell.); and adults of the peanut litter bug, Elasmolomus sordidus (F.) (Lygaeidae), from a laboratory culture maintained on peanuts. When injected into the haemolymph, D-glucose (universally  $^{14}\text{C}$ -labelled), glycerol- $1\text{-}^{14}\text{C}$ , and amino acids [DL-phenyl (alanine- $1\text{-}^{14}\text{C}$ ), DL-tryptophan (methylene- $^{14}\text{C}$ ), and D-tryptophan (methylene- $^{14}\text{C}$ )], rapidly appear in the watery saliva of phytophagous Heteroptera, Pentatomorpha; and glucose, glycerol, and some amino acids are also incorporated into the precursor of the component of the saliva that solidifies to form the 'stylet sheath'. Injection of a large amount of an amino acid into the haemolymph also causes the compound to appear in unusual concentration in the watery saliva, apparently due to excretory activity by the accessory gland of the salivary apparatus. Radioisotopic and chromatographic evidence is given for the occurrence in the various salivary secretions of carbohydrate and amino- and phospholipid.

- 193 Miles, P.W., Lloyd, J. SYNTHESIS OF A PLANT HORMONE BY THE SALIVARY APPARATUS OF PLANT-SUCKING HEMIPTERA. Nature, Lond. 213 (1967) 801-802.

A number of experiments were carried out to test the possible synthesis of salivary  $\beta$ -indolyl acetic acid (IAA) in Hemiptera, using Eumecopus punctiventris Stål (Pentatomidae) and Elasmolomus sordidus (F.). Injection of  $1.5\ \mu\text{Ci}$  of DL-3-phenyl (alanine- $1\text{-}^{14}\text{C}$ ) in  $3\ \mu\text{l}$  of Martignoni and Scallion's salt solution (without ascorbic acid) into the haemolymph of larvae of Eumecopus at the 5th instar resulted in the appearance in the salivary glands,  $1\text{-}1\frac{1}{2}$  h later, of subequal quantities of labelled phenylalanine and other radioactive compounds with the same  $R_f$  as dihydroxyphenylalanine (DOPA). Results support an earlier suggestion that DOPA is the substrate for the salivary polyphenol oxidase, and indicates that phenylalanine is a precursor. Further experiments were done with the much smaller Elasmolomus. In another experiment, DL-tryptophan (methylene- $^{14}\text{C}$ ), in  $0.5\ \mu\text{l}$  salt solution containing  $2\ \mu\text{g}$  L-phenylalanine (unlabelled), was injected. Autoradiography showed that in the homogenate of the salivary glands (including the accessory gland and ducts), a small amount of the tryptophan had been converted to a labelled compound with the same  $R_f$  as IAA. Chromatography of the haemolymph of untreated insects showed that free tryptophan and phenylalanine are normal constituents; IAA synthesis is considered to occur naturally during salivation of these insects.

- 194 Minks, A.K. BIOCHEMICAL ASPECTS OF JUVENILE HORMONE ACTION IN THE ADULT Locusta migratoria. Archs. nêrl. Zool. 17, 2 (1967) 175-257.

An extensive report is given of the biochemistry of juvenile hormone action in the adult L. migratoria, including respiration experiments, experiments with radioactive compounds, agar-gel electrophoresis of proteins, and electron microscopy. The role of juvenile hormone in respiratory metabolism is discussed, and a comparative study of the effects of allatectomy and some other operations is presented. The results are in accord with the concept that the fat body produces a considerable fraction of the proteins required for oogenesis, and with the ideas of K.C. Highnam et al. (J. Ins. Physiol. 9: 1963, 587) that the neuro-secretory cells activate protein synthesis in the fat body. However, a more independent role is suggested

for the corpora allata in this process. The data are more in accord with the idea that juvenile hormone exerts its activities in the presence of active protein synthesis and induces the formation of specific vitellogenic proteins. The change of lipid and glycogen metabolism in the fat body may be explained in the same way. (CA 68; 1968, 1150q)

- 195 Muramatsu, M., Otomo, K., Shimura, K. THE BIOSYNTHESIS OF GLYCINE IN THE SILKWORM. III. FORMATION OF GLYCINE FROM GLYCOLIC- $^{14}\text{C}$  ACID AND THE OCCURRENCE OF GLYCOLATE OXIDASE. Seikagaku Zasshi (J. Biochem., Tokyo) 59, 3 (1966) 304-309. (In English)

$^{14}\text{C}$ -labelled glycolate was converted to labelled glycine in the silkworm in vivo. Less, but significant amounts of serine and alanine were also formed. Glycolate oxidase activity was found in the homogenate of silkworm fat body. Other tissues, such as silk glands and digestive tracts, showed no detectable enzyme activity. An enzyme system from the fat body also catalysed the conversion of glycolate to  $\alpha$ -ketoglutarate and pyruvate. Glycolate oxidase activity was stimulated about 2-fold by the addition of  $5 \times 10^{-4} \text{ M NH}_4\text{OH}$  to the system, while the formation of  $\alpha$ -ketoglutarate was significantly inhibited. (CA 65; 1966, 1093e)

- 196 Nishizawa, T. FIBROIN SYNTHESIS OF POSTERIOR SILK GLANDS. VII. FIBROUS NUCLEIC ACID IN THE FIFTH INSTAR LARVAE. Seikagaku 38 (1966) 224-233. (In Japanese)

From posterior silk glands of silkworm (*Bombyx mori*) at the 5th-instar larval stage, fibrous nucleic acid preparation was isolated, consisting mostly of RNA and a slight amount of DNA. It was speculated from analytical data that DNA and RNA make up a complex in this nucleic acid preparation. Actinomycin inhibited the synthesis of this specific nucleic acid, which stimulated the incorporation of glycerine- $^{14}\text{C}$  into protein using the supernatant fraction obtained by centrifugation at 105 000 g for 60 min. (CA 65; 1966, 9405g)

- 197 Norman, C. QUANTITATIVE STUDY OF DISTRIBUTION OF SULPHYDRYL GROUPS IN THE DEVELOPING GRASSHOPPER (*Melanoplus differentialis*) EMBRYO. Physiol. Zool. 27 (1954) 141-156.

The distribution of sulphhydryl groups was studied quantitatively in the grasshopper egg which, during the course of its development, goes into a mitotically blocked or diapause state in which metabolic and other cellular activities are greatly diminished. In one series of experiments, 4-d-old eggs were irradiated at 130 kV, at 125 R/min for 6 min (total dose 750 R), incubated at 25°C and subsequently lots were removed for amperometric analyses. The dose prevents embryo development. The curve representing the yield of -SH of irradiated eggs (without embryos) maintains a fairly constant level of -SH content from the 10th day of prediapauses to the 6th day of diapause. This level of -SH is comparable to that found in normal zero-day prediapauses eggs. The yield of -SH curve for the controls (with embryos) shows a progressive decrease in the number of titratable sulphhydryl groups. Nonprotein yield extracts of irradiated as well as control eggs, when treated with 10-15 mg of sodium borohydride for 30 min at room temperature and titrated amperometrically, gave negative results. This indicates the absence of oxidized as well as reduced soluble free mercapto groups in the yolk. Summarizing it may be concluded that: The increase of titratable -SH groups in prediapauses, a levelling-off during diapause, and the resumption of a rapid rise in the post-diapause period parallel the metabolic, mitotic, and morphogenetic activity of the embryo during these phases of development. The increase of embryo -SH occurs at the expense of the yolk -SH. There is no direct relationship between the sulphhydryl content and the "blocked" or diapause state in the grasshopper embryo. The excessive, abrupt rise in the titer of -SH groups during postdiapause is associated with morphogenetic growth and differentiation. The embryo is probably the site of origin of the enzyme responsible for the breakdown of protein -SH in the yolk. The nonprotein -SH (probably glutathione) enters the embryonic cells, serving as a substrate for protein -SH resynthesis, as well as acting as a regulator of cell metabolism. Cytoplasmic -SH consists of protein and nonprotein thiols, with all the non-protein -SH in the reduced state. The yolk and nuclei do not contain oxidized or reduced soluble free thiol groups. The histochemical demonstration of protein-bound -SH in the embryo qualitatively confirms the quantitative data and indicates the localization of protein-bound mercaptans in nerve and muscle tissue of postdiapause embryos.

- 198 Okada, T., Goto, M. SYNTHESIS OF 2-AMINO-4-HYDROXY-6-HYDROXYMETHYLPTERIDINE-10-C- $^{14}$  AND 2-AMINO-4-HYDROXYPTERIDINE-10-C- $^{14}$  AND THEIR METABOLISM IN *Drosophila melanogaster*. Seikagaku Zasshi (J. Biochem., Tokyo) 58, 5 (1965) 458-462.

The biosynthesis of sepiapterin by D. melanogaster, mutant sepi, was investigated with 2-amino-4-hydroxy-6-hydroxymethylpteridine-10-<sup>14</sup>C and reduced 2-amino-4-hydroxypteridine-10-<sup>14</sup>C. It was found that after growth with both labelled compounds radioactive isoxanthopterin and sepiapterin could be isolated by paper chromatography from the adult flies. The postulated hypothesis concerning the biosynthesis of Drosophila pteridines was thus proved to be correct. (From auth. summ.)

- 199 Passama-Willaume, M., Barbier, M. SUR LA BIOSYNTHESE DE LA BILIVERDINE IX $\alpha$  PAR LA MANTE Mantis religiosa ET LE CRIQUET Locusta migratoria. C.r. hebdomadaire. Séances Acad. Sci., D 263 (1966) 924-925.

Glycocoll-1,2-<sup>14</sup>C<sub>2</sub>, dissolved in Ringer solution, was injected in the 4th abdominal ring of green larvae of M. religiosa and L. migratoria; after 32 h and 72 h, respectively, larvae were killed in dry ice; green pigments were extracted with water and Me esters were prepared after lyophilization of these extracts. Chromatographic study shows that the injected glycocoll-1,2-<sup>14</sup>C<sub>2</sub> is incorporated in the biliverdin, which is the hypodermal and blood pigment of these two species. (CA 66: 1967, 17350z)

- 200 Pettit, B.J., Rasch, R.W. TRITIUM- LABELED HISTIDINE INCORPORATION INTO GIANT SALIVARY CHROMOSOMES. J. Cell Biol. 23, 2 (1964) 73A. Abstr. 148, at "4th Annual Meeting of the American Society for Cell Biology, Cleveland, Ohio, USA, 11-13 Nov. 1964".

The role of protein synthesis in the structure of giant chromosomes was followed by feeding <sup>3</sup>H-labelled histidine to larvae for a 2-h period 24 - 48 h before puparium formation. The pattern of incorporation into chromosome "puffs" was followed by incubation of explanted Drosophila salivary glands in a Ringer's solution containing the <sup>3</sup>H-labelled amino acid. Following a short exposure to the isotope, one lobe of the gland was fixed immediately, as a control, while the other lobe was incubated in "cold" medium for periods up to 2 h. The "developmental stage" of the larva was determined by the number of hours that had elapsed from the sealing of the operculum to the time of explantation. The incorporated isotope was visualized by the application of autoradiographic stripping film to chromosome smears. The autoradiographic patterns of incorporation will be presented and discussed. (Abstr.)

- 201 Pettit, B.J., Rasch, R.W. TRITIATED HISTIDINE INCORPORATION INTO Drosophila, Drosophila virilis, SALIVARY CHROMOSOMES. J. cell.comp. Physiol. 68, 3 (1966) 325-333.

An autoradiographic study of <sup>3</sup>H-histidine incorporation into non-histone protein of explanted larval salivary gland chromosomes of D. virilis showed patterns of incorporation that were dependent upon the stage of larval development. The sequence of changes in the development of several puffs in a specific chromosomal region was followed using the appearance of pigment in the anterior spiracles as a means of larval staging. <sup>3</sup>H-histidine incorporation into these puffs in prepupae occurred as the puffs were regressing in size and protein staining. Acid extraction of histone and nucleic acid failed to alter the character of the autographs; presumably a non-histone protein is involved in the <sup>3</sup>H-histidine incorporation. Other puff sites in the same prepupal chromosomes showed various patterns of isotopic amino acid incorporation indicating that the pattern reported for a specific region may not be true for all puff sites. (Auth.)

- 202 Poremska, Z., Gorzkowski, B., Jezewska, M.M. UTILIZATION OF OROTATE-<sup>14</sup>C IN THE BIOSYNTHESE OF PYRIMIDINES IN Helix pomatia AND Celerio euphorbia. Acta biochim. pol. 13 (1966) 107-111.

An attempt was made to ascertain whether orotate can serve as precursor for synthesis of pyrimidine nucleotides in those uricotelic invertebrates in which so far the presence of carbamoylphosphate synthase has not been demonstrated. After injection of radioactive orotate to C. euphorbia pupae, active UMP\* and CMP\*\* were found in RNA isolated from the fat body and muscle. In RNA from H. pomatia hepatopancreas, radioactive UMP and CMP were found after administration of labelled orotate, aspartate, or bicarbonate. The presence of aspartate carbamoyltransferase in snail hepatopancreas was demonstrated. In the two invertebrates studied, the biosynthesis of the pyrimidine ring probably follows the same pathway as in vertebrates. (CA 65: 1966, 13018h)

\* uridine monophosphate.

\*\* cytosine monophosphate.

- 203 Price, G.M. THE IN VITRO INCORPORATION OF [U-<sup>14</sup>C] VALINE INTO FAT BODY PROTEIN OF THE LARVA OF THE BLOWFLY, Calliphora erythrocephala. J. Insect Physiol. 12 (1966) 731-740.
- The incorporation of [U-<sup>14</sup>C] L-valine into fat body isolated from the larva of the blowfly, C. erythrocephala, has been studied in vitro. The effect of various concentrations of L-valine and of a mixture of amino acids was studied at pH 6.5. The highest incorporation rates were obtained with fat body isolated from 4-d-old larvae, the rate falling off rapidly as the larvae became older. It was found that the fat body synthesised protein which was then released into the surrounding incubation medium. During the course of incubation the specific activity of this released protein increased rapidly until its level was many times that of the structural protein of the fat body. (Auth.)
- 204 Price, G.M., Bosman, T. THE ELECTROPHORETIC SEPARATION OF PROTEINS ISOLATED FROM THE LARVA OF THE BLOWFLY, Calliphora erythrocephala. J. Insect Physiol. 12 (1966) 741-745.
- The proteins in the haemolymph isolated from blowfly larvae of different ages were separated by electrophoresis on acrylamide gel. Their pattern was compared with that of proteins released in vitro by blowfly fat body and found to be almost identical. Fat body was also incubated in the presence of [U-<sup>14</sup>C] valine, and the distribution of <sup>14</sup>C-activity among the labelled proteins was measured. (Auth.)
- 205 Price, G.M. THE EFFECT OF DIFFERENT IONS ON THE INCORPORATION OF [U-<sup>14</sup>C] VALINE INTO FAT BODY PROTEIN OF THE LARVA OF THE BLOWFLY, Calliphora erythrocephala. J. Insect Physiol. 13 (1967) 69-79.
- Fat body from blowfly larvae was incubated with a mixture of amino acids, [U-<sup>14</sup>C] valine and various Ringer's solutions. The metabolism of phenylalanine was further examined by incubating fat bodies in the presence of [U-<sup>14</sup>C] phenylalanine. Incorporation of [U-<sup>14</sup>C] valine into protein was considerably affected by changes in the level of Ca, Mg, K, and Na in the incubation medium. During 60 min incubation there was a fall in the levels of alanine, aspartate, glutamate, and phenylalanine in the medium and a rise in glutamine and tyrosine. An examination of the in vivo distribution of free amino acids between various tissues of the larva showed that aspartate and glutamate were concentrated in tissues other than the haemolymph and that phenylalanine and tyrosine were concentrated in the fat body in particular.
- 206 Rao, D.R., Ennor, A.H., Thorpe, B. THE ISOLATION AND IDENTIFICATION OF L-LANTHIONINE AND L-CYSTATHIONINE FROM INSECT HEMOLYMPH. Biochemistry 6, 4 (1967) 1208-1216.
- Lanthionine and cystathionine have been isolated in crystalline form from the deproteinized haemolymph of Bombyx mori (silkworm) and Antheraea pernyi (Japanese oak moth) by ion-exchange chromatography. Both compounds have been characterized as the L-enantiomorphs by enzymic and physical methods. A preliminary survey of the distribution of lanthionine and cystathionine throughout a number of phyla has been carried out and the results of this, together with some preliminary experiments on the in vivo incorporation of isotopically labelled materials, is reported. The presence of free L-lanthionine in insect tissues is invariably associated with a complete absence or, at the best, barely detectable traces of cysteine, cystine, and methionine. (Auth.)
- L-[<sup>35</sup>S] cystine, L-[<sup>35</sup>S] methionine, and L-[3-<sup>14</sup>C] serine were used.
- 207 Ray, J.W. THE FREE AMINO ACID POOL OF COCKROACH (Periplaneta americana) CENTRAL NERVOUS SYSTEM. p.31-38 of "The Physiology of the Insect Central Nervous System". Treherne, J.E., Beament, J.W.L., Eds. 1965.
- 208 Razet, P. END PRODUCTS OF NITROGEN CATABOLISM IN INSECTS, Année Biol. 5 (1-2) (1966) 43-73.
- A review of the origin and fate of uric acid, degradation of nucleic acids, nonuric acid N excretion, urea, ammonia, amino acids, creatine, creatinine, and pteridines.
- 209 Rembold, H. ZUM STOFFWECHSEL DES BIOPTERINS IN DER HONIGBIENE, (Bioprotein metabolism in the honey bee.) Naturw. Rdsch., Stuttg. 12, 11/12 (1959) 432-433. (In German) Also presented at "17. Internationaler Kongress für Reine und Angewandte Chemie. 30 Aug. - 6 Sep. 1959".

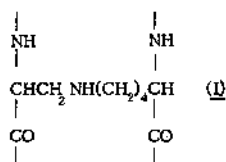
Biopterin metabolism was studied in workers and queens, using  $^{14}\text{C}$ -biopterin. One quarter of the radioactivity was found to remain in the insect. An equal amount was determined in the pupa and the imago. The rest is eliminated as faeces prior to pupation. Biopterin scarcely metabolises into other fluorescent compounds.

- 210 Rembold, H. STOFFWECHSEL DES BIPTERINS UND POLAROGRAPHISCHE CHARAKTERISIERUNG VON PTERIDINEN. (Biopterin metabolism and the polarographic characterization of pteridines.) p.485-484 of "3rd International Symposium on Pteridine Chemistry. Proceedings. Stuttgart, Federal Republic of Germany. 1962". New York, Macmillan Co. 1964. (In German)

Biopterin (I) is a characteristic component of royal jelly. Its metabolism was studied by following the development of queen larvae. Synthesis, separation, and characterization of the 6- and 7-L-erythro-1,2-dihydroxypropyl-derivatives of 2-amino-4-hydroxypteridin are described.  $^{14}\text{C}$ -I (purified chromatographically via phosphorus cellulose) was obtained as analogue. Its metabolism was investigated in rat, *Drosophila melanogaster*, and the bee. These insects metabolize I only slightly. In rat, the bulk of the injected I is eliminated within a few days, having undergone only slight degradation. In a growth test on *Cribidia fasciculata*, a markedly lower activity than in I was observed in various pteridines and pyrimidines. 6-polyhydroxyalkyl-pteridines which have an OH-group in the 2-position of the side chain in the L-erythro component are an exception. The possible application of polarographic methods for determining the structure of pteridines is demonstrated by various examples.

- 211 Robson, A., Zaidi, Z.H. THE FORMATION OF LYSINOALANINE DURING THE TREATMENT OF SILK FIBROIN WITH ALKALI. *J. Text. Inst. Trans.* 58, 6 (1967) 267-269.

Mulberry leaves with  $^{14}\text{C}$ -labelled cystine were fed to silkworms (*Bombyx mori*), and silk fibroin obtained contained  $^{14}\text{C}$ -labelled cystine. The fibroin was treated with  $\text{K}_2\text{CO}_3$ , acid hydrolysed to give lysino-alanine of specific activity  $2.84 \times 10^{-3} \mu\text{Ci}/\mu\text{M}$ , and oxidized with performic acid to give cysteic acid having a specific activity of  $2.98 \times 10^{-2} \mu\text{Ci}/\mu\text{M}$ . The findings indicate that <10% of the lysinoalanine (lysinoalanine residue (I)) formed,



has its origin in cystine. (CA 67:1967, 51505j)

- 212 Rock, G.C., King, K.W. AMINO ACID REQUIREMENTS OF LARVAE OF THE RED-BANDED LEAF ROLLER DETERMINED WITH GLUCOSE- $\text{U-}^{14}\text{C}$ . *Bull. ent. Soc. Am.* 13, 3 (1967) 193. Abstr. 135. "New York Meeting of the Entomological Society of America. New York, N.Y., USA. 27-30 Nov. 1967".

Larvae of the insect *Argyrotaenia velutinana* were reared aseptically on a synthetic medium containing glucose- $\text{U-}^{14}\text{C}$ . Specific activity measurements of the amino acids indicated that the insect was capable of synthesising certain amino acids from glucose. These results agreed with those obtained by the amino acid deletion technique. (Abstr.)

- 213 Rodríguez, J.G., Hampton, R.E. ESSENTIAL AMINO ACIDS DETERMINED IN THE TWO-SPOTTED SPIDER MITE, *Tetranychus urticae* Koch (ACARINA, TETRANYCHIDAE) WITH GLUCOSE- $\text{U-}^{14}\text{C}$ . *J. Insect Physiol.* 12 (1966) 1209-1216.

Amino acids were determined by the indirect method in young adult *T. urticae* females. The mites were maintained on bean plants in the greenhouse, starved overnight, then fed for 24 h on a chemically defined diet containing glucose- $\text{U-}^{14}\text{C}$ . During the starvation period the concentrations of the amino acids dropped to about 10% of those in mites taken directly off bean plants and analysed. 18 protein amino acids and three non-protein amino acids were detected. The relatively high labelling found in alanine, aspartic acid, cysteic acid, cystine, glutamic acid, glycine, proline, serine, and threonine indicated that the mite is capable of synthesising these amino acids from glucose; therefore these are classified as nutritionally non-essential. Arginine, histidine, isoleucine, leucine, lysine, methionine,

phenylalanine, tyrosine, and valine showed sufficiently low activity to be classified as essential in the diet of T. urticae. Ornithine, citrulline, and  $\alpha$ -amino butyric acid were present but not labelled. (Auth.)

- 214 Rogers, J.E., Jr. STUDIES ON THE BIOGENESIS OF p-BENZOQUINONES IN THE DEFENSIVE SECRETION OF A TENEBRIONID BEETLE (Eleodes longicollis). Diss. Abstr. 28, 4 (1967) 1399-B - 1400-B.

Little attention has been given to the biogenesis of the repellent components, including the simple p-benzoquinones. Potential precursors of the three p-benzoquinones isolated from the defensive secretion of the Arizona desert beetle E. longicollis were examined using  $^{14}\text{C}$ -labelled compounds. The compounds were administered to small groups of beetles orally or by subcutaneous injection. Several days later the quinones were collected by provoking the beetle to discharge its defensive secretion from abdominal glands. Derivatives of the quinones, obtained by treatment of the crude secretion with 2,4-dinitrophenylhydrazine, were separated and purified by paper chromatography. The activity of the quinone monohydrazone derivatives was determined on a gas flow counter and corrected by comparison with a calibrated standard source. The results of these incorporation studies indicate that two separate pathways exist to the quinones. The most efficient precursors of p-benzoquinone (I) were the aromatic amino acids phenylalanine and tyrosine (uniformly labelled or specifically ring labelled). Shikimic acid was also incorporated into I, but no significant incorporation of simple aliphatic compounds was observed. Methyl-p-benzoquinone (II) and ethyl-p-benzoquinone (III) were most efficiently formed from acetate and malonate. Propionate was incorporated into III preferentially. D-Glucose and pyruvate were less efficient precursors of the alkylated quinones. These results suggest that p-benzoquinone is synthesised from the aromatic ring of phenylalanine and tyrosine, perhaps by a route analogous to that reported for ubiquinone biogenesis in the rat. The alkylated quinones (II and III) would appear to arise via the acetate pathway. The methyl group of II is probably formed from acetate, while propionate is the probable source of the ethyl group of III. This apparent biogenesis of an aromatic ring from smaller fragments was observed in entire, intact beetles. Thus, any symbiotic organisms normally present in the beetle could participate in these postulated biogenetic schemes. (From DA)

- 215 Sekeris, C.E. SCLEROTIZATION IN THE BLOWFLY IMAGO. Science, N. Y. 144 (1964) 419-420.

N<sub>1</sub>-acetyldopamine has been identified as a dihydroxyphenylalanine metabolite in the blow fly imago during eclosion. The activity of the dihydroxyphenylalanine decarboxylase, the main enzyme responsible for its formation, which is minimal during the pupal stage, increases 1 d before eclosion. In mature larvae N-acetyldopamine-glucoside accumulates. Its concentration was determined by labelling it with 2- $^{14}\text{C}$ -dopamine. It remained constant during pupal life until 1 d before eclosion. Metabolism of radioactive tyrosine and DOPA during eclosion followed the same pathway as in the prepupa. The similarity of dihydroxyphenylalanine metabolism in Calliphora during eclosion and during puparium formation justifies the assumption that N-acetyldopamine can be regarded as the sclerotizing agent of the imaginal cuticle. It is concluded that the formation of this substance is under the control of ecdysone.

- 216 Sekeris, C.E., Herrlich, P. ZUM TYROSINSTOFFWECHSEL DER INSEKTEN. XVII. DER TYROSINSTOFFWECHSEL VON Tenebrio molitor UND Drosophila melanogaster. (The tyrosine metabolism of insects. XVII The tyrosine metabolism of Tenebrio molitor and Drosophila melanogaster.) Hoppe-Seyler's Z. physiol. Chem. 344, 3/4 (1966) 267-275. (In German, with German and English summary)

In Tenebrio and Drosophila, as in Calliphora, there are two main pathways of tyrosine metabolism: firstly the hydroxylation to Dopa and the synthesis of N-acetyl-dopamine and secondly the degradation to phenol carboxylic acids. The analogy with sclerotising metabolism in Calliphora and the appearance of N-acetyl-dopamine at the time of sclerotisation suggest a mechanism similar to that found in the cuticle of Calliphora. Max. activities of Dopa decarboxylase in Tenebrio are also found before pupation and at the beginning of the imaginal stage. The curve of transaminase activity follows the opposite course. In Drosophila, the decarboxylase activity can be shown in vitro too. These findings are discussed in relation to results with other insects. - ( $\text{U-}^{14}\text{C}$ ) tyrosine,  $\beta$ -(3,4-dihydroxyphenyl)-( $\alpha$ - $^{14}\text{C}$ )-alanine (=Dopa), and  $\beta$ -(3,4-dihydroxyphenyl)-( $\alpha$ - $^{14}\text{C}$ )ethylamine (=dopamine) were the labelled compounds used and injected, in aqueous solution, either laterally into the abdomen or subcutaneously into the larvae.

- 217 Sekeris, C.E. THE EFFECT OF ECDYSONE ON RNA AND PROTEIN METABOLISM IN INSECTS. p.388-394 of "Regular Nucleic Acid Protein Biosynthesis. Proceeding of an International Symposium. Lunteren, The Netherlands, 1966". Published 1967.
- Enzyme induction by ecdysone (I), the effects of I on RNA metabolism in the insect epidermis, and the action of I on isolated epidermis nuclei are reviewed. I initiates sclerotization by producing the sclerotizing agent N-acetylcholine indirectly through stimulation of mRNA synthesis and by induced enzyme formation. The primary site of action of I is on the nuclei epidermal cells. (CA 68:1968, 27722c).
- 218 Sekeris, C.E., Karlson, P. BIOSYNTHESIS OF CATECHOL AMINES IN INSECTS. Pharmac. Rev. 18, 1 (1966) 89-94.
- After injection of glycine-2-<sup>14</sup>C and aminoimidazolecarboxamide-4-<sup>14</sup>C into the butterfly Pieris brassicae in the chrysalid stage, the rate of incorporation into leucopterin (I), guanine, and adenine was detected. Similar activities were observed after xanthine-2-<sup>14</sup>C, showing that the biogenesis of I was not independent from purine (II) biogenesis, and that II was converted to pteridine with a min. of structural changes. (CA 64:1966, 16343a)
- 219 Shigematsu, H., Koyasako, T. (The article is concerned with the incorporation of <sup>14</sup>C-glycine into the posterior silk gland of Bombyx mori.) Bull. seric. Exp. Stn. Japan 17 (1961) 295-.
- 220 Shigematsu, H. RECENT ADVANCE IN STUDIES ON THE FIBROIN SYNTHESIS BY THE POSTERIOR SILKGAND OF THE SILKWORM. SABCO J. 1 (1964) 21-29.
- The <sup>32</sup>P-RNA in the posterior silk gland of Bombyx mori was analysed by MAK column chromatography. The chromatographic patterns obtained were essentially the same as those from Philosamia fat body RNA (see 348).
- 221 Shigematsu, H., Takeshita, H., Onodera, S. EFFECT OF ACTINOMYCIN AND MITOMYCIN ON FIBROIN SYNTHESIS IN THE POSTERIOR SILK GLAND OF Bombyx mori. Seikagaku Zasshi (J. Biochem., Tokyo) 58, 6 (1965) 604-606. (In English)
- The posterior silk glands of B. mori larvae were removed after a single injection of actinomycin or mitomycin in various concentrations, and the incorporation of glycine-<sup>14</sup>C into duct fibroin was measured. Inhibition of fibroin synthesis was observed between 3 h and 2 d after actinomycin, while mitomycin was apparently not an active inhibitor. The DNA-directed synthesis of a specific RNA appears to be an obligatory 1st step in fibroin synthesis. (CA 64: 1966, 11602d)
- 222 Shigematsu, H., Takeshita, H., Onodera, S. INCORPORATION OF GLYCINE-<sup>14</sup>C INTO FIBROIN IN SUBCELLULAR FRACTIONS OF THE POSTERIOR SILKGANDS OF THE SILKWORM, Bombyx mori L. Seikagaku Zasshi (J. Biochem., Tokyo) 60, 2 (1966) 140-146.
- Posterior silkglands of the silkworm were collected from larvae on the 4th to 6th day of the 5th-instar and after incubation in vitro with glycine-<sup>14</sup>C, incorporation of radioactivity into fibroin in sub-cellular fractions was studied. The greatest and most rapid incorporation was observed in the supernatant (105S) after centrifugation at 105 000 g for 60 min. After deoxycholate (DOC) treatment, the soluble part (M-S) of the fraction M (microsome) (precipitate after centrifugation at 105 000 g for 60 min) showed an incorporation curve similar to that of 105S, but the incorporation to the DOC-insoluble (M-P) of M was less and similar to that of the DOC-soluble part (LM-S) of fraction LM (large microsomes) of fraction LM (precipitate after centrifugation at 20 000 g for 30 min). The rate of labelling of fibroin in the insoluble fraction (LM-P) after treatment of fraction LM with DOC was, as it was also the case with fraction CD, almost linear during the reaction period. After 15 min incubation, the specific radioactivity of fibroin in fraction R (particulate) was much higher than that in fraction S, which was obtained by further centrifugation of fraction 105 S for 180 min at 105 000 g. Sucrose density-gradient sedimentation analysis of fraction R and M indicated that fraction R mainly consisted of free ribosomes, while fraction M contained large, heterogeneous particles. In fraction R, the radioactivity was found in the ribosomal peak. There was unexpectedly low labelling of fraction M. The effect of ribosomes in a free state on fibroin synthesis was discussed. (Auth. summary)

- 223 Simon, H., Wacker, H., Walter, J. WEITERE UNTERSUCHUNGEN ZUR BIOGENESE DES LEUKOPTERINS, (Further studies on the biogenesis of leucopterin (in *Pieris brassicae*). ) p.327-341 of "Pteridine Chemistry. Proceedings of the 3rd International Symposium, Stuttgart, Federal Republic of Germany, Sep. 1962". New York, Macmillan Co. 1964. (In German, with English summary)

In a continuation of our experiments to elucidate the biogenesis of leucopterin, glycine-2-<sup>14</sup>C and aminoimidazolecarboxamide-4-<sup>14</sup>C were injected at the initial and nearly final stages of development of the cabbage butterfly pupae. The incorporation in leucopterin, guanine, and adenine and the ratios of the specific radioactivities of these substances were determined. These ratios in the wings of the butterflies always approximate to one independent of the time of application. The amount of precursors incorporated in the purines and the leucopterin increases steadily, reaching a max. at the 6th-day of development. After application of xanthine-2-<sup>14</sup>C, guanine, adenine and leucopterin show very similar specific activities. From these results we conclude that the biogenesis of leucopterin is not independent from that of purine biogenesis and that the conversion purine → pteridine goes via a minimum of structural changes. 2-Amino-4-hydroxypteridine-2-<sup>14</sup>C is incorporated into isoxanthopterine, but guanine and adenine also show a significant radioactivity. 2,4,5-Triamino-6-hydroxypyrimidine and 2-amino-4-hydroxypteridine-6-aldehyde are incorporated but not very efficiently. On the other hand, 2,4-diamino-5-formylamino-6-hydroxypyrimidine and 2-amino-4-hydroxy-6-(1',2',3',4'-tetrahydroxybutyl) pteridine-2-<sup>14</sup>C are not used by the organism. After the application of ribose-1-<sup>14</sup>C to the 4,5,6,7,8 and 9-day-old pupae, the ratio of <sup>14</sup>C incorporated into C-6 and C-7 of the leucopterin goes from 42:58 to 14:86. In the butterflies, relatively large amounts of 2,8-dihydroxyadenine are found. All these results obtained fit into the general biogenesis scheme which was previously proposed by this laboratory. (Auth.)

- 224 Simon, H. THE BIOGENESIS OF LEUKOPTERIN AND ITS RELATION TO PURINE METABOLISM. Beitr. biochem. Physiol. Naturstoffen, Festschr. (1965) 467-476. (In German)

The pterins, as a group, are widely distributed in plants and animals and have a variety of metabolic functions and have parallels with purine metabolism. Leucopterin was isolated from *Pieris brassicae* (white cabbage butterfly) grown in the laboratory and was studied to determine where <sup>14</sup>C was found when the <sup>14</sup>C was given as specifically labelled compounds of purine, pyrimidines, HCO<sub>2</sub>H, glycine, acetate, and ribose to the caterpillars and pupae. It was concluded that purine and pteridine formation must follow the same pathways in this species. (CA 65: 1966, 5942b).

- 225 Skinner, D.M. PROTEIN SYNTHESIS IN THE *Cecropia* SILKMOTH. Anat. Rec. 138 (1960) 383. Abstr. See also II/289.

The in vivo incorporation of valine-1-<sup>14</sup>C into the proteins of various tissues of the *Cecropia* silkmoth during its life cycle and under various physiological conditions was investigated. Tissues excised from diapausing pupae 24 h after injection of labelled valine contained small amounts of the label. The specific activities (cpm) of the tissues were as follows: blood, 2; fat body, 100; epidermis, 340; midgut, 500. With the onset of adult development incorporation increased two-, 5-, 8- and 200-fold in midgut, fat body, epidermis and blood respectively. 2 d before adult emergence, all tissues were highly labelled except the fat body, which is being resorbed during that period. The specific activity of egg proteins of the old adult (8 d after emergence) was the highest obtained during these studies. In view of Harvey and Shappiro's work on injury metabolism in *Cecropia* and since the amino acid was injected into the silkmoths, the incorporation of valine into injured brainless diapausing pupae was also investigated. Injury raised incorporation into all tissues to the level of developing adults. An equal increase was also caused by injection of the moulting hormone ecdysone. Indeed, the hormonally treated animals showed the first signs of adult development, retraction of the epidermis. The in vivo synthesis of a single protein was also studied. Reduced cytochrome c was extracted from thoracic muscles. Its absorbency was measured at 550 m in the reduced and oxidized states. Although muscle formation begins on the 11th day after the initiation of adult development, with these methods, cytochrome c was first detected on the 17th day. It increased in amount during the next 5 d and levelled off in the adult. (Abstr. of paper read at meeting)

- 226 Stidham, J.D. THE FREE AMINO ACID COMPOSITION AND CERTAIN ASPECTS OF THE METABOLIC FATE OF CARBON-14 LABELLED ALPHA ALANINE AND ASPARTIC ACID IN THE AGING FEMALE MOSQUITO, *Aedes aegypti* (L.). Diss. Abstr. 27 (1967) 3348-B.

The free amino acids of the adult female were investigated at seven different ages in mosquitoes maintained on two different dietary regimens (sucrose, and sucrose and blood). In addition, certain

aspects of the metabolic fate of  $^{14}\text{C}$ -labelled  $\alpha$ -alanine and aspartic acid were studied (0.1 mCi per 1.0 ml of 0.01 N HCl). Three tissue fractions (amino acid extract, lipid fraction, and protein fractions) were analysed for radioactivity. Sixteen free amino acids were found to be consistently present in mosquitoes from the two dietary regimens: these sixteen amino acids included  $\alpha$ -alanine, aspartic acid, glutamic acid, glycine, serine, threonine, proline, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, and arginine. Four other amino acids found but in rather low concentrations included  $\delta$ -alanine, taurine, cysteic acid and methionine sulfoxide. Statistical procedures applied to the data on free amino acids indicated that diet alone had less effect on individual amino acids than it did when time (age) was considered, or diet was primarily significant when time was included. Throughout the 45 d experimental period the concentrations of free amino acids in those mosquitoes given sucrose and blood were consistently higher than the concentrations in those mosquitoes given only sucrose as adults. The amino acid found to be present in the highest concentration in both sucrose fed, and sucrose and blood fed mosquitoes over the 45 d period was  $\alpha$ -alanine, comprising from 34-52% of the total amino acid concentration. The total amino acid concentration in both sucrose fed, and sucrose and blood fed mosquitoes was found to be highest at 30 d. Mosquitoes fed sucrose and blood were found to be slightly heavier per individual mosquito than those mosquitoes maintained on sucrose alone but mosquitoes from both groups reached their heaviest weight at 16 d. Analyses of tissue fractions from mosquitoes fed  $^{14}\text{C}$ -labelled  $\alpha$ -alanine and aspartic acid revealed radioactivity in the amino acid extract, lipid fraction, and protein fraction. Mosquitoes fed  $^{14}\text{C}$ -labelled  $\alpha$ -alanine and aspartic acid were found to expire  $^{14}\text{C}-\text{CO}_2$  at a rather high rate shortly after uptake of the labelled compound followed soon after by a sharp decline to a low but steady rate of release. Analysis of excreta from mosquitoes fed  $^{14}\text{C}$ -labelled alanine and aspartic acid revealed a very low activity indicating very little loss of  $^{14}\text{C}$  by this route. The significance of free amino acids and the high percentage of alanine as well as the radioactivity of the three tissue fractions are discussed.

- 227 Sugiura, K., Goto, M. BIOSYNTHESIS OF PTERIDINES IN *Drosophila melanogaster*. *Biochem. biophys. Res. Commun.* 28, 5 (1967) 687-691.

The biosynthesis of pteridines was studied in *D. melanogaster*. The 2-amino-4-hydroxy-6-(D-erythro-1',2',8',-trihydroxypropyl)pteridine was a bipterin precursor. 2-Amino-4-hydroxypteridine (reduced) was also incorporated into bipterin. A possible metabolic pathway of pteridines was discussed. (CA 87: 1967, 98039a)

- 228 Telfer, W.H. THE MECHANISM AND CONTROL OF YOLK FORMATION. *A. Rev. Ent.* 10 (1965) 161-184.

Review article divided into sections dealing with extraovarian contributions to vitellogenesis (evidence for the uptake of blood proteins by the oocytes, protein yolk spheres as depositories of blood protein, the route of entry, formation of yolk spheres from blood proteins), ovarian contributions to yolk formation (the role of the follicle cells, of nurse cells and trophic tissues, and of the oocyte), and the control of yolk formation. Numerous studies are cited in which radioisotopes had been used (p.164-6, 169, 171-4).

- 229 Thomas, K.K., Nation, J.L. CONTROL OF A SEX-LIMITED HEMOLYMPH PROTEIN BY CORPORA ALLATA DURING OVARIAN DEVELOPMENT IN *Periplaneta americana*. *Biol. Bull.* 130, 2 (1966) 254-264.

The relation between corpora allata and protein synthesis during ovarian development in *P. americana* was studied. In all female roaches, allatectomized within 48 h after imaginal moult, the ovary remained small, and the oocytes neither grew nor deposited yolk. The haemolymph protein concentration of allatectomized roaches was low (1.09 g/100 ml) 35 d after operation, compared with the levels in the sham-operated controls (1.71 g/100 ml). Electrophoretic study of the haemolymph proteins revealed a gradual decrease and final disappearance of one of the five fractions in the experimental insects. In males, the concentration of this fraction was low compared with the females, suggesting that the formation of this group of proteins is sex-linked. Allatectomized insects showed a lower rate of incorporation of  $^3\text{H}$ -labelled amino acids into haemolymph proteins (1400 cpm/mg protein) compared with the controls (2100 cpm/mg protein). In ovarietomized roaches, the total haemolymph protein concentration increased (2.59 g/100ml) 14 d after operation compared with the controls (1.82 g/100 ml). The concentration of the sex-linked fraction increased after ovarietomy. (CA 65: 1966, 4320h)

- 230 Threadgold, J., Sheldon, H. ON THE FINE STRUCTURE OF A NEW CYTOPLASMIC COMPONENT IN A SECRETING CELL. *J. Cell Biol.* 23, 2 (1964) 96A-97A. Abstr. 199, at "4th Annual Meeting of the American Society for Cell Biology. Cleveland, Ohio, USA, 11-13 Nov. 1964".

Electron microscope observations on the aciniform and ampullaral silk glands of the spider (*Tegenaria derhamii*) fixed in  $\text{OsO}_4$  or glutaraldehyde and  $\text{OsO}_4$  show that cells of this tissue, like cells of the exocrine pancreas, plasma cells, and osteoblasts, have well defined arrays of granular reticulum, a large Golgi complex, and mitochondria. The nucleus, nucleolus, and nuclear envelope are not remarkable. The silk, which has a stippled or reticulate appearance in the lumen of the glands, can be recognized in large vacuoles of the cytoplasm of these cells. In addition to these structures there are aggregates of particles (which look identical with the RNP particles) in the cell sap. These aggregates differ from the usual "rosettes" in that usually they are partly or completely surrounded by a membrane. Often the particles are very regularly arranged along the inner aspect of the membrane, in contrast to the arrangement of RNP particles of the granular reticulum, which lie along the outer surface of the cisternal membrane. The centre of this membrane-bounded new cytoplasmic component usually contains material with an appearance which resembles the silk, but in some instances the contents have an intense osmiophilia. Though a sequence of forms starting with the aggregation of several small particles and progressing through a membrane-enclosed particle-studded body to a large secretory droplet can be constructed from electron photomicrographs, the identity of the contents and the significance of the particles on the inner surface of the membrane is not clear. In order to identify the nature of these particles, alanine- $^3\text{H}$  was injected into the coelomic cavity of spiders. Autoradiographs for light microscopy show labelling of the cytoplasm and silk. One interpretation of these new cytoplasmic structures is that they represent polyribosome-messenger RNA complex *in situ* in a cell which is secreting a material that includes a large proportion of protein, silk fibroin. (Abstr.)

- 231 Travis, J., McElroy, W.D. ISOLATION AND SEQUENCE OF AN ESSENTIAL SULFHYDRYL PEPTIDE AT THE ACTIVE SITE OF FIREFLY LUCIFERASE. *Biochemistry* 5, 7 (1966) 2170-2176.

The reaction of the competitive inhibitor dehydroluciferin with ATP in the presence of firefly luciferase has been reported to result in the masking of two of the six sulphhydryl groups of the enzyme. Because the titration of all of the sulphhydryl groups in luciferase results in total inhibition, it was of interest to determine the amino acid sequence in the vicinity of the protected sulphhydryl groups. Labelling of the essential sulphhydryl groups with N-ethylmaleimide- $^{14}\text{C}$  followed by tryptic digestion resulted in the isolation of a single radioactive decapeptide whose sequence was determined. In view of these results molecular weight studies of native and denatured luciferase together with amino terminal analyses and tryptic peptide patterns of the enzyme were undertaken. The results indicate that firefly luciferase is composed of two like monomeric units of molecular weight 50 000. (CA)

- 232 Tsiapalis, C.M., Hayashi, Y., Chefurka, W. POLYRIBOSOMES FROM HOUSEFLIES. *Nature*, Lond. 214 (1967) 358-361.

Investigations of the properties of ribosomes and polyribosomes isolated from adult house fly, *Musca domestica*, throw new light on the part played by these structures in protein biosynthesis and suggest that nascent protein may be an important constituent that holds a polyribosome together.  $^{14}\text{C}$ -leucine was used in the study.

- 233 Watanabe, H. PROTEIN SYNTHESIS IN THE TISSUES OF THE SILKWORM, *Bombyx mori*, INFECTED WITH NUCLEAR-POLYHEDROSIS VIRUS. *J. invertebrate Path.* 9, 3 (1967) 428-429.

$^3\text{H}$ -tyrosine was used as a protein precursor to analyse protein synthesis in *B. mori* infected with nuclear polyhedrosis. The virus was administered perorally to 4th-instar larvae. On the 5th day after inoculation the larva was given a subcutaneous injection of  $^3\text{H}$ -tyrosine (specific activity 185 mCi/mM, 20  $\mu\text{Ci/g}$  body weight), with subsequent examination of various tissues. Protein synthesis was not found to be appreciably enhanced in diseased cells, up to a point just prior to polyhedra formation. Subsequently, active protein synthesis was very pronounced around the newly developed polyhedra and continued with polyhedral growth.

- 234 Watt, W.B. PTERIDINE BIOSYNTHESIS IN THE BUTTERFLY *Colias eurytheme*. *J. biol. Chem.* 242, 4 (1967) 565-573.

Quantitative data are presented on the distribution of pteridines, primary xanthopterin, leucopterin, erythropterin, and sepiapterin, in adults and developing pupae of the butterfly *C. eurytheme*. The

pteridine biosynthetic pathway in the developing wings of Colias was studied by the use of radioactive precursors injected into whole pupae and incubated in Ringer's solution with excised, developing wings. By comparison of the isotope incorporation by pupae and isolated wings of adenosine- and guanosine- $U-^{14}C$  (I) and guanosine- $8-^{14}C$ , it was concluded that Colias forms the pteridine ring from guanosine (or a phosphorylated derivative) with loss of the C-8 of guanosine. Pteridine interconversions were examined by studying the kinetics of isotope incorporation by the pteridines from I immediately after its injection into whole pupae, and by studying the incorporation patterns obtained upon incubation of isolated wings with xanthopterin- $^{14}C$ , leucopterin- $^{14}C$ , and erythropterin- $^{14}C$ . The pteridine pathway is bipartite, one branch leading to position 6 side chain-bearing pteridines, the other leading to pteridines not alkylated at position 6. Within the latter branch, xanthopterin serves as precursor of leucopterin and erythropterin. The origins of the position 6 side chain of sepiapterin and the position 7 side chain of erythropterin were studied by incubation of isolated wings with radioactive pyruvate, malate, lactate, serine, threonine, and  $\beta$ -hydroxybutyrate. There was no incorporation into the sepiapterin side chain from any of these, but there was marked incorporation into the erythropterin side chain from pyruvate, malate, and lactate. In addition, sepiapterin made by Colias from I was oxidized to 2-amino-4-hydroxypteridine-6-carboxylic acid, and the specific radioactivities of the parent compound and oxidation product were compared. The observed loss of specific activity agrees quantitatively with that expected if the sepiapterin side chain arises by modification of substitution on the initial pteridine position 6 side chain derived from guanosine ribose. The predictions of the alternative hypothesis of cleavage and replacement of the initial side chain are not compatible with the values observed. (CA 66: 1967, 58257h).

- 235 Wyss-Huber, M., Lüscher, M. ÜBER DIE HORMONALE BEEINFLUSSBARKEIT DER PROTEINSYNTHESE IN VITRO IM FETT KÖRPER VON Leucophaea maderae (INSECTA). (The effect of hormones on protein synthesis in vitro in the fat body of Leucophaea maderae (Insecta)). Revue suisse Zool. 73,3 (1965) 517-521. (In German)

Adult female Leucophaea fat body was incubated in a Warburg apparatus in the presence of  $^{14}C$ -alanine with and without corpora cardiaca. Corpora cardiaca taken from animals in the stage of oocyte maturation stimulated oxygen consumption and synthesis of proteins in fat body of insects taken during the same phase of the sexual cycle. Incubation of fat body of pregnant females with corpora cardiaca of oocyte maturation-animals resulted in increased oxygen consumption, but had no effect on protein synthesis. The fat body responds phase-specifically to the hormone of the corpora cardiaca, which is probably stored neurosecretory material. (Auth. summary).

See also:

- 40 Some biochemical aspects of insect metamorphosis. (Gilbert, L.I. et al., 1961)  
 43 Metabolic control mechanisms in insects. (Harvey, W.R. et al., 1966)  
 92 Glycogen accumulation during oogenesis and its premature release by blocking of the RNA supply. (Study on Musca domestica L.) (Engels, W. et al., 1967).  
 95 Trehalases from the cockroach, Blaberus discoidalis: activation, solubilization and properties. (Gilby, A.R. et al., 1967)  
 98 Action of ecdysone on the conversion of  $^{14}C$ -glucose in dauer pupa of the silkworm, Bombyx mori. (Kobayashi, M. et al., 1967)  
 99 Action of insect hormones on the fat of  $^{14}C$ -glucose in the diapausing, brainless pupa of Samia cynthia pyri (Lepidoptera: Saturniidae). (Kobayashi, M. et al., 1967)  
 104 Effect of DDT on incorporation of  $^{14}C$ -labelled glucose into protein and soluble intermediates of nymphal Triatoma infestans. (Villar, E. del et al., 1967)  
 243 Control of synthesis of RNA and protein in diapausing and injured cecropia pupae. (Berry, S.J. et al., 1964)  
 244 Effects of hormones and injury on RNA synthesis in saturniid moths. (Berry, S.J. et al., 1967)  
 253 The induction by ecdysone of puff modifications in the salivary gland chromosomes of Chironomus tentans. (Clever, U. et al., 1960).  
 256 Induction and repression of a puff in Chironomus tentans. (Clever, U., 1966)  
 257 Gene activity patterns and cellular differentiation. (Clever, U., 1966)  
 268 Radioisotope incorporation and nucleic acid synthesis in dipteran embryos. (Eudy, W.W. et al., 1967)  
 280 The role of the nucleus in RNA and protein metabolism of the Chrysopa perla oocytes. (Gruzova, M.N., 1966)

- 285 Study on the structure and function of the lampbrush Y-chromosome in Drosophila. (Henning, W., 1967)
- 286 Effects of substrates on gene-controlled enzyme activities in cultured embryonic cells of Drosophila. (Horikawa, M. et al., 1967)
- 288 Effects of actinomycin D on nucleic acid metabolism and protein biosynthesis during metamorphosis of Tenebrio molitor. (Ilan, J. et al., 1966)
- 298 Regulation of gene action in insect development. (Kroeger, H. et al., 1966)
- 309 RNA and protein synthesis in puffs of isolated salivary gland chromosomes of Chironomus. (Lezzi, M., 1967)
- 311 Insect embryogenesis: macromolecular syntheses during early development. (Lockshin, R.A., 1966)
- 312 Variations in metabolic activity during the larval development of Rhynchosciara. (Mattingly, E. et al., 1966)
- 313 Variations in metabolic activity during the larval development of Rhynchosciara. (Mattingly, E.M., 1966)
- 321 Juvenile hormone and RNA synthesis in pupal tissues of saturniid moths. (Oberlander, H. et al., 1966)
- 332 Incorporation of uridine and leucine in vitro by Cecropia silkworm wing epidermis during diapause and development. (Reddy, S.R.R., 1967)
- 334 Aspects of structure of polytene chromosome puffs of Drosophila busckii derived from experiments with antibiotics. (Ritossa, F.M. et al., 1963)
- 346 Nucleic acid and protein synthesis in saturniid pupae. (Spencer, J.B., 1967)
- 349 RNA, protein, and uric acid content of body tissues of Periplaneta americana (L.) as influenced by corpora allata during ovarian development. (Thomas, K.K. et al., 1966)
- 356 "Conference on Insect Endocrines, Brno, Czechoslovakia, 22-26 Aug., 1966". (Wyatt, G.R., 1966)
- 359 Etudes de la formation de l'acide ribonucléique et des protéines chez les insectes. (Zalokar, M., 1965)
- 363 Isolation of diglyceride-bound lipoprotein from insect hemolymph. (Chino, H. et al., 1967)
- 416 Study on the function of nurse cells in meristotic insect ovaries, with special reference to oogenesis in aedeagous Coleoptera. (Bier, K., 1965)
- 417 Oogenesis, the growth of giant cells. (Bier, K., 1967)
- 418 Structure and function of oocyte chromosomes and nucleoli and extra-DNA during the oogenesis of panoistic and meristotic insects. (Bier, K. et al., 1967)
- 419 The deposition of endocuticle in an insect, Calpodex ethlius Stoll (Lepidoptera, Hesperidae). (Condoullis, W.V. et al., 1966)
- 446 Hormonal control of reproduction. - I. Initiation of oocyte development in the isolated abdomen of Leucophaea maderae. (Chambers, D.L. et al., 1967)
- 453 Oogenesis in Hyalophora cecropia. (King, R.C. et al., 1965)
- 458 Metabolism of proline in insect flight muscle and its significance in stimulating the oxidation of pyruvate. (Sacktor, B. et al., 1967)
- 462 Biochemical changes in the larvae of the variegated cutworm, Peridroma saucia, after infection with a nuclear polyhedrosis virus. (Van der Geest, L.P.S., 1967)
- 568 Use of radioisotopes for studies on the ecology of tick vectors of disease. Progress Report, April 1, 1966 - January 1, 1967. (Sonshtine, D.E., 1966)
- 597 Cerebral amino acid transport in vitro: effect of hypoxia induced by cyanide in vivo. (Levi, G. et al., 1967)

### 1.2.5. Nucleic Acids. Nucleotides.

- 236 Akai, H. RNA SYNTHESIS OF THE SILK GLAND IN THE SILKWORM, Bombyx mori. J. Electron Microsc., Chiba Cy 14, 4 (1965) 336. (In English)

By light microscopic and electron microscopic autoradiography, the synthesis and intracellular shifts of RNA were studied in the silk gland of 5-d-old larvae. Each animal was injected with uridine-<sup>3</sup>H and 4  $\gamma$  of thymidine. From 5 min to 24 h after injection a part of the gland was removed for microscopic examination. The result indicated that the bulk of RNA in the posterior silk gland was

synthesized in the nucleus in about 30 min after the injection of precursor and transported on rough endoplasmic reticulum in the cytoplasm in 24 h. (CA 65; 1966, 14161f)

- 237 Arnold, D. G. AN AUTORADIOGRAPHIC STUDY OF RNA SYNTHESIS IN ISOLATED SALIVARY GLANDS OF *Drosophila hydei*. Dis. Abstr. 28, 3 (1967) 1260-B - 1261-B.

The characteristics of RNA synthesis in *D. hydei* have been studied using autoradiographic techniques. A method for correcting grain counts for self-absorption of B-particles is discussed. The correction factor was calculated using determinations of dry mass made by interference microscopy. It was found that the uncorrected grain counts for nucleolus were too low by a factor of 12, those for nucleoplasm, by a factor of 3.6, and for the cytoplasm, by a factor of 7.4. When isolated salivary glands were exposed to uridine-<sup>3</sup>H for a 15-sec interval, label was localized primarily over the nucleolus; with a 1-min exposure to isotope, RNA in the nucleoplasm also was labelled. It was concluded that some portion of the nucleolar RNA is synthesized in the nucleolus, and is not transferred from other regions of the nucleus. However, an examination of the time-course of incorporation does not exclude the possibility of a chromosomal origin of some fraction of nucleolar RNA. After extraction of RNA with N HCl after incorporation of uridine-<sup>3</sup>H, a small percentage of the label was retained; this label was assumed to be incorporated in DNA, possibly by conversion of uridine-<sup>3</sup>H to thymidine-<sup>3</sup>H. In Feulgen-stained nuclei, this non-extractable label was earliest apparent over the nucleolus, and with longer intervals in isotope, also appeared over the chromatin. In glands which had been pre-incubated in Actinomycin D before exposure to uridine-<sup>3</sup>H, it was found that the non-extractable label was present only over the chromatin; the antibiotic inhibited incorporation of label into the intra-nucleolar DNA. A 15-min preincubation with 10<sup>-7</sup> M Actinomycin D, followed by a 3-min exposure to uridine-<sup>3</sup>H, revealed an 80% inhibition of RNA synthesis in both nucleolus and nucleoplasm. With longer exposures to uridine-<sup>3</sup>H, a gradual increase in the amount of isotope incorporated in both nucleolus and nucleoplasm was apparent. Glands which were incubated 15 min in isotope after Actinomycin pretreatment incorporated about 50% of the control level of label in both nucleolus and nucleoplasm. A delayed appearance of label in cytoplasmic RNA indicated a dependence of this labelling on some nuclear process which is affected by Actinomycin D. Incorporation patterns of uridine-<sup>3</sup>H and cytidine-<sup>3</sup>H were compared by exposing isolated salivary glands to labelled precursors, followed by transfer to a solution of unlabelled nucleosides. The data suggest that two different RNA's are present in the nucleolus. One fraction is characterized by a higher uridine incorporation, a rapid synthesis and rapid disappearance from the nucleolus. The second fraction incorporated less uridine, was synthesized more slowly, and remained associated with the nucleolus for longer periods of time. Similarities in the isotope incorporation patterns of the rapidly turning over nucleolar fraction and the RNA of nucleoplasm suggest that this might be a messenger type of RNA. (DA)

- 238 Barigozzi, C., Dolfini, S., Fraccaro, M., Rezzonico Raimondi, G. IN VITRO STUDY OF THE DNA REPLICATION PATTERNS OF SOMATIC CHROMOSOMES OF *Drosophila melanogaster*. Exptl Cell Res. 43, 1 (1966) 231-234.

Embryonic cells were treated with thymidine-<sup>3</sup>H and radioautographed. Exposure for 6 h to thymidine-<sup>3</sup>H resulted in 90% labelling of the metaphases. After 3.5 - 4 h of exposure, 60% of the metaphases were labelled. All of the chromosomes were labelled in most species, indicating that the cells picked up the label at various stages of the 2nd half of the S period. In male cells, the Y chromosome was either late in duplicating or the last to terminate synthesis along its entire length. The IV pair of autosomes was frequently labelled together with the sex chromosomes, while the other 2 pairs had little or no label. The data suggest that the pattern of late DNA replication coincides with the gross distribution of heterochromatin. (CA 65; 1966, 18902c)

- 239 Barr, H. J., Plaut, W. NUCLEOLAR DNA IN *Drosophila*. J. Cell Biol. 31, 2 (1966) 10A. Abstracts of Papers Presented at the "6th Annual Meeting of the American Society for Cell Biology, Houston, Tex., USA. 17-19 Nov. 1966". Abstr. 15.

Elements containing DNA have been found in association with nucleoli of third instar larval salivary gland cells of various *Drosophila* species. This DNA is defined by its secondary fluorescence when stained with acridine orange or cotriphosphine O, its sensitivity to nuclease digestion, and, in the cases tested, its capacity to incorporate <sup>3</sup>H-thymidine or to show a positive Feulgen reaction. It is seen within or on the surface of nucleoli in standard squash preparations. Although there may be variations in the appearance of this material with larval age, its morphology is to a large extent

species specific. The striking differences in morphology in different species make it highly unlikely that the material is randomly removed from the chromosomes and fused with the nucleolus during preparation. In some species, e.g. *D. virilis* and *D. hydei*, a single large chunk of DNA is found, often accompanied by smaller fibres and flecks. In *D. repleta* the nucleolar DNA takes the form of swirls which normally do not extend to the periphery of the nucleolus. DNA-containing fibres running between nucleoli and chromosomes can be seen in all species studied; the chromosomal regions involved include, but are not restricted to, the chromocenters. In *D. melanogaster* there is no obvious difference in amount of nucleolar DNA between cells known to have 1, 2, and 3 nucleolar organizing regions. Nor is there an obvious difference in this respect between males and females of *D. ananassae*, known to have 3 and 2 nucleolar organizing regions, respectively. (Abstr.)

- 240 Benz, G. NUCLEIC ACID METABOLISM OF HEALTHY AND VIRUS-INFECTED LARVAE OF *Diprion hercyniae*. *Vjschr. naturf. Ges. Zürich* 112, 1 (1967) 29-70. (In German)

The metabolism of nucleic acids in healthy and polyhedrosis-infected larvae of *D. hercyniae* was studied, employing autoradiographic, biochemical, and histological methods. Infection by the virus stimulates the synthesis of nuclear DNA (multiplication of chromosomes and enlargement of nuclei), possibly as a defense reaction of the cell. By this process, enzymes necessary for DNA replication and virus multiplication are produced. After a short lag period, the virus interferes with chromatin synthesis. Although the virus does not depend on the building stones derived from host DNA, the breakdown of host chromatin is induced. At the same time, virogenic stromata are formed, and the synthesis of virus DNA begins at a very high rate in these structures. Shortly after the host DNA has been completely broken down, the synthesis of viral DNA stops. (CA 68: 1968, 10715c)

- 241 Berendes, H.D. DIFFERENTIAL REPLICATION OF MALE AND FEMALE X-CHROMOSOMES IN *Drosophila*. *Chromosoma* 20 (1966) 32-43.

The replication patterns of larval salivary gland chromosomes of *D. hydei* and *D. melanogaster* were studied by autoradiography with  $^3\text{H}$ -thymidine injected in mid third-instar larvae. Larvae were injected with 1  $\mu\text{l}$  Ringer's solution containing 1 mCi/ml  $^3\text{H}$ -thymidine (specific activity 14.5 Ci/mM); the effective thymidine concentration in *D. hydei* larvae was 0.4  $\mu\text{Ci}/\text{mg}$  body wt. The male X-chromosome showed a different replication behaviour in comparison to that of the female X-chromosome and autosomes. It is concluded that the male X-chromosome finishes its replication earlier than the female X-chromosome. Moreover, the time needed for a complete replication cycle of individual identical replication units was found to be shorter in the male than in the female X-chromosome. Although the whole X-chromosomes behave differently there were no differences observed in the sequence of the discontinuous labelling patterns of the two types of X-chromosome. One autosomal replication unit was observed which showed a different replication behaviour in males and females. The possible origin of the differential behaviour of the two X-chromosomes is discussed in terms of their difference in degree of polyteny.

- 242 Berendes, H.D., Keyl, H.G. DISTRIBUTION OF DNA IN HETEROCHROMATIN AND EUCHROMATIN OF POLYTENE NUCLEI OF *Drosophila hydei*. *Genetics* 57, 1 (1967) 1-13.

The brain ganglion of *Drosophila* contains diploid as well as polytene cells. This tissue is therefore particularly suitable for a study of the replication and quantitative distribution of heterochromatin and euchromatin in early polytenic stages. Thymidine- $^3\text{H}$  autoradiography revealed that the heterochromatin and the euchromatin showed an asynchronous, but in most cases overlapping, period of DNA synthesis in the diploid cells. Cytophotometric DNA measurements showed that the total values for nuclear DNA of the nuclei measured could not be arranged into a geometric series of classes originating from the diploid value. When the values for chromocentral heterochromatin and the euchromatin were separated, a good geometric distribution of the values of each of the types of chromatin was established. The polytene nuclei generally showed different classes of heterochromatin, which were sometimes but not always in accordance with the class expected on the basis of the euchromatin value. It is assumed that the process of polytenization involves an independent replication of euchromatin and heterochromatin, and that the number of replication steps completed by each of the types of chromatin can be different. (Auth. summary)

- 243 Berry, S.J., Krishnakumaran, A., Schneiderman, H.A. CONTROL OF SYNTHESIS OF RNA AND PROTEIN IN DIAPAUSING AND INJURED CECROPIA PUPAE. *Science*, N.Y. 146 (1964) 938-940.

$^3\text{H}$ -uridine, 10  $\mu\text{Ci}/\text{g}$  wet weight, was injected at various times after injury to determine which tissues showed an increased synthesis of RNA in response to injury. Injury could be shown to stimulate RNA

synthesis in all pupal tissues, also the synthesis of several blood proteins. A precocious synthesis of a protein ("injury protein") which normally appears in the blood during adult development was also found to be stimulated by injury. This was confirmed by experiments involving the injection of  $^{14}\text{C}$ -labelled algal protein hydrolysate (1.48 mCi/mg). Actinomycin D, injected as 2  $\mu\text{g/g}$  of body weight, blocks the injury-stimulated increase in blood protein synthesis and the injury-induced synthesis of injury protein. At concentrations of 0.5  $\mu\text{g/g}$ , however, it prevents the induction of injury-protein synthesis but does not prevent the increased synthesis of other blood proteins. These results suggest that low concentrations of actinomycin may inhibit the synthesis of new kinds of messenger RNA but still permit the continued synthesis of mRNA's already in production at the time the actinomycin is injected.

- 244 Berry, S. J., Krishnakumaran, A., Oberlander, H., Schneiderman, H. A. EFFECTS OF HORMONES AND INJURY ON RNA SYNTHESIS IN SATURNIID MOTHS. *J. Insect Physiol.* 13, 10 (1967) 1511-1537.

The rate of RNA synthesis during post-embryonic life was studied in related silkmoths (Saturniidae): *Hyalophora cecropia*, *Samia cynthia walkeri*, and *Antheraea polyphemus*, which have a pupal diapause, and *S. c. ricini* which normally has no diapause. Autoradiographic determinations were made of the rate of incorporation of  $^3\text{H}$ -uridine in various tissues during normal development and in insects subjected to endocrinological and surgical manoeuvres. The effects of inhibitors of RNA and protein synthesis during the life cycle were also analysed. The effects of inhibitors of RNA and protein synthesis during various stages of the life cycle were also analysed. The rate of RNA synthesis in many tissues roughly paralleled the rate of respiration during various stages of the life cycle, but there were significant differences in temporal patterns of RNA synthesis not only between tissues but within a tissue. During larval moult cycles chitogenous epithelia such as epidermis, tracheae, and foregut showed temporal patterns of RNA synthesis which correlated directly with the process of moulting. In other tissues the temporal patterns of RNA synthesis did not parallel the events of the moult cycle. The rate of RNA synthesis was higher during larval life than in any other stage. After pupation RNA synthesis decreases in most tissues and is extremely low during diapause at which time only a few tissues such as haemocytes, brain, oenocytes, Malpighian tubules, and perigonadal fat body continue to synthesise RNA at significant rates. During the first half of the pupal-adult transformation, the rate of RNA synthesis increased initially, but thereafter it decreased in many tissues. Integumentary injury to diapausing pupae stimulated RNA synthesis. A systematic stimulation of RNA synthesis which roughly paralleled the increased respiration stimulated by injury was observed in all tissues except the gonads. A pupal cuticle was synthesised by the regenerating epidermis in the absence of juvenile hormone. When RNA synthesis was blocked in diapausing pupae with actinomycin D, pupae survived and continued to synthesise proteins for 24 d, suggesting that messenger RNAs survive for many days. When protein synthesis was blocked with puromycin, diapausing pupae died within 17 d. During periods of rapid RNA synthesis such as larval life, early stages of adult development and after injury, the insects were promptly killed by low doses of actinomycin. When RNA synthesis was low, such as during diapause, in late states of adult development, and during adult life, they were insensitive to large doses of actinomycin. Experiments on developing adults indicated that the mRNA for many specific developmental events was made many days prior to the event.

- 245 Bier, K., Ribbert, D. STRUKTUR UND GENETISCHE AKTIVITÄT DES DNA-KEIMBAHNKÖRPERS VON *Dytiscus*. (Structure and genetic activity of the DNA mass in evidence during germ cell development.) *Naturwissenschaften* 53, 4 (1966) 116. (In German)

A mass of extrachromosomal DNA appears in the ovary of *Dytiscus* during germ cell development until it finally reaches the nucleus of the oocyte. It consists of fine fibrils. In *Dytiscidae*  $^3\text{H}$ -uridine was found to be incorporated into the entire region within the oocyte nucleus taken up by these fibrils. Such incorporation was not confined to the early stages but also continued in the later stages of oogenesis. The possible function of the DNA mass is discussed at this admittedly preliminary stage of the investigation.

- 246 Binstiel, M. L., Jacob, J., Sirlin, J. L. ANALYSIS OF NUCLEOLAR RNA SYNTHESIS IN DIPTERAN SALIVARY GLANDS. *Archs Biol. Liège* 76, 1/4 (1965) 563-587.

Salivary glands of larvae of *Smittia parthenogenetica* removed at the 5th week were incubated with uridine (I)- $2\text{-}^{14}\text{C}$ ,  $1\text{-U-}^{14}\text{C}$ ,  $1\text{-}^5\text{-}^3\text{H}_2$ ,  $1\text{-}^5\text{-}^3\text{H}$ , L-methionine (II)-methyl- $^{14}\text{C}$ , and II- $^{35}\text{S}$ . Sucrose

gradient analysis of RNA synthesised by the glands showed the newly formed RNA to be highly heterogeneous with labels in soluble RNA, ribosomal RNA, and RNA larger than 28S. In the presence of DRB (5,6-dichloro-1 ( $\beta$ -D-ribofuranosyl) benzimidazole) and TRB (4,5,6-trichloro-1 ( $\beta$ -D-ribofuranosyl) benzimidazole) the incorporation of labelled material into the RNA of the chromosomes and cytoplasm, but not the nucleolus, was inhibited. Radioactivity in this case was mainly in low-molecular-weight RNA (4S). Label from II-methyl- $^{14}\text{C}$  was transferred almost specifically to the nucleolar 4S RNA, the transfer of  $^{14}\text{C}$  representing transmethylation to the RNA. The amount of methionyl- $^{14}\text{C}$ -RNA was very small. When incubated with I-U- $^{14}\text{C}$  the nucleolar RNA contained a significant amount of ribothymidine- $^{14}\text{C}$ , further supporting the theory that transmethylation is to newly synthesised 4S RNA. (CA 65: 1966, 14160b)

- 247 Camargo, E. Plessmann, Plaut, W. THE RADIOAUTOGRAPHIC DETECTION OF DNA WITH TRITIATED ACTINOMYCIN D. *J. Cell Biol.* 35, 3 (1967) 713-716.

Salivary glands were derived from 3rd-instar larvae of *Drosophila melanogaster* and *D. hydei*.  $^3\text{H}$ -actinomycin D was used (0.3 ml of 10  $\mu\text{Ci/ml}$  of water). Acid and enzyme extractions preceded exposure to actinomycin D. The specificity of  $^3\text{H}$ -actinomycin D binding for DNA is evident from (1) label only being detected at known DNA sites, and (2) a total absence of label following pre-digestion of preparation with DNase. RNase has no measurable effect on subsequent binding of actinomycin D. The amount of binding is affected by the method of fixation. Hydrolysis of salivary gland squash preparations with HCl results in complete suppression of subsequent binding. - Attempts to demonstrate in vivo binding of actinomycin D are discussed.

- 248 Caspari, E. W. SOMATIC MUTATIONS IN THE MOTH *Ephesia*. Report on Research, August 1, 1964 - September 1, 1967. NYO-2902-11, Rochester Univ., N. Y. 46p.

Progress is reported on studies on the induction of somatic mutations in the moth *Ephesia* by the injection of 5-bromodeoxyuridine, 5-fluorouracil, or DNA from *Ephesia* during various developmental stages.  $^3\text{H}$ -thymidine was used in conjunction with 5-BDU.

- 249 Caspari, E. W. SOMATIC MUTATIONS IN THE MOTH *Ephesia*. NYO-2902-10, Rochester Univ., N. Y. 14 Sep. 1967, 18p.

The influence of 5-BDU on the formation of abnormal scales was studied. Labelled 5-BDU injected into last-instar larvae is taken up by the nuclei of the wing buds. 5-BDU and thymidine, injected into last-instar larvae, induce an immediate increase in the number of mitoses, followed already after 3 h by a decided drop. Labelled thymidine is not found to be incorporated into mitotic figures up to 2 h after injection, and only a small proportion of metaphases are labelled up to 24 h after injection. It is concluded that the G-2 period is very long or irregular, so that there is no relation between DNA synthesis and the time of the next mitosis.

- 250 Chaudhary, K. D., Lecomte, A. IN VIVO SYNTHESIS AND BREAKDOWN OF DEOXYRIBONUCLEIC ACID IN *Tribolium confusum* Duval. *Can. J. Biochem.* 44, 12 (1966) 1571-1575.

The in vivo synthesis of DNA, as shown by the rate of incorporation of  $^{14}\text{C}$ -thymidine, has been investigated at different stages in the life cycle of *T. confusum*. During the larval period, a close similarity is observed between the rate of DNA synthesis and the pattern of growth. The pupal stage, which is a non-growth phase, is characterized by a cessation of DNA synthesis. During the larval growth phase, although the 3-d-old larvae have the lowest and the 13-d-old have the highest rate of DNA synthesis, the rate of DNA degradation in the older larvae is almost twice as great as that of the younger larvae. These findings are consistent with the observed total concentration of DNA of the insect at these stages. (Auth.)

- 251 Clarke, K. U., Gillott, C. STUDIES ON THE EFFECTS OF THE REMOVAL OF THE FRONTAL GANGLION IN *Locusta migratoria* L. - II. RIBONUCLEIC ACID SYNTHESIS. *J. exp. Biol.* 46, 1 (1967) 27-34.

A cytochemical investigation was made of the RNA content of the cells of the mid-gut, fat body and epidermis in 3rd- and 4th-instar *L. migratoria* L. from which the frontal ganglion had been removed, in control operated and in starved animals. In operated locusts the nucleus was smaller, the nucleoli small or absent, and the cytoplasmic RNA much less than that found in operated controls. No differences

were observed in the DNA content of the cells. No "all-or-none" effect has ever been noted following the removal of the frontal ganglion. For example, there was some synthesis of mid-gut protease, some incorporation of uridine into RNA within the nucleus, and some incorporation of  $^{14}\text{C}$ -glycine into protein. Autoradiograph studies were made of the uptake of  $^3\text{H}$ -uridine into the cells of operated and control operated locusts. In operated locusts the appearance of labelled uridine in the nucleus was delayed, the rate of uptake slower, and the total amount incorporated less (never more than 25%) than in the controls. Studies were made of the uptake of  $^{14}\text{C}$ -uridine into the nuclei of operated and control operated locusts. Nuclei were isolated from locusts killed at known times after the isotope had been injected and their radioactivity was measured. The results confirmed those found in the radioautographic studies. The significance of these results is considered in the light of the Jacob & Monod model of the control of protein synthesis.

- 252 Claypool, C. J., Bloch, D. P. SYNTHESIS OF RIBONUCLEIC ACID AND HISTONE CHANGE DURING SPERMATOGENESIS IN THE GRASSHOPPER Chortophaga viridifasciata. Nature, Lond. **215** (1967) 986-987.

The correlation between RNA synthesis and changes in staining reaction with fast green and eosin was investigated in spermatids. Nymphs were injected with  $6\ \mu\text{Ci}$  of  $^3\text{H}$ -uridine (specific activity  $1.15\ \text{Ci/mM}$ ), killed 5 or 6 h after injection, and subsequently fixed and stained. A striking negative correlation between RNA synthesis and eosinophilia was observed during spermiogenesis. Decline in RNA synthesis during meiotic chromosome condensation and during the later spermatid stages was accompanied by an increase in eosinophilia and a masking of the fast green staining. The nature of the eosinophilic protein is not known, but is probably a histone because it is a basic nuclear protein which requires the removal of DNA before staining. The eosinophilic histone, probably rich in lysine, may play the part of a non-specific regulator of RNA synthesis in the nucleus as a whole.

- 253 Clever, U., Karlson, P. INDUKTION VON PUFF-VERÄNDERUNGEN IN DEN SPEICHELDRÜSEN-CHROMOSOMEN VON Chironomus tentans DURCH ECDYSON. (The induction by ecdysone of puff modifications in the salivary gland chromosomes of Chironomus tentans.) Exptl Cell Res. **20**, 3, Pt. 2 (1960) 623-626.

$^3\text{H}$ -ecdysone was injected into last-instar larvae, and the chromosomes autoradiographed. The modifications observed in the chromosomes are apparently a morphological indication of gene activity in the particular regions involved.

- 254 Clever, U., Beermann, W. STUDIES OF NUCLEO-CYTOPLASMIC INTERRELATIONS IN GIANT CHROMOSOMES OF DIPTERA. Int. Congr. Zool. **4** (1963) 210-215.

The distribution of active sites within the genome as well as the relationship of their activity to other physiological processes within the cell has been studied in different laboratories, making use of the "puff" phenomena which occur in the giant chromosomes of Diptera and which have been accepted to represent synthetically active genes. Work in this field is reviewed, and numerous studies quoted in which autoradiographic techniques had been used.

- 255 Clever, U. CHROMOSOMAL CHANGES ASSOCIATED WITH DIFFERENTIATION. p. 242-253 of "Genetic Control of Differentiation", Presented at the "18th Brookhaven Symposium, Upton, N. Y., USA, 7-9 Jun. 1965". BNL-931, Brookhaven National Lab., Upton, N. Y. 275p.

Special attention is paid to puffing phenomena. Various differences in chromosomal structure and changes in differentiation are discussed, also the action of ecdysone. Autoradiographic methods are used routinely in much of this work.

- 256 Clever, U. INDUCTION AND REPRESSION OF A PUFF IN Chironomus tentans. Devl Biol. **14** (1966) 421-438.

Two puffs in salivary glands, I-18-C and IV-2-B, seem to be controlled by the concentration of ecdysone. Towards the end of the moulting periods, IV-2-B regresses while I-18-C is still of maximal size. Injection of ecdysone into old prepupae does not re-induce IV-2-B, whereas this locus in larvae of all other stages rapidly responds to the hormone. Haemolymph from old prepupae without IV-2-B induces puffs I-18-C and IV-2-B when injected into last-instar intermoult larvae, and thus stimulates the effect of injected ecdysone. This ability is not lost by heating the haemolymph to  $80^\circ\text{C}$ . Haemolymph from intermoult larvae does not have any effect, whereas haemolymph from

very young last-instar larvae and from young prepupae apparently has a slight effect on I-18-C, but no effect on IV-2-B. Treatment with cycloheximide results in the reappearance of IV-2-B in old prepupae, although it has no effect on puffing at this locus in larvae of other stages. To examine the effect of cycloheximide on protein synthesis,  $^3\text{H}$ -leucine and  $^3\text{H}$ -valine ( $\sim 50 \mu\text{Ci}/\text{ml}$ ) were injected into larvae after various periods of treatment and incorporation was allowed for 1 or 6 h. To examine the effect on RNA synthesis, glands were explanted at various intervals and incubated in a  $^3\text{H}$ -uridine-sucrose medium ( $10 \mu\text{Ci}/\text{ml}$ ) containing  $10 \mu\text{g}/\text{ml}$  cycloheximide. Almost no incorporation of  $^3\text{H}$ -amino acids into cytoplasm or TCA precipitating material was detected after 4 h of cycloheximide treatment, while incorporation of  $^3\text{H}$ -uridine into chromosomes and nucleoli was unchanged after even 24 h. The  $^3\text{H}$ -uridine incorporation pattern in cycloheximide-treated glands and in control glands were compared. Puffing is prevented by actinomycin D. It is concluded that the titer of ecdysone is high at the end of the pupal moult. This implies an antagonistically acting factor at this stage which represses IV-2-B. The apparent relationship of this factor to protein synthesis is considered.

- 257 Clever, U. GENE ACTIVITY PATTERNS AND CELLULAR DIFFERENTIATION. Am. Zool. 6 (1966) 33-41.

Puffing in giant chromosomes of Diptera is considered to reflect the pattern of active gene loci in these chromosomes. In any one tissue only a relatively small portion of the total bands (about 10 to 20%) have been observed to form a puff at some time or another in larval development. These patterns of "potentially active" loci are tissue specific, though greatly overlapping. The actual rate of activity at these loci is controlled independently of each other and independently in each tissue by factors of the extranuclear metabolism. Puffing at some loci seems to be related to specific cellular functions, such as secretion of the salivary glands. The activity of others may be related to more basic metabolic processes. In relation to larval development, puffing patterns may change with changing cell functions or with developmental processes in the cells themselves. In salivary glands of *Chironomus* activity of DNase and of acid phosphatase seems to change in relation to cell breakdown at the end of the pupal moult. Changes of acid phosphatase activity begin early in the last larval instar, but the enzyme is bound to lysosomes until metamorphosis. This suggests that the genes specifically active during metamorphosis have to interact with a long-term control-system of development. The induction of metamorphosis is a sequential process, gene activations being among the first steps in this sequence. The activation of these genes by ecdysone is independent of protein synthesis. It is only the reaction of these genes that leads to the subsequent events in the cell, including the subsequent puff activations. This is shown by the fact that they depend on early RNA synthesis as well as on protein synthesis.\* These results on puffing are discussed with regard to the general problem of the relationships between patterns of gene activity and differentiation. (Auth.)

\* Numerous supporting studies in which autoradiographic techniques had been used are cited.

- 258 Daillie, J. THYMIDINE METABOLISM IN THE SILK GLAND OF THE SILKWORM. 1. THE MAIN PATHWAYS OF THE PRECURSOR IN THE IN VITRO-INCUBATED GLAND. Annls Biol. anim. Biochim. Biophys. 7, 2 (1967) 115-129. (In French)

Thymidine metabolism of the silk gland secretory tube was examined during in vitro incubation of glands taken on the 4th day of the 5th stage. The cell walls showed a high permeability to thymidine, and the nucleoside was converted to nucleotide in the glandular cells; catabolic reactions were negligible. The total amount of nucleotide formed increased to a max. within a 30 min incubation, and this max. level was maintained for at least 30 min. Some synthesised nucleotides were released into the incubation medium. Thymidine mono-, di-, and tri-phosphates were formed, the latter during the 1st 5 min of incubation. The equilibrium between the various enzymic reactions changed during the incubation. Although the total quantity of nucleotides increased, the relative proportions of thymidine triphosphate and monophosphate, but not of thymidine diphosphate, varied. The phosphorylation reaction appeared to be blocked at the initial stages after the 1st 5 or 10 min of incubation, although the production of thymidine triphosphate increased during the 2nd 15 min of incubation. Thymidine was incorporated into DNA at a constant rate, and the labelling of DNA appeared to be insensitive either to variations in the amount of thymidine triphosphate or to the increase in the amount of labelled nucleotides. (CA 68: 1968, 1149w)

- 259 Dailite, J. THYMIDINE METABOLISM IN THE SILK GLAND OF *Bombyx mori*. II. UTILIZATION OF RADIOACTIVE NUCLEOTIDES IN DNA SYNTHESIS BY THE IN VITRO-INCUBATED GLAND ON THE 4TH DAY OF THE 5TH INSTAR. *Annls Biol. anim. Biochim. Biophys.* 7, 3 (1967) 227-243.
- In vitro incubations, radioactive thymidine in the medium was exchanged with part of the endogenous thymidine in the silk glands obtained from silkworms in the 4th day of the 5th instar. However, not all of the precursor was directly available for DNA synthesis. A reserve compartment and an active compartment within the cell are postulated. (CA 68:1968, 76076d)
- 260 Dailite, J. METABOLISME DE LA THYMIDINE DANS LA GLANDE SERICIGENE DU VER A SOIE III. INCORPORATION DE LA THYMIDINE RADIOACTIVE DANS LA GLANDE SERICIGENE PRELEVEE AU 6<sup>e</sup> JOUR DU 5<sup>e</sup> AGE ET INCUBEE "IN VITRO". *Annls Biol. anim. Biochim. Biophys.* 7, 4 (1967) 347-354. (With English summary)
- <sup>3</sup>H-methyl thymidine was used. Though the DNA synthesis in the silk gland on day 6 of the 5th stage is slowed down, thymidine easily penetrates into the in-vitro incubated gland. The activity of the phosphorylating kinases is lower than in younger glands. However, the rate of production of the nucleotides is higher than their rates of utilization by DNA, which incorporates the precursor at a very low rate. On increasing the thymidine concentration of the medium, the incorporation of the precursor into DNA soon reaches a max. (0.05% nucleic acid synthesis/h). The amount of endogenous thymidine precursors available could not be accurately estimated; it is ~0.01% of the thymidilic contents of the gland DNA, and does not seem to be capable of ensuring more than a 15 min synthesis. The possible parts played by thymidine kinases and endogenous precursors in the regulation of DNA synthesis are discussed.
- 261 Dailite, J. METABOLISME DE LA THYMIDINE DANS LA GLANDE SERICIGENE DU VER A SOIE. IV. ETUDES SUR LA GLANDE "IN SITU". *Annls Biol. anim. Biochim. Biophys.* 7, 4 (1967) 355-372. (With English summary)
- The transport towards the silk gland of the nucleoside injected in the haemocoel is very fast. Experiments were carried out on days 3 and 4 of the 5th instar. At both ages, the main part of the thymidine disappears from the haemolymph within 10-15 min, due to immediate metabolism of the nucleoside. On day 3, 15 min after injection of labelled thymidine, radioactivity in the gland is due to nucleotides or degraded compounds. The labelled nucleotides (thymidine-2-<sup>14</sup>C and <sup>3</sup>H-methyl-thymidine) are soon incorporated in the pool of endogenous precursors. The rate of disappearance of labelled nucleotides may therefore be roughly equalled to the rate of renewal of the endogenous pool, i.e. 0.6 times/h on day 3. Under these conditions, the precursor is very quickly incorporated in DNA, from the start of the experiment. The rate of labelling then slows down; peak DNA radioactivity is reached within 2-8 h, on days 3 and 4. The decrease in specific radioactivity allows DNA synthesis in the gland to be estimated. On day 6, a slowing up of the various processes was observed. - Findings are compared with in vitro results.
- 262 Danieli, G. A., Rodino, E. LARVAL MOLTING CYCLE AND DNA SYNTHESIS IN *Drosophila hydei* SALIVARY GLANDS. *Nature*, Lond. 213 (1967) 424-425.
- Larvae from the late 2nd to the late 3rd instar were given <sup>3</sup>H-thymidine in their diet during 1 of 11 8-h periods comprising the developmental span studied. Autoradiography of the salivary glands showed an initial intensive synthesis to 4.5 times the original DNA after which the labelled nuclei decreased rapidly to ~20% of the amount observed with the moult. At 132 h, a new period of synthesis occurred increasing to the puparium. The DNA content per gland during a restricted period in the 3rd instar corresponded to at least two doublings. The data relate the cycle with physiological events in moulting but not with ecdysone. (CA 66:1967, 83513f)
- 263 Desai, L., Ficq, A., Gérard, C., Lacroix, N., Tencer, R. Laboratoire de Morphogénèse expérimentale et de la Physiologie cellulaire. 6. Cellules différenciées - Divers. 8.2. Chromosomes géants des larves de Diptères. p.67-68 of "Applications radiobiologiques de la physiologie cellulaire, Rapport Final 1961-1966". EUR 3607 f, European Atomic Energy Community, Brussels (Belgium). 1967, 137p.
- A group consisting of L. Desai, A. Ficq, C. Gérard, N. Lacroix and R. Tencer have between them been investigating the action of histones on giant chromosomes of *Chironomus* (using <sup>3</sup>H-thymidine and <sup>3</sup>H-uridine).

Desai (cf. 6.2.1) has found that basic proteins (various fractions of histones have been tested) strongly inhibit DNA and RNA synthesis in all bands. Microinjection of trypsin into larvae has caused an increase in the size of existing puffs, and increased incorporation of uridine into the chromosomes. Tencer and Lacroix (6.2.2) have attempted a direct demonstration, of the role of nucleolar organizer in r-RNA synthesis, by autoradiography, without obtaining positive results so far.

Ficq (cf. 6.2.3) found that in Rhynchosciara  $^3\text{H}$ -actinomycin tended to "colour" giant chromosomes much more uniformly than the Feulgen reaction, so that interbands may also contain DNA. Lacroix, Desai and Tencer found bandwise labelling with actinomycin in Chironomus which becomes homogeneous when RNase treatment is given.

- 264 Dion, A. S., Herbst, E. J. THE LOCALIZATION OF SPERMIDINE IN SALIVARY GLAND CELLS OF Drosophila melanogaster AND ITS EFFECT ON URIDINE- $^3\text{H}$  INCORPORATION. Proc. natn. Acad. Sci. U.S.A. 58, 6 (1967) 2367-2371.

The autoradiographic localization of spermidine (I) and the effects of polyamines on RNA synthesis in situ in polytene chromosomes of D. melanogaster were reported. An initial decrease in RNA level during the prepupal stage was followed by a significant increase in RNA on pupation. A 2nd increase in RNA coincided with the maturation of the adult organs of the imago. The increase in polyamine content, most notably of putrescine, during the prepupal stage was followed by a decrease upon pupation. At approx. the midpupal period, i.e., when the imaginal eyes were pigmented, the prepupal polyamine levels were re-established. Upon eclosion, the level of putrescine dropped markedly, and concurrently small increases in the levels of I and spermine were observed. I at  $5.0 \times 10^{-5}$  and  $10^{-4}$  M substantially inhibited  $^3\text{H}$ -labelled uridine incorporation into the nuclei in salivary gland cells of D. melanogaster. I, at  $6.4 \times 10^{-4}$  M, produced concomitant changes in nuclear structures. All spermine concentrations investigated ( $0.29 - 4.6 \times 10^{-4}$  M) strongly inhibited the incorporation of  $^3\text{H}$ -labelled uridine. Although the nuclear to cytoplasmic labelling ratio of tritiated I in distal cells of the salivary gland is approx. 2, the label appears uniformly distributed in proximal cells. (CA 68: 1968, 47373r)

- 265 Durand, M., Favard-Sereno, C. NUCLEIC ACID METABOLISM DURING OVOGENESIS IN CRICKETS, Gryllus bimaculatus. p. 331-395 of the "5th Symp. Int. Hist., Histochim. Cytochim. Lipides. Sofia, 1963". Published 1966.

Autoradiography of eggs of G. bimaculatus, labelled with thymidine- $^{14}\text{C}$  and adenine- $^{14}\text{C}$  show 30% of the cells are synthesizing DNA. Traces of radioactivity were observed in the cytoplasm just before cessation of follicle epithelial activity. Analysis shows  $9-12 \times 10^{-3}$   $\mu\text{g}$  of DNA/egg compared to  $5.7 \times 10^{-6}$   $\mu\text{g}$ /haploid sperm, confirming the occurrence of endomitosis. The A/T ratio of DNA of eggs plus follicle cells is 0.58, of eggs alone 0.38, and 1 in the DNA of testicles. The low egg ratio increases during embryonic development to 1 at the blastula stage. Autoradiography shows that RNA is synthesised by 5 d on chromosomes, passing to nucleoli and cytoplasm in 40 d. RNA is transferred from follicle cells to the oocyte. (CA 68: 1968, 66851p)

- 266 Erdström, J. E., Daneholt, B. SEDIMENTATION PROPERTIES OF THE NEWLY SYNTHESIZED RNA FROM ISOLATED NUCLEAR COMPONENTS OF Chironomus tentans SALIVARY GLAND CELLS. J. molec. Biol. 28, 2 (1967) 331-343.

$^3\text{H}$ -uridine and  $^3\text{H}$ -cytidine were used. Sedimentation analyses of labelled RNA from isolated nuclear components of C. tentans salivary gland cells are presented. It is shown that nucleoli form a 38 S component which is converted to one 30S and one 20S component. The 30S fraction is probably composed of the heavy ribosomal RNA fraction and an intermediate precursor, slightly larger than 30S. The finished light ribosomal 17S RNA molecule, to which the 20S component is believed to be a specific intermediate precursor, was never observed in the nucleolus. Label due to preribosomal and ribosomal components was absent from chromosomes and nuclear sap. The nucleoli do not form a labelled 4S RNA peak, in contrast to chromosomes and nuclear sap. The distribution of 4S RNA label among the chromosomes suggests its formation from a large number of loci. The chromosomes also produce polydisperse RNA in the sedimentation range of 10 - 90S. The polydispersity is not a function of heterogeneity in band origin but is found in the RNA of the single Balbiani ring. The polydisperse RNA has a sedimentation range that is higher than that reported for messenger RNA. (Essentially auth.)

- 267 Ermokhina, T. M., Mekhanik, M. L., Zaitseva, G. N., Belozerskiĭ, A. N. PHENYLALANYL-RNA SYNTHETASE AND PHENYLALANINE SOLUBLE RNA (s-RNA) IN YEAST AND INSECTS. Dokl. Akad. Nauk SSSR 170, 4 (1966) 974-977.

RNA synthetases from fly larvae and yeast were fractionated on DEAE-cellulose columns. The insect-derived aminoacyl-RNA synthetase gave two phenylalanyl-synthetases,  $E_1$  and  $E_2$ , differing in eluability by aqueous NaCl, which corresponded to the 2 phenylalanine types of s-RNA in the fly larvae, as shown by aminoacylation with phenylalanine- $^{14}C$ . The yeast-derived synthetase material gave but one fraction of active enzyme in  $E_2$  fraction eluted from DEAE-cellulose by aqueous NaCl. This was identical with the 1st fraction of phenylalanine type of s-RNA of the fly larvae. Thus, there is a universal type of phenylalanine s-RNA. The other insect RNA-synthetase was apparently specific for the insect. (CA 66: 1967, 35659m)

- 268 Eudy, W. W., Dobrogosz, W. J. RADIOISOTOPE INCORPORATION AND NUCLEIC ACID SYNTHESIS IN DIPTERAN EMBRYOS. J. Cell. Biol. 35, 2 Pt.2 (1967) 37A. Abstr. 73. "7th Annual Meeting of the American Society for Cell Biology. Denver, Colo., USA, 13-15 Nov. 1967".

Owing to the fact that dipteran insect embryos are closed systems and exhibit high impermeability to water-soluble metabolites, it was difficult in the past to determine the biochemical changes that occur during embryogenesis in these organisms. Studies in our laboratory with Phormia regina have shown that these difficulties can be overcome, and that isotopically labelled metabolites can in fact be used in studying dipteran embryo development. Eggs are collected for 1 h, sterilised and de-chlorinated with NaOCl, transferred with sterile Ringer's solution to flasks containing sodium lauryl sulfate, and then vigorously aerated by shaking during the entire developmental period. Viability is high and good synchronous development is obtained. Aqueous solutes such as  $^3H$ - or  $^{14}C$ -leucine, -uridine, -thymidine, and -glucose, and  $^{32}P_i$  are readily taken up, and, as indicated by chemical fractionation experiments, are assimilated into the expected macromolecular components. With this information at hand, studies were undertaken to delineate the nature of nucleic acid formation in these embryos. The embryos were incubated with  $^3H$ -uridine,  $^3H$ -thymidine, or  $^{32}P_i$  for various periods. This was followed by extraction of the nucleic acids with phenol and purification by alcohol precipitation and chromatography on columns of methylated albumin-kieselguhr. The oligonucleotide fraction was rapidly labelled at all stages of development. The synthesis of soluble RNA and ribosomal RNA, however, did not occur until approx. 25% of the total development period (18 h) had elapsed - a time corresponding approx. to the onset of gastrulation. A nucleic acid fraction containing  $^3H$  from both  $^3H$ -uridine and  $^3H$ -thymidine was detected during the early stages of nucleic acid synthesis and presumably represents RNA-DNA hybrid material. The formation of  $\beta$ -galactosidase and the pattern of protein biosynthesis were also measured in this system. (Abstr.)

- 269 Firshein, W., Berry, S. J., Swindlehurst, M. THE BIOSYNTHESIS OF DNA BY INSECTS. I. THE UTILIZATION OF DEOXYCYTIDINE BY DEVELOPING ADULTS OF THE CECROPIA SILKWORM. Biochim. biophys. Acta 149, 1 (1967) 190-198.

The pathway of deoxycytidine utilization was investigated in developing pupae of Hyalophora cecropia. The following intermediates were identified in a single assay system starting with deoxycytidine: deoxyuridine, deoxyuridine monophosphate, thymidine monophosphate, thymidine diphosphate, and thymidine triphosphate. The existence of this pathway was confirmed in several ways in addition to the detection of the actual intermediates. First, injection of [ $^{14}C$ ]deoxycytidine or [ $^{14}C$ ]deoxyuridine into developing pupae resulted in the localization of the label in the thymine moiety of DNA and no significant radioactivity was detected in the cytosine moiety. Second, omission of various components from the complete assay system produced a predictable loss of certain intermediates. Third, the activity of other enzymes which might compete with this pathway were not detected or detected in trace amounts, e.g., deoxycytidine monophosphate deaminase, deoxycytidine kinase, thymidine synthetase, deoxyuridine monophosphate kinase and deoxycytidine degradative enzymes. The enzyme which converts deoxyuridine monophosphate to deoxythymidine monophosphate (thymidylate synthetase) was not detected in diapausing or injured diapausing pupae, but was present in significant amounts in developing pupae. The injection of [ $^{14}C$ ]thymidine into developing pupae also resulted in the association of the radioactivity with DNA-thymine, but little or no thymidine kinase activity could be detected in the extracts. (Auth. summary)

- 270 Gabrusewycz-Garcia, N., Kleinfeld, R. G. A STUDY OF THE NUCLEOLAR MATERIAL IN Sciara coprophila. J. Cell Biol. 29, 2 (1966) 347-359.

In the polytene chromosomes of *S. coprophila*, in addition to a nucleolus, large numbers of nucleolar-like structures or micronucleoli are formed. A detailed mapping localized the nucleolar organizer at one end of the X-chromosome and revealed that ~18% of the bands of each chromosome are potentially capable of producing micronucleoli. Most of these sites are in regions known from a previous study to show asynchronous DNA replication: DNA puffs and certain heterochromatic regions. Micronucleoli are rarely found in association with bulbs. The RNA metabolism of the polytene chromosomes during late 4th instar was studied using radioautographic techniques. Isolated glands were incubated in  $^3\text{H}$ -uridine for 10 - 30 min, and radioautographs were made of crushed preparations. Despite the wide range of variation found among different larval cultures, the following pattern was observed. Just prior to and at the beginning of DNA puff formation, a period of intense extrachromosomal nucleolar and micronucleolar RNA synthesis occurs. After max. development of the DNA puffs, the synthesis of extrachromosomal RNA is at a low point, while incorporation into bulbs and DNA puffs remains high. With the onset of the prepupal stage, all nuclear RNA synthesis ceases. (Auth.)

- 271 Gabrusewycz-Garcia, N. CYTOLOGICAL AND AUTORADIOGRAPHIC STUDIES ON *Sciara coprophila* SALIVARY GLAND CHROMOSOMES. Diss. Abstr. 27, 12 Pt. I (1967) 4246-B.

Detailed revised cytological maps of the three autosomal salivary elements of *S. coprophila* were prepared, using several distinct larval stages from mid 4th to late 4th instar. The sites of DNA puffs and bulbs, as well as their extent at maximal development were determined and represented in these maps. A method of evaluating the age of larvae within the last 3 - 4 d of 4th instar was worked out by means of eyespot size determinations (counts). This is particularly important since the eyespot sizes are correlated with the development of the salivary gland nuclei in which dramatic changes in puffing pattern occur within a short period of time during these stages. The positions of the centromere regions in salivary chromosomes of *S. coprophila* were confirmed by observing special replicative features of these regions. High resolution autoradiography was used to follow  $^3\text{H}$ -thymidine incorporation into DNA of salivary gland chromosomes of larvae from the last 5 - 6 d of 4th instar. Earlier studies in *Sciara* indicated an all-or-none type of chromosomal replication. However, we observed differential labelling of bands and groups of bands. The centromere regions, certain chromosomal ends, as well as a number of deeply-staining bands showed a parallel behaviour in replication and form one group designated group C. The second group P includes a number of light-staining bands, bulbs, and, depending on the stage of development, either the puffs or the pre-puff sites. These two groups show a markedly different behaviour in relation to precursor uptake. When group C shows a high rate of incorporation, group P shows no incorporation, and vice versa. However, during a considerable part of the replication period, most or all of the bands display uniform labelling (pattern E). Pattern E is found with high frequency in relation to the other two. From extensive observations on these replication patterns, we have constructed a probable sequence of  $P \rightarrow E \rightarrow C$ . A statistical procedure was chosen to demonstrate the existence of the two patterns of unequal labelling. It is significant to note that in *Sciara* all chromosomes from the same nucleus show the same pattern. They are either a) all uniformly labelled (pattern E), b) all show the C pattern, or c) all show the P pattern. Replication patterns occurring at the time of DNA puff formation are easily followed, since a large proportion of the nuclear population is undergoing DNA synthesis at this time. The sequence of patterns at this stage differs from that of the earlier replication in that the C pattern fails to occur and also in that the P sites show incorporation continuously; they are not only the first but also the last sites to incorporate the isotope. There are several cytological criteria by which the so-called "heterochromatin" may be identified. Using all of these criteria bands of group C may be considered as heterochromatic. (From DA)

- 272 García, F.J.T. ESTUDIO DE LA FRECUENCIA DE LETALES EN DIFERENTES LOCALIDADES DEL DISTRITO FEDERAL EN *Drosophila melanogaster* Y ALELISMO. (Study on the frequency of lethals in *Drosophila melanogaster* encountered in different locations, and on allelism.) Thesis, Universidad Nacional Autónoma de México, México City. 1967, 38p. (In Spanish)

In order to determine which of two autosomes carry the lethal gene the chromosomes were isogenized. This consists of producing a homozygous individual from a heterozygous one by means of the chromosome balance, when the lethal factor is manifested. Two x-ray-induced translocations, Cy/Pm in chromosome II and Ubx/Sb in III, were used. In order to analyse the wild flies, an isogenization process was applied to each of the captured individuals, and chromosomes II and III were studied. From the experimental data obtained, the occurrence of recessive lethal and semilethal autosomal genes could be deduced in the heterozygous state in chromosomes II and III of *D. melano-*

gaster in the various localities studied in the Federal District of Mexico. By comparing the occurrence of lethal and semilethal genes in localities far apart and also in localities close to one another, a balance in the population dynamics of D. melanogaster could be deduced. The mean results obtained indicate a greater incidence of lethal and semilethal genes in chromosome II. The causes of this greater incidence are unknown. Due to the fact that the techniques applied in this experiment differ from those used in other parts of the world, the findings obtained in Mexico cannot be compared with any others, but it may be concluded that even under different climatological conditions the same lethal mutations exist in various wild populations throughout the world.

- 273 Gaudecker, B. von. RIBONUCLEIC ACID SYNTHESIS IN THE NUCLEOLUS OF Chironomus thummi (Tendipes thummi), AS STUDIED BY HIGH-RESOLUTION AUTORADIOGRAPHY. Z. Zellforsch. mikroskop. Anat. 82, 4 (1967) 536-557. (In English)

Full-grown 4th-instar larvae of T. thummi were injected with uridine-<sup>3</sup>H, and the salivary glands were fixed after 13 min - 46 h. Autoradiographs of sections examined in the light and electron microscopes showed a transposition of label from the pars fibrosa to the pars granulosa of the nucleolus. After short uridine incorporation, label was also found over chromosomal material, extending into the nucleolus. The label over the nucleolus organizer, extending into the nucleolus, indicates ribosomal RNA synthesis at these chromosomal sites. This RNA, which is first located in the pars fibrosa, subsequently breaks down within the nucleolus into two different components which combine with protein. After 20 min the smaller of these RNA particles (40 S) is found in the cytoplasm, whereas the bigger one (60 S) migrates to the pars granulosa of the nucleolus. With time these appear in the cytoplasm, combining with the 40 S particles to form the functional ribosomes. (CA 68: 1968, 47428p)

- 274 Gaulden, M. E., Jones, G. A., III. EXPERIMENTAL CONTROL OF NUCLEIC ACID SYNTHESIS IN INDIVIDUAL DIVIDING CELLS. J. Cell Biol. 31, 2 (1966) 38A. Abstracts of Papers Presented at the "6th Annual Meeting of the American Society for Cell Biology, Houston, Tex., USA. 17-19 Nov. 1966". Abstr. 73.

Chromatin of living neuroblasts of the grasshopper embryo, Chortophaga viridifasciata de Geer, is visible in some form throughout the entire cell cycle. At interphase, prophase, and telophase, it can be quickly and reversibly condensed by culture medium hypertonic to the cells or extended by medium hypotonic to them. Synthesis of both DNA and RNA (uptake of <sup>3</sup>H-thymidine and <sup>3</sup>H-uridine, respectively) is known to begin in the neuroblast at the mid-point of telophase; DNA synthesis ends in very early prophase and RNA synthesis continues into mid-prophase. Radioautographs of neuroblasts exposed to media of varying tonicity containing <sup>3</sup>H-thymidine (<sup>3</sup>HT) reveal the following points. (1) If cells are placed in hypertonic medium at a stage immediately preceding the beginning of DNA synthesis (anaphase or early telophase), initiation of synthesis is prevented as long as the chromatin is kept in a condensed state (control cells in labelled isotonic medium for comparable times progress into the synthesis period). (2) If the cell has just begun DNA synthesis (mid-telophase) before being placed in hypertonic medium, a very small amount of <sup>3</sup>HT uptake is observed. (3) If a cell is well into the synthesis period (late telophase or interphase) when the chromatin is condensed by hypertonic medium, uptake of <sup>3</sup>HT is reduced by about 50%. Results are reported of experiments in progress, designed to determine whether the extension of mid- and late prophase chromatin by hypotonic medium to a state resembling interphase chromatin is accompanied by <sup>3</sup>HT uptake and whether RNA synthesis is affected by tonicity-induced changes in the physical state of chromatin.

- 275 Gaulden, M. E. INFLUENCE OF THE PHYSICAL STATE OF CHROMATIN ON NUCLEIC ACID AND PROTEIN SYNTHESIS AND ON RADIATION SENSITIVITY OF CELL DIVISION. Progress Report. ORO-3546-3, Texas Univ., Dallas. Southwestern Medical School. 24 Feb. 1967, 12p.

Eggs of grasshoppers (Chortophaga) were subjected to temperatures of 3 - 4°C; embryonic growth was stopped, and after six months of storage the eggs failed to hatch. New techniques for making serial paraffin sections of grasshopper embryos and techniques for making autoradiographs of <sup>3</sup>H-thymidine labelled chromosomes of neuroblast cells were developed. The degree of expansion of condensation of the chromosomes in relation to DNA synthesis is discussed. The effects of colchicine on RNA metabolism are being studied. Immunological studies on rapidly dividing neuroblast cells are being conducted. (NSA 21: 1967, 22241)

- 276 Gay, H. ORGANIZATION OF CHROMOSOMES IN HIGHER FORMS. Carnegie Institution of Washington. Cytogenetics Laboratory. Carnegie Inst. Wash. Yb. 64 (1964/65) 537-552.

In one section the fine structure of chromosomes, the development of salivary-gland chromosomes, and the perichromatin in vesicles of *Tradescantia* mitotic chromosomes are discussed. A further section is devoted to the mitotic cycle and DNA replication in *Haplophragma gracilis*, another to the DNA of mitotic chromosomes of *Drosophila* (p. 546-547); radioautographic analysis now in progress of  $^3\text{H}$ -thymidine incorporation into mitotic chromosomes of neuroblast cells of *D. melanogaster* may give a value to the origin of their polynemic nature. The base composition of heterochromatic DNA in *D. melanogaster* is examined (p. 548-550).

- 277 Greenberg, J. R. SEDIMENTATION STUDIES ON *Drosophila virilis* SALIVARY GLAND RNA. *J. Cell Biol.* 35, 2 Pt. 2 (1967) 49A-50A. Abstr. 99, "7th Annual Meeting of the American Society for Cell Biology. Denver, Colo., USA. 13-15 Nov. 1967".

RNA synthesised by salivary glands of 3rd-instar larvae or prepupae incubated in an artificial culture medium containing  $^3\text{H}$ -uridine has been compared with RNA from whole larvae of similar age labelled by injection of  $^3\text{H}$ -uridine. RNA was extracted by cold phenol-SDS and examined by sucrose density gradient zonal centrifugation. *Drosophila* RNA having major components of about 28S, 18S, and 4S was used as a marker. The first distinct component labelled in whole larvae sedimented at 38S. Label next appeared simultaneously in 30S and 18S components, and eventually in a 28S component, together with a relative decline in the 38S and 30S components. By 4 h, only 28S, 18S, and 4S RNA were labelled. It seems probable that the 38S molecule is the precursor of the 30S molecule and 18S ribosomal RNA, the 30S molecule being in turn the precursor of 28S ribosomal RNA. RNA from isolated salivary glands labelled for 15 min also showed a peak at 38S, the remainder being polydisperse and as fast as 70S. Upon further incubation, label appeared in a 30S component, but radioactivity peaks coinciding with the 28S and 18S OD peaks were never obtained. The radioactive salivary gland RNA was always polydisperse, although the proportion of heterogeneously sedimenting RNA was diminished by transferring labelled glands to "chase" medium for 3 h. Upon prolonged labelling, or labelling followed by a chase, a relatively stable 32S peak which is presumably an intermediate in ribosomal RNA formation was found in salivary gland RNA, although this component was not detected in whole larval RNA. It is concluded that salivary glands labelled in vitro make ribosomal precursor RNA, but not finished ribosomal RNA. Apparently, the fraction of the 38S molecule normally destined to become 18S ribosomal RNA is degraded, while a remaining fraction accumulates as a 30S molecule. However, salivary glands in vivo make ribosomal RNA normally.

- 278 Greenberg, J. R. DEVELOPMENTAL VARIATION IN THE UPTAKE OF A RNA PRECURSOR BY *Drosophila virilis* SALIVARY GLANDS. *J. Cell Biol.* 35, 2 Pt. 2 (1967) 167A-168A. Abstr. 346. "7th Annual Meeting of the American Society for Cell Biology. Denver, Colo., USA. 13-15 Nov. 1967".

The kinetics of incorporation of  $^3\text{H}$ -uridine into salivary glands at mid to late 3rd instar (feeding stage) and prepupa has been examined. Incorporation was measured by removing explanted glands at intervals from an artificial culture medium containing the labelled precursor and counting cold trichloroacetic acid-insoluble radioactivity. Results were expressed as cpm per salivary gland, cpm/ $\mu\text{g}$  protein, or cpm/ $\mu\text{g}$  DNA. The last method was the most satisfactory. 3rd-instar glands showed kinetics which were linear, or of a higher order, for 90 min or more (3 of 3 experiments). Prepupal glands showed an initially higher rate of incorporation, but the rate decreased rapidly after 30 min (10 of 11 experiments). It is thought that the kinetics of incorporation into the prepupal glands represents an approach to a steady state of RNA synthesis, rather than a cessation of synthesis, for the following reasons: (1) The same kinetics was obtained regardless of how long the glands were kept in vitro before labelling (2-4 h). (2) Glands kept in vitro as long as 20 h before labelling incorporated readily. (3) If glands were labelled, then placed in medium containing unlabelled nucleosides or actinomycin D, there was a decline in the level of trichloroacetic acid-insoluble radioactivity. When RNA made in vitro at both stages was examined by sucrose gradient centrifugation, no consistent differences were found between them after either 15 min or 3.5 h of labelling. Both stages made much heterogeneously sedimenting RNA and precursors of ribosomal RNA, though neither made finished ribosomal RNA. The kinetics observed for prepupal salivary glands may be explained by one or more of the following possibilities: (1) an increase in the rate of synthesis of unstable heterogeneously sedimenting RNA; (2) a decrease in the rate of synthesis of normally stable RNA species - ribosomal and transfer; (3) a decrease in the stability of usually stable RNA species. (Abstr.)

- 279 Grelt, R. F. DNA REPLICATION AND CROSSING-OVER IN THE *Drosophila* OOCYTE. p.85 of "Research and Development in Progress - Biology and Medicine. Issue No.4". TID-4204, Division of Biology and Medicine (AEC), Washington, D.C. Apr.1968, 229p. Abstr. BIA 2589.  
<sup>3</sup>H-thymidine to be used in the autoradiographic part of the study.
- 280 Gruzova, M.N. THE ROLE OF THE NUCLEUS IN RNA AND PROTEIN METABOLISM OF THE *Chrysopa perla* OOCYTES. *Tsitologiya* 8, 6 (1966) 713-718. (In Russian)  
 Autoradiographic experiments on the incorporation of adenine-<sup>14</sup>C, phenylalanine-<sup>14</sup>C, and tyrosine-<sup>14</sup>C were undertaken to explain the role of the oocyte nucleus in the RNA and protein metabolism in ovarioles of *C. perla*. In the shortest time intervals of incubation (5 and 15 min) adenine-<sup>14</sup>C was incorporated mainly in the oocyte nuclei. After 1 h the oocyte cytoplasm became radioactive. The most intense incorporation of oocytes coincided with the period of fragmentation of the nucleoli in the middle and later stages of previtellogenesis. Tracers were not observed in the karyosphere. Tyrosine-<sup>14</sup>C and phenylalanine-<sup>14</sup>C were observed to be incorporated both in the oocyte cytoplasm and in the oocyte nuclei at 15, 30, and 60 min. The most active stages in the incorporation of amino acids were also seen to be the middle and late stages of previtellogenesis. The nucleoli of young oocytes did not incorporate these amino acids. It was assumed that the karyosphere incorporated the protein precursors like the karyolymph, without any specific accumulation of these precursors. (CA 66: 1967, 73640f)
- 281 Hansen-Delkeskamp, E., Sauer, H. W., Duspiva, F. RIBONUCLEINSÄUREN IN DER EMBRYO-GENESE VON *Acheta domestica* L. (Ribonucleic acids in the embryogenesis of *Acheta domestica* L.) *Z. Naturf.* 22b, 5 (1967) 540-544. (In German)  
 The total RNA contents (~0.1 µg/embryo) were extracted from cricket embryos by means of the sodium dodecylsulphate - phenol method and separated by zone gradient centrifugation. Hardly any labelling of RNA could be observed in the synchronous cleavage stages, following <sup>14</sup>CO<sub>2</sub> - incubation of 0.5 g-eggs. Incorporation of rRNA (~10 S-fraction) begins simultaneously in the heterochronous cleavage divisions and coincides with the prolongation of interphase, the occurrence of nucleoli and the appearance of cleavage nuclei in the posterior portion of the egg. A net synthesis of rRNA only occurs during the formation of the basic physical features. During the period of development under consideration the 18 S-rRNA always clearly showed a much higher rate of <sup>14</sup>CO<sub>2</sub>-incorporation than the 28 S-rRNA.
- 282 Harris, S. E., Forrest, H. S. RNA AND DNA SYNTHESIS IN DEVELOPING EGGS OF THE MILK-WEED BUG, *Oncopeltus fasciatus* (Dallas). *Science*, N. Y. 156 (1967) 1613-1615.  
 [<sup>3</sup>H-5T]-uridine (23.0 Ci/mM) and [<sup>3</sup>H-6T]-thymidine (14.5 Ci/mM) were used to study RNA and DNA synthesis, respectively. Ribosomal RNA synthesis in developing eggs of *O. fasciatus* was turned on at gastrulation (44 h). A rapid burst of ribosomal RNA synthesis occurred during the 68 - 118 h period, when almost all of this type of RNA found in the 188-h-old egg was synthesised. Synthesis was essentially turned off with the beginning of organogenesis ~72 h later. The pattern for DNA synthesis is similar although less pronounced. The rate of DNA synthesis in the 20-h blastula stage is high but does not account for the increase in DNA and cell numbers which occurs then. From gastrulation up to 92 h, DNA is synthesised at a constant rate which decreases, subsequently.
- 283 Heinonen, L., Halkka, O. EARLY STAGES OF OOGENESIS AND METABOLIC DNA IN THE OOCYTES OF THE HOUSE CRICKET, *Acheta domestica*. *Annls Med. exp. Biol. Fenn.* 45, 1 (1967) 101-109.  
 The development and metabolism of a spherical Feulgen-positive body (FPB) was investigated in the early stages of oogenesis in the house cricket, *A. domestica*. The FPB is first seen in premeiotic interphase and begins to disintegrate in diplotene. Features of its metabolism and especially its mode of disintegration suggest that the FPB contains metabolic DNA. Thymidine is incorporated into the oocyte nucleus only at preleptotene and leptotene. The chromosomal DNA synthesis begins and is completed slightly earlier than that of FPB, but they partially overlap in time. The FPB of *Acheta* forms micronucleoli and releases them into the nucleoplasm. The micronucleoli incorporate tritiated uridine and presumably synthesise ribosomal RNA. A hypothesis to explain the kinetics of rapid vitellogenesis in *Acheta* is presented. (CA 67: 1967, 71419n)

- 284 Hennig, W. CHROMOSOMENFORSCHUNG UND GENPHYSIOLOGIE. (Chromosome research and gene physiology.) Umschau 66, 13 (1966) 435-437. (In German)

Short review article, essentially of work on the giant chromosomes of Chironomus tentans. The usefulness of autoradiographic methods is evident in the study of DNA content of the chromosomes, the puffing phenomenon and its relation to RNA synthesis, the characterization of the newly synthesised RNA (messenger-, transfer-, and ribosomal RNA) and its role in protein synthesis, DNA and protein synthesis, regulation processes and gene localization.

- 285 Hennig, W. UNTERSUCHUNGEN ZUR STRUKTUR UND FUNKTION DES LAMPENBÜRSTEN-Y-CHROMOSOMES IN DER SPERMATOGENESE VON Drosophila. (Study on the structure and function of the lampbrush Y-chromosome in Drosophila.) Chromosoma 22, 3 (1967) 294-357. (In German, with English summary)

The following labelled precursors were used:  $^3\text{H}$ -uridine (specific activity 13.0 - 31.0 Ci/mM),  $^3\text{H}$ -cytidine (27.2 Ci/mM),  $^3\text{H}$ -thymidine (7.2 and 15.0 Ci/mM),  $^3\text{H}$ -L-histidine (38.1 Ci/mM),  $^3\text{H}$ -D/L-leucine (10.9 Ci/mM), and D/L-leucine-1- $^{14}\text{C}$  (36.6 mCi/mM). For single labelling 0.1  $\mu\text{l}$  of a mixture of 2  $\mu\text{Ci}$   $^3\text{H}$ -uridine and 2  $\mu\text{Ci}$   $^3\text{H}$ -cytidine or 3  $\mu\text{Ci}$   $^3\text{H}$ -D/L-leucine/ $\mu\text{l}$  Drosophila Ringer were used. For double labelling for simultaneous measurement of RNA and protein synthesis 1  $\mu\text{l}$  of a solution of 3  $\mu\text{Ci}$   $^3\text{H}$ -uridine + 3  $\mu\text{Ci}$   $^3\text{H}$ -cytidine + 1  $\mu\text{Ci}$   $^{14}\text{C}$ -D/L-leucine in 1  $\mu\text{l}$  Drosophila Ringer were injected. Incorporation studies with radioactive precursors have shown that the lampbrush-like loops of the Y-chromosome in the spermatocytes of D. hydei contain axial DNA and actively synthesise RNA. Uridine-incorporation, at least in some of the loops, appears to be polarized. In most of the loops, the amount of the label increases with incubation time. Studies of the life cycle of spermatocytes indicate that labelled RNA is stored in the loops for about 20 - 30 h, while the loops themselves persist for about 120 h. Following incubation with labelled amino acids, an uptake of labelled proteins from the cytoplasm into the nucleus was observed. The labelled nuclear proteins apparently leave the nucleus within a few hours, without long-term binding to the Y-structures, for even a 40-h incubation does not result in preferentially labelled Y-structures. These data, along with data on the action of antimetabolites, suggest that the Y-structures are dynamic structures: their form seems to be maintained by an equilibrium between the accumulation and outflow of matrix material surrounding the DNA axis. The possible role of the functional structures of the Y-chromosome for messenger utilization in the postmetotic stages of spermiogenesis is discussed.

- 286 Horikawa, M., Ling, L. L., Fox, A. S. EFFECTS OF SUBSTRATES ON GENE-CONTROLLED ENZYME ACTIVITIES IN CULTURED EMBRYONIC CELLS OF Drosophila. Genetics 55 (1967) 569-583.

Active RNA and DNA synthesis occurs in primary cultures of embryonic cells from D. melanogaster. The incorporation of  $^{32}\text{P}$  into phospholipid, acid-soluble, and nucleic acid fractions of such cells was studied as a measure of their synthetic activities.  $^{32}\text{P}$  was introduced in the form of carrier-free  $(^{32}\text{PO}_4)^{3-}$ . Such cells, when cultured in basal medium, exhibit very low levels of alkaline phosphatase, alcohol dehydrogenase, and xanthine dehydrogenase activities. During normal development, alkaline phosphatase activity reaches a peak in 3rd-instar larvae. The addition of enzyme substrates,  $\beta$ -glycerophosphate or phenylphosphate, to the medium has no effect on the alkaline phosphatase levels exhibited by embryonic cells in primary cultures. Alcohol dehydrogenase activity reaches a peak during the pupal period. The addition of ethanol to the medium in appropriate concentrations has no marked effect on the growth rate of embryonic cells, but results in a doubling of alcohol dehydrogenase activity. Methanol has a similar, although less marked, effect. The response of cultured cells to the addition of ethanol is rapid, and removal of ethanol from the medium results in a rapid decline in alcohol dehydrogenase activity. Alkaline phosphatase and xanthine dehydrogenase levels are not affected. Xanthine dehydrogenase reaches a peak in adults. The addition of hypoxanthine, xanthine or uric acid to the medium results in a significant depression of xanthine dehydrogenase activity extractable from cultured embryonic cells. Studies with larval enzyme demonstrate that uric acid is a noncompetitive inhibitor of xanthine dehydrogenase, suggesting that the depression of enzyme activity in embryonic cells by hypoxanthine and xanthine results from their conversion to uric acid and enzyme inhibition.

- 287 Howard, E. F., Plaut, W. ORDERED CHROMOSOMAL DNA SYNTHESIS IN Drosophila melanogaster. J. Cell Biol. 35, 2 Pt. 2 (1967) 59A. Abstr. 119. "7th Annual Meeting of the American Society for Cell Biology. Denver, Colo., USA. 13-15 Nov. 1967".

DNA synthesis has been studied in three polytene chromosome segments from the larval salivary gland of *D. melanogaster*. The segments are the distal one-fifth of chromosomes 2 R, 3 L, and X. Each segment contains cytological regions or replicons whose individual DNA synthetic activity can be resolved radioautographically from that in adjacent replicons. When excised salivary glands are exposed for short periods of time to <sup>3</sup>H-thymidine, different combinations of replicons are labelled in different nuclei. Each unique combination of labelled replicons in a chromosome segment constitutes a labelling pattern. Two kinds of analysis of all the patterns observed in individual chromosomes and all the combinations of patterns observed in more than one chromosome in single nuclei reveal that the DNA synthetic activity of the chromosomal replicons is coordinated. First, it is shown statistically that the various patterns and combinations of patterns could not be produced with the observed frequencies by the random association in single nuclei of independently replicating replicons. Second, if it is assumed that once DNA synthesis begins in a replicon it goes to completion without stopping, the various patterns and combinations of patterns can be arranged in ordered arrays which reflect the temporal sequence of DNA synthesis among chromosomal replicons. These arrays predict that in any one chromosome DNA replication begins and ends at very few loci and that simultaneous synthesis at a larger number of points occurs at an intermediate time. The chromocentre is resolvable as a unit of DNA synthesis in radioautographs. Labelled chromocentres can be seen in nuclei which contain unlabelled chromosome arms. However, labelled chromosome arms are never seen with unlabelled chromocentres. It is concluded that the time span of DNA synthesis in the chromocentre is longer than that in the chromosomal replicons. (Abstr.)

- 288 Han, J., Han, Judith, Quastel, J.H. EFFECTS OF ACTINOMYCIN D ON NUCLEIC ACID METABOLISM AND PROTEIN BIOSYNTHESIS DURING METAMORPHOSIS OF *Tenebrio molitor*. *Biochem. J.* 100, 1 (1966) 441-447.

Injection of 0.16 µg of actinomycin D into pupae of the beetle *T. molitor*, results in the development of modified adults in which the head and thorax are essentially adult, while the abdomen and wings remain pupal-like. It is suggested that the messenger RNA for the development of head and thorax is present in the animal from first day of pupation. Injection of 0.16 µg of actinomycin D brings about 51-67% inhibition of labelled uridine incorporation into RNA. When thymus DNA is mixed with actinomycin D before injection into pupae the latter develop into normal adults. This protection does not occur when DNA and actinomycin D are injected separately. The inhibition of incorporation of labelled uridine into RNA by actinomycin is diminished to some extent when DNA and actinomycin D are injected separately, and abolished if they are injected together. Inhibition of RNA synthesis by actinomycin D in vitro is fully reversible. DNA or deoxyguanosine can reverse the effect of actinomycin D. Incorporation of labelled glycine into protein is not affected by actinomycin D injection during the first 6 d of pupation. On the 7th day it becomes diminished in control pupae but this effect is prevented by actinomycin D. It is suggested that the template for protein synthesis is stable during the first 6 d of metamorphosis and that on the 7th day there is a qualitative change in the protein synthesised on the template. (CA 65: 1966, 5944e).

- 289 International Lab. of Genetics and Biophysics, Naples (Italy). REPORT OF ACTIVITIES OF THE INTERNATIONAL LABORATORY OF GENETICS AND BIOPHYSICS, JULY 1, 1963 - JUNE 30, 1964. EUR-2589.e. 16 Aug. 1965, 52p.

Some work on dipteran puff RNA synthesis involving autoradiography is also reported by the research group on animal genetics.

- 290 Ishizaki, H. PUFFING (OF SALIVARY GLAND CHROMOSOMES). *Tampakushitsu Kakusan Koso* 11, 11 (1966) 946-951. (In Japanese)

A review with 22 references. The title phenomenon is peculiar to Diptera insects. In the puffing state, DNA becomes diffuse and RNA becomes dense.\* This is discussed in relation to the development of insects. (CA 68: 1966, 10606v).

\* It is assumed that radioisotope studies are considered here. (It was not possible to consult the original.) (Compiler's note).

- 291 Jacob, J. AN ELECTRON MICROSCOPE AUTORADIOGRAPHIC STUDY OF THE SITE OF INITIAL SYNTHESIS OF RNA IN THE NUCLEOLUS OF *Smittia*. *Expl Cell Res.* 48, 2 (1967) 276-282.

The study was carried out on explants of salivary glands from late larvae. These were incubated for 10 - 12 min in Morgan Morton and Parker's medium 199 with added  $^3\text{H}$ -uridine (100  $\mu\text{Ci/ml}$ , specific activity 3.0  $\text{Ci/mM}$ ), fixed in (4°C) 6.5% glutaraldehyde in phosphate buffer at pH 7.5, the glands being subsequently embedded in glycol methacrylate and silver sections mounted on carbonized formvar-coated Ni-grids, and covered with an Ilford L4 nuclear emulsion. Results from pulse labelling are consistent with the interpretation that in the salivary gland cells of the late larval stage of *Smittia*, the synthesis of nucleolar RNA is primed by the intranucleolar DNA dispersed in the inner part of the nucleolus. From the limited evidence so far available, it is postulated that the nucleolar organizer complex may consist of an internal and an external entity. Such a distinction finds support in what appears to be a functional divisibility of the organizer. These postulates require confirmation.

- 292 Kaplan, W. D., Oftedal, P. GENETIC AND RADIOAUTOGRAPHIC EVIDENCE FOR A DNA-CONTAINING BODY IN THE CYTOPLASM OF THE ADULT TESTES OF *Drosophila melanogaster*. *Genetics* 56, 3 Pt.2 (1967) 569. Presented at the "1967 Meetings of the Genetics Society of America, Stanford, Calif., USA. 31 Aug.-2 Sep. 1967".

Genetic and radioautographic studies following larval feeding with  $^3\text{H}$ -thymidine or  $^3\text{H}$ -deoxycytidine indicated that the genetic effects of the  $^3\text{H}$   $\beta$ -emissions are confined to the nuclei of origin. On the basis of these observations one would expect that following the infection of adult males with  $^3\text{H}$ -thymidine, daily brooding techniques would show a correlation between elevated mutation rates and labelled sperm heads that would appear at about the tenth postinfection day. However, this did not turn out to be so. Mutation rates above control appeared in early broods for which the sperm heads were unlabelled. Radioautographs showed, moreover, the presence of silver grains over cytoplasmic areas of the tests and also over sperm tails. This activity, by definition, derived from DNA inasmuch as the preparations had been treated by acid hydrolysis before the slides were filmed. Furthermore, the activity disappeared upon treatment with DNase but not after RNase digestion. The genetic effects arose, presumably, from the action of  $\beta$ -rays originating in the cytoplasm of individual spermatogonia or spermatocytes. (Abstr.)

- 293 Keyl, H.G. A DEMONSTRABLE LOCAL AND GEOMETRIC INCREASE IN THE CHROMOSOMAL DNA OF *Chironomus*. *Experientia* 21, 4 (1965) 191-193.

After a discussion of various data obtained by autoradiographic methods, the quantitative results of the present study are described. Microspectrophotometric measurements carried out on the salivary gland chromosomes of the cross between two sub-species of *C. thummi* showed differences in DNA content in homologous bands. These differences followed the relation  $1:2^n$  (where  $n = 1, 2, 3$  or  $4$ ). Various degrees of doubling of DNA contents were observed in certain bands. Mechanisms are discussed which might explain this duplication phenomenon.

- 294 Keyl, H.G. LOKALE DNS-REPLIKATIONEN IN RIESENCHROMOSOMEN. (Localized DNA replication in giant chromosomes.) p.55-68 (Discussion p.68-69) of "Probleme der biologischen Reduplikation". Sitte, P., Ed. Berlin, Springer Verlag. 1966. (In German, with English summary)

Data on the significance of localized DNA synthesis in salivary gland chromosomes of *Sciara coprophila*, *Rhynchosciara angelae*, and *Glyptotendipes barbipes* are reviewed. Puff formation and DNA synthesis were studied by means of  $^3\text{H}$ -thymidine. In some experiments on *Chironomus thummi*  $^{14}\text{C}$ -thymidine was used in addition. The possibility of a local increase in DNA content, other than during a replication phase, may be considered proved for the chromosomes of *Sciara* and *Rhynchosciara*. This type of localized increase in DNA is, however, a less general event than might be concluded from Pavan's work. The occurrence of this type of localized DNA increase (metabolic DNA) could not be confirmed for the chromosomes of *Glyptotendipes*. At the end of each replication phase of the giant chromosomes a number of loci of DNA synthesis can be observed. However, such local DNA synthesis is based on differential properties (DNA content) of the individual replication units. A hypothesis for the buildup of such replication units is proposed. It presents a plausible basis for the occurrence and the origin of homologous replication units of different DNA content during evolution.

- 295 Krishnakumaran, A., Schneiderman, H.A. DEVELOPMENT CAPACITIES OF THE CELLS OF AN ADULT MOTH. *J. exp. Zool.* 157, 3 (1964) 293-306

$^3\text{H}$ -thymidine and -uridine were injected into adults in order to assess the rates of DNA and RNA synthesis in intact adults, in fragments of adults, and in adults grafted to various types of partners.

Biosynthetic capabilities of tissues of the short lived adults were studied using surgical, histological, and autoradiographic techniques. Adults of *Samia cynthia* can have their life span of 11 d prolonged to four months by parabiosis to pupal partners, which presumably supply nutrients. Parabiosis of adults or isolated abdomens to developing pupae caused supernumerary moults in the adults (sometimes two supernumerary adult-adult moults). When an adult is parabiosed to a developing pupal partner with active prothoracic glands the ecdysone produced by the developing pupae induced DNA synthesis in several tissues of the adult including the epidermis, midgut, and gonadal sheath, not just the haemocytes. The gradual decrease in RNA synthesis from emergence until death in all tissues of intact adults and isolated adult abdomens can be prevented in isolated abdomens by grafting them to isolated pupal abdomens. Many of the tissues of the adult moth are considered to have the same biochemical defect as the tissues of the diapausing pupa, i.e., an inability to synthesise DNA. In both, this defect is repaired by ecdysone.

- 296 Krishnakumaran, A., Oberlander, H., Schneiderman, H.A. RATES OF DNA AND RNA SYNTHESIS IN VARIOUS TISSUES DURING A LARVAL MOULT CYCLE OF *Samia cynthia ricini* (LEPIDOPTERA). *Nature*, Lond., 205 (1965) 1131-1133.

The rate of synthesis of DNA and RNA in various tissues during larval moulting of a silkworm is described and the control of these processes examined in *S. cynthia ricini*, a non-diapausing saturniid moth.  $^3\text{H}$ -thymidine (10  $\mu\text{Ci/g}$ ) or  $^3\text{H}$ -uridine (10  $\mu\text{Ci/g}$ ) was injected into anesthetized larvae on each of the 7 d of the 4th instar and during the moult to the 5th- and final larval instar. Ecdysone (I) controls DNA synthesis in most larval tissues but both DNA synthesis and the rate of RNA synthesis in chitogenous epithelium. In some larval tissues like fat body, Malpighian tubules, dermal glands, nervous tissues, and chitogenous epithelium, the threshold level of I required for DNA synthesis is high, and during part of a larval instar the I titer falls so low that DNA ceases in these tissues. In other tissues (midgut and imaginal wing disks) the threshold level of I required for DNA synthesis is low and during larval life the hormone titer never falls below this threshold; hence these tissues synthesise DNA continuously and respond to increased I secretion with increased DNA synthesis. Although the haemocytes also respond to increased I with increased DNA synthesis, unlike other tissues they are capable of considerable DNA synthesis in the absence of I. The prothoracic gland RNA synthesis and cyclic secretion of hormone is under control of the brain hormone but DNA synthesis may be controlled by I.

- 297 Krishnakumaran, A., Berry, S.J., Oberlander, H., Schneiderman, H.A. NUCLEIC ACID SYNTHESIS DURING INSECT DEVELOPMENT. - II. CONTROL OF DNA SYNTHESIS IN THE CECROPIA SILKWORM AND OTHER SATURNIID MOTHS. *J. Insect Physiol.* 13 (1967) 1-57.

The present report examines factors that control the rate of DNA synthesis during larval life, metamorphosis, pupal diapause, and adult life of Cecropia and other Saturniid moths.\* Autoradiographic determinations were made of the rate of incorporation of  $^3\text{H}$ -thymidine in various tissues during the life history. DNA synthesis was also studied in insects subjected to diverse endocrinological and surgical manoeuvres and treated with juvenile hormone. Using labelling techniques, the careers of epidermal cells were followed from one instar to the next. In addition, various tissues were labelled with  $^3\text{H}$ -thymidine, implanted into unlabelled hosts, and their developmental fate determined. DNA synthesis in adult moths was compared with DNA synthesis in other adult insects representing six orders.\*\* The effects of a potent inhibitor of DNA synthesis on development and moulting were also analysed. During each larval moult cycle and during metamorphosis of Saturniid moths there is a definite temporal pattern of DNA synthesis in each tissue and in regions within many tissues. During larval moults in some tissues such as chitogenous epithelia, Malpighian tubules, and nervous system, DNA synthesis occurred soon after the secretion of ecdysone. In other tissues such as imaginal wing disks and muscles, DNA synthesis during larval life was not correlated with ecdysone secretion. Almost every epidermal cell appeared to synthesise DNA during every normal moult cycle, but some tissues such as Malpighian tubules and prothoracic glands did not synthesise DNA during the pupal-adult moult although they did so during earlier moults. Apparently after experiencing a moult in the absence of a high concentration of juvenile hormone these tissues differentiate and ordinarily do not synthesise DNA again. However, two experimentally induced pupal-pupal moults in the presence of juvenile hormone and ecdysone restored the capacity of pupal Malpighian tubules to synthesise DNA. In insects with a pupal diapause DNA synthesis ceased after pupation in all tissues except haemocytes and to a

\* *Hyalophora cecropia*, *Samia cynthia walkeri*, *S.c. ricini*, and *Antheraea polyphemus*.

\*\* Lepidoptera, Diptera, Coleoptera, Hemiptera, Dictyoptera, and Odonata.

small extent the gonads and fat body. This is not the result of low metabolism because the metabolic processes of a pupa could be increased up to 15-fold by integumentary injury without stimulating DNA synthesis. The absence of DNA synthesis in most tissues appears to be a fundamental characteristic of diapause and results from the absence of ecdysone. DNA synthesis was also absent in adult moths and in surgical preparations deprived of a source of ecdysone such as isolated larval abdomens, 'dauer' larvae, and non-diapausing pupae which had their brains extirpated. The addition of ecdysone stimulated DNA synthesis in many tissues of both diapausing pupae and adults. In contrast, experimental administration of brain hormone or juvenile hormone alone and in the absence of ecdysone failed to stimulate DNA synthesis in any tissues examined. Injury to the integument of a diapausing pupa provoked DNA synthesis only in epidermal cells adjacent to the wound but did not stimulate general DNA synthesis. Injury to diapausing pupae also released an 'injury factor' which stimulated DNA synthesis in haemocytes but not in other tissues. Non-diapausing insects appeared to telescope into the larval-pupal transformation a number of the changes normally associated with adult development in diapausing insects such as DNA synthesis in certain muscles and parts of the epidermis. Artificial diapause could be imposed in a non-diapausing pupa either by brain removal or by injection of mitomycin C - an inhibitor of DNA synthesis - before or after some DNA synthesis had begun in the epidermis. However, after epidermal DNA synthesis was well along, an artificial diapause could no longer be imposed. Thus injecting mitomycin C into pupae that had already initiated adult development prevented further DNA synthesis and the cell divisions necessary for scale formation, but did not block the synthesis of an adult cuticle and moulting. No adult insect among six orders examined showed DNA synthesis in epidermis, tracheae, or fat body more than 3 d after ecdysis. This absence of DNA synthesis is correlated with the absence of ecdysone, and renewed DNA synthesis can be induced with ecdysone. Adult insects which live only a short time showed no DNA synthesis in any tissues except haemocytes. However, long-lived insects such as cockroaches, mealworms, dragon flies, and the Monarch butterfly showed DNA synthesis in several tissues during adult life. The developmental fate of various cells was determined by labelling experiments. It was shown that larval epidermal cells and their immediate descendants persisted in significant numbers from one larval instar to the next. However, most pupal epidermal cells appeared to be the remote descendants of larval cells. Similarly, the developmental fate of haemocytes and fat body was determined by implanting labelled pupal cells into unlabelled pupae which were allowed to transform into adults. The relation between DNA synthesis and subsequent synthetic activities of cells differed in various kinds of insects at different developmental stages. In Saturniid moths DNA synthesis seems to be an early obligatory step in the differentiation of most epidermal cells. In other insects (e.g. *Rhodnius*, *Oncopeltus*) some larval epidermal cells form adult structures without prior DNA synthesis. In the general case, the metamorphosis of epidermal cells may or may not require DNA synthesis and may be viewed in some insects as a progressive differentiation which is punctuated incidentally by moults and DNA synthesis. Although DNA synthesis may not be necessary for the metamorphosis of certain cells it appears to be necessary for the reversal of metamorphosis. Apparently DNA replication erases certain biases and enables cells to be reprogrammed. The mechanism of action of ecdysone was examined. Experimentally induced moulting in adult silkmooths is not preceded by DNA synthesis in most epidermal cells, hence stimulation of DNA synthesis cannot be the primary effect of ecdysone even though it is an early effect in many tissues at several developmental stages. From an analysis of available data it is concluded that ecdysone should be viewed primarily as a moulting hormone that initiates biosynthetic activities which lead to moulting. Its primary effect is to stimulate the synthesis or translation of certain messenger RNA's which were previously non-existent or non-functional and which are necessary for moulting. The nature of the proteins coded by these new messenger RNA's was considered. (Auth.)

- 298 Kroeger, H., Lezzi, M. REGULATION OF GENE ACTION IN INSECT DEVELOPMENT. A. Rev. Ent. 11 (1966) 1-22.

This review concentrates on the phenomenon of puffing. The morphology and biochemistry of the puff is described, followed by an analysis of the sequential appearance and disappearance of puffs and of biochemical changes in the tissues as development proceeds. In a 2nd section, the experimental agents, hormonal and non-hormonal, which change puffing patterns are enumerated and their mode of action and the sequence of events leading to the appearance of specific combinations of puffs during normal development are discussed. - Numerous studies are cited in which radioisotopes had been used. - The literature survey which lists 92 references was concluded in March 1965.

- 299 Kuliyev, P., Mamedniyazov, O.N. BIOSYNTHESIS OF NUCLEIC ACIDS IN THE SILK GLAND OF THE SILKWORM. Izv. Akad. Nauk. turkmen. SSR, Ser. Biol. No.3 (1967) 3-7. (In Russian)

The nucleic acid content in the silk gland of the silkworm increased in the 1st half of the 5th developmental stage. When enforced synthesis of silk precursors was started, the amount of nucleic acids in the gland remained constant. (CA 68: 1968, 66790t)

- 300 Kuliyev, P., Mamedniyazov, O.N. BIOSYNTHESIS OF NUCLEIC ACIDS IN THE SILK GLAND OF THE SILKWORM. II. Izv. Akad. Nauk. turkmen. SSR, Ser. Biol. No.4 (1967) 3-9. (In Russian)

RNA synthesis was studied, utilizing centrifugation in a sucrose gradient, separation on a column of methylated albumin, and autoradiography. The incorporation rate of labelled precursors into RNA of the silkworm silk gland on various days of the 5th instar of caterpillars showed that all kinds of RNA's were synthesised during the first half of this stage. The rate of  $^{32}\text{P}$  incorporation into RNA on the 2nd day of the 5th instar was 8-10-fold higher than before becoming a pupa. In the early stages of the 5th instar, the biosynthesis of RNA took place in the nucleus and was connected with the DNA template; during the later stages of the 5th instar, however, the incorporation of adenine involved only the exchange of terminal nucleotides in rRNA. The problem of mRNA for the synthesis of fibroin in the silk gland of the silkworm was not resolved. (CA 68: 1968, 10712b)

- 301 Kunz, W. ZUR CHROMOSOMENSTRUKTUR IN DEN OOCYTEN DER HEUSCHRECKE Locusta migratoria L. (Study of chromosome structure in the oocyte of the grasshopper, Locusta migratoria L.) Naturwissenschaften 53, 1 (1966) 23. (In German)

The oocyte nucleus contains  $\alpha$ -spheres of varying sizes, presumably a part of the beaded chromosomes observed, and varying numbers of  $\beta$ -spheres which do not lie along such chromosomes. To date, autoradiographs taken after injecting  $^3\text{H}$ -uridine ( $\sim 0.28 \mu\text{Ci/grasshopper}$ , incubated for  $\approx 7.5$  min) showed radioactivity to be primarily localized in the beaded chromosomes. Uridine was also incorporated by the fine threads in the nucleus, but to a lesser extent.

- 302 Kunz, W. FUNKTIONSTRUKTUREN IM OOCYTENKERN VON Locusta migratoria. (Functional structures in oocyte nuclei of Locusta migratoria.) Chromosoma 20, 3 (1967) 332-370. (In German, with English abstract)

Examination of living oocyte nuclei of L. migratoria has revealed the presence of thread-like structures. They are paired and are thought to be the uncoiled chromosomes since they are broken into fragments by treatment with DNase. The greater part of the threads carries lateral loops like the lampbrush chromosomes of Amphibia. A smaller part has no loops but bears a series of conspicuous granules with bright appearance under positive phase contrast optics (beaded segments). The visibility of the chromosomes has been investigated in solutions with several ions. In hypertonic media the chromosomes contract, the granules fuse, and the beaded segments become lumpy. In nitrogenous atmosphere and if kept at low temperature the beaded structures are also transformed into several lumps. After return to normal conditions they reconstitute their characteristic beaded appearance. In autoradiographs obtained by injection of  $^3\text{H}$ -uridine (specific activity up to  $24400 \text{ mCi/mM}$ ,  $0.3 \text{ mCi/animal}$ ) into the body cavity and by incubation of isolated nuclei in vitro, rather uniformly distributed labelling occurs in the oocyte nuclei for up to 30 min incubation time. With prolonged incubation the activity of the beaded segments becomes more intense than the labelling of the lampbrush chromosomes. After treatment with actinomycin RNA synthesis stops, the axes of beading and the lampbrush chromosomes contract, and the granules disappear more and more. The beaded structures may have nucleolar function in Locusta as they have in Amphibia. If so, the only difference from the Amphibian nucleoli would be the continued attachment to the lampbrush chromosomes. (From abstr.)

- 303 L'Helias, C. INDUCTION DE DESORDRES TISSULAIRES CHEZ LES INSECTES PAR ALTERATION DE L'EQUILIBRE ENTRE FACTEURS DE CROISSANCE PTERINIQUES ET HORMONES DE CROISSANCE ET MECANISME DE CETTE INDUCTION. (Induction of tissue disorders in insects by altering the equilibrium between pterinic growth factors and growth hormones, and the mechanism of this induction.) Annals Endocr. 27, Suppl. 3 (1966) 343-352. (In French)

The injection of a pterinic growth factor (follic acid) to insects in diapause in hormone-deficient conditions induces the formation of tumours. From these tumours, artificially induced in chrysalids of Pieris brassicae, a factor causing tumours is extracted, which can be isolated by centrifugation. It

is infectious to other species when given by injection and is transmissible hereditarily. A study of Drosophila shows that it is transmitted through the cytoplasm by modification of sex ratio: reduction of the  $\delta$  which (carry?) the lethal tumours. Experiments proved that the factor induced by the pterines is made up of light particles which contain DNA. It is DNA which is the active constituent. This DNA, labelled by  $^3\text{H}$ -thymidine, is run on a sucrose gradient at 25 000 rpm. The radioactive and biologically active factor is a 4S component after the breakdown of light particles. This fraction is chromatographed on ECTEOLA cellulose column using a continuous gradient of CINA for elution. The new radioactive and biologically active fraction is incubated with DNase or RNase. The RNase incubate alone is still biologically active. Then, the biological factor induced by a disturbed rate of pterines-growth hormones is really DNA. (Auth.)

- 304 L'Helias, C. INDUCTION DE DESORDRES TISSULAIRES CHEZ LES INSECTES PAR ALTERATION DE L'EQUILIBRE FACTEURS DE CROISSANCE PTERINIQUES/HORMONES DE CROISSANCE ET MECANISME DE CETTE INDUCTION. (Induction of tissue disorders in insects by altering the equilibrium between pterinic growth factors and growth hormones, and the mechanism of this induction.) C. r. Séanc. Soc. Biol. 160, 3 (1966) 461-465.

Injection of folic acid into chrysalids in diapause modified the pterine/growth hormone equilibrium and caused tissue disorganization. A factor isolated from the injected insects by prolonged centrifugation at 26 000 g was interspecific and produced tissue hyperplasia, increased the number of blood cells, produced testicular or intestinal melanomas, reduced the number of males, and caused larval death when injected into Drosophila. Injection of folic acid or 2-amino-4-hydroxypteridine carboxylic acid into female Carausius morosus did not alter these females, but increased the intersexuality of their descendants and caused death of the adult male offspring. Injection of the factor into aphids similarly did not affect the injected insects, but only affected the 3rd generation, producing a cuticular hardening in males. Crossing experiments indicated the involvement of cytoplasmic inheritance in transmitting the factor from treated Drosophila females. The isolation of the factor by homogenization of treated larvae, centrifugation, sucrose density gradient, and chromatography on ECTEOLA-cellulose is described; since the biologically active fraction coincides with that incorporating  $^3\text{H}$ -thymidine and since DNase, but not RNase, abolished biological activity, the factor is apparently DNA. (CA 65: 1966, 19048d)

- 305 Lara, F.J.S. THE CONTROL OF NUCLEIC ACID SYNTHESIS IN THE SALIVARY GLANDS OF Rhynchosciara angelae. Final report Aug. 1965 - Jul. 1966. AD-640929, Sao Paulo Univ. (Brazil). Biochemistry Lab. 1966, 27p.

Methods were developed for extraction of RNA from salivary glands of Rhynchosciara, yielding high quality preparation as judged by hydrodynamic properties. By this method and pulse labelling the synthesis of RNA in the glands throughout larval development was studied. The principal class of RNA synthesised before the appearance of the large puffs is a 35 S RNA. This RNA is subsequently transformed into ribosomal RNA. After appearance of the large puffs one observes almost complete inhibition of synthesis of ribosomal precursor. This correlation indicates the material produced in puffs is important in control of synthesis of RNA. (Auth.)

- 306 Lara, F.J.S., Hollander, F.M. CHANGES IN RNA METABOLISM DURING THE DEVELOPMENT OF Rhynchosciara angelae. Natn. Cancer Inst. Monogr. No. 27 (1966) 235-242, Published 1967.

A study was made of the nucleic acids (I) produced at the puffs in the giant polytene chromosomes of the salivary glands of R. angelae during developmental stage 3 and early and late stage 5 (prepupa), corresponding, respectively, to times before, during, and after the appearance of the giant chromosomal puffs. I were isolated in bulk and assessments were made of possible correlations between alterations of their biological, chemical, and physicochemical properties and the appearance of the puffs. The levels of DNA and RNA determined throughout the larval development appeared to attain max. corresponding to definite periods (late stage 4 and early stage 5) and coinciding in time with the appearance of many large puffs in the chromosomes. Prior to the appearance of large puffs, I labelling in larvae sacrificed 40 min after the injection of uridine- $^3\text{H}$  took place in an RNA having a sedimentation constant of approx. 35 S; incorporation also took place in other places, with peaks being observed at 18 and 4 S. The specific activity of the 18 S RNA was about twice that observed in the 28 S RNA. The rapidly labelled 35 S material matured into RNA having the sedimentation characteristics of ribosomal RNA. Incorporation of  $^3\text{H}$  during 80 min led to the labelling of all classes of RNA present in the sedimentation profile, as well as of the 35 S RNA. Results differed in larvae of the age at which the giant

puffs were present in the salivary chromosomes, the specific activities being generally lower than those observed at the earlier developmental stage, not much difference being observed between incorporation during 40 and 80 min. Practically no incorporation of  $^3\text{H}$  was observed in larvae at the end of the 5th developmental stage, when the giant puffs had regressed, indicating that the inhibitory processes, already apparent at the stage where puffs were present, reached their max. intensity at this age. It could not be decided whether the observed inhibition was at the level of synthesis or resulted from a rapid destruction of the rapidly labelled RNA by degradative enzymes which might increase in late larval life, but it could be shown that such degradative enzymes were not operative during the RNA extraction process. Since no particularly distinct class of molecules sedimenting at 28 S was observed in the 40-min incorporation experiments, whereas the 18 S class was always very distinct and had a specific activity higher than that observed in the 28 S region, it appeared that the processes leading to the formation of two classes of ribosomal RNA had different velocities, as had been reported for rat liver, Hela cells, and bacteria. It was suggested that the control of RNA synthesis might depend on the concentration of amino acids available to the cells, as had been found with bacteria. The findings indicated a correlation in the occurrence of important molecular events in the cells and of morphological alterations in the chromosomes. (CA 68: 1968, 68864 v)

- 307 Leach, W.M. FORMATION OF TRITIUM POOLS DURING MITOSIS. *J. Cell Biol.* 23, 2 (1964) 52A-52A. Abstr. 104. "4th Annual Meeting of the American Society for Cell Biology. Cleveland, Ohio, USA, 11-13 Nov. 1964".

Synthesis of DNA in grasshopper neuroblasts, determined by incorporation of  $^3\text{H}$ -labelled thymidine ( $^3\text{HTdR}$ ) into acid-insoluble DNase-digestible material, extends from middle telophase to very early prophase. To test the possibility that  $^3\text{H}$  derivatives accumulate in stages of the cell cycle during which DNA synthesis does not occur, neuroblasts in different stages between early prophase and early telophase were exposed to  $^3\text{HTdR}$  and "mapped" for subsequent reidentification, then rinsed in culture medium which contained excess unlabelled thymidine. Mitotic progress of mapped cells was observed by bright-field microscopy. Mapped cells were fixed 10 - 15 min after the start of DNA synthesis and reidentified in autoradiograms. Incorporation of  $^3\text{H}$  into DNA was less in neuroblasts exposed to  $^3\text{HTdR}$  during prometaphase and metaphase than in those exposed in early, middle, and late prophase, or early telophase. Ranges of grain numbers were similar for neuroblasts rinsed in excess unlabelled thymidine for 5 or 20 min after exposure to  $^3\text{HTdR}$  during metaphase. A possible interpretation of these observations is that intracellular retention of a "pool" of  $^3\text{H}$  derivatives is dependent on the presence of the nuclear membrane.

- 308 Leach, W.M., Carlson, J.G. ULTRAVIOLET-INDUCED INCORPORATION OF TRITIATED THYMIDINE INTO *Chortophaga* NEUROBLASTS DURING PROPHASE. *J. Cell Biol.* 35, 2, Pt. 2 (1967) 77A. Abstr. 157. "7th Annual Meeting of the American Society for Cell Biology. Denver, Colo., USA. 13-15 Nov. 1967".

Synthesis of deoxyribonucleic acid in neuroblasts of the grasshopper *C. viridifasciata* (De Geer) has been determined by radioautography to end during very early prophase. Middle and late prophase neuroblasts, however, incorporate  $^3\text{H}$ -thymidine ( $^3\text{H-TdR}$ ) into acid-insoluble deoxyribonuclease-digestible material, as detected by radioautography. At the 2804-Å wavelength, middle prophase neuroblasts incorporated  $^3\text{H-TdR}$  after 256 erg/mm<sup>2</sup>, an exposure level at which mitotic delay is not detected. Incorporation is detected after 512 erg/mm<sup>2</sup> at each of the wavelengths examined: 2483, 2537, 2650, 2804, and 2967 Å; but the wavelengths are not equally effective. As the cell progresses through middle prophase, the most effective wavelength in inducing  $^3\text{H-TdR}$  incorporation shifts from 2537 Å at the beginning of middle prophase to 2804 Å during the terminal part of middle prophase. During late prophase, however, a similar shift in effective wavelengths is not observed. (Abstr.)

- 309 Lezzi, M. RNS- UND PROTEIN-SYNTHESE IN PUFFS ISOLIERTER SPEICHELDRÜSEN-CHROMOSOMEN VON *Chironomus*. (RNA and protein synthesis in puffs of isolated salivary gland chromosomes of *Chironomus*.) *Chromosoma* 21, 1 (1967) 72-88. (In German, with English abstract)

Isolated salivary gland chromosomes of *C. tentans* incubated in a simple salt or sucrose solution are able to synthesise not only RNA but also protein in their puffs. Two different methods were used to expose the chromosomes to the radioactive medium which contained  $^3\text{H}$ -cytidine-5'-triphosphate ( $^3\text{H-CTP}$ ) or L-amino acids uniformly labelled with  $^{14}\text{C}$ .  $^3\text{H-CTP}$  was primarily incorporated into puff RNA, particularly in the Balbiani rings. The migration of radioactively labelled material from

isolated nucleoli to isolated chromosomes suggests that not all protein in the puffs is synthesised by the puffs themselves but that part of the puff protein stems from the nucleoli. It is concluded that besides bound RNA polymerase a puff contains ribosomes attached to the growing mRNA molecules.

- 810 Lima-de-Faria, A., Moses, M.J. ULTRASTRUCTURE AND CYTOCHEMISTRY OF METABOLIC DNA IN *Tipula*. *J. Cell Biol.* 30, 1 (1966) 177-192.

A DNA body is present in the females of the fly *T. oleracea* and is formed in contact with the sex chromosomes in the oögonial interphases. At each oögonial mitosis, the DNA body follows the chromosomes to one anaphase group and is included in one of the telophase nuclei. The body increases appreciably in size during the interphase of meiosis. All oöcytes have the body, but only a few nurse cells possess it. The DNA body synthesises its DNA at a different time from the chromosomes, as is shown by incorporation of tritiated thymidine, and contains 59% of the DNA of the nucleus, as is disclosed by spectrophotometric measurements. At late diplotene the DNA body disintegrates, releasing its DNA into either the nucleus or the cytoplasm. When studied in the electron microscope, the DNA body appears composed of a tight mass of intertwined fibrils. Demonstration that the main mass of the body is composed of DNA is obtained from cytochemical tests which reveal that the DNA body is Feulgen positive, stains green with azure B, incorporates  $^3\text{H}$ -thymidine, and after digestion with DNase is Feulgen negative. The DNA of the body is complexed with histone, like the DNA of the chromosomes, as is revealed by an intense alkaline fast green staining. Electron microscope examination of oöcytes reveals that one side of the DNA body is in close contact with the nuclear envelope and that the other side possesses an outer shell composed mainly of particles 150 - 250 Å in diameter. Between the outer shell and the chromosomes there is a band of low electron opacity, 4000 - 7000 Å thick. In the light microscope, this light band together with the outer shell is Feulgen negative and stains violet with azure B; this is confirmation of the presence of RNA. In the oöcytes the nucleoli are found inside the DNA body. These nucleoli have a nucleolonema composed mainly of particles 150 - 250 Å. The nucleoli are Feulgen negative, alkaline fast green negative, stain violet with azure B, and do not stain with azure B after RNase digestion, thus confirming their RNA content. The presence of the nucleoli inside the DNA body and of a band of RNA between the body and the chromosomes is indicative of a high RNA synthetic activity. Since the DNA of the body is complexed with histone, as in the chromosomes, and the nucleoli are located inside the body, the simplest interpretation of the DNA body is that it represents hundreds of copies of the operations of the nucleolar organizing region or neighbouring regions. The situation found in *Tipula* has several basic features in common with the polytene chromosomes of other Diptera and with the hundreds of nucleoli present in *Triturus* oöcytes. In all three cases, genes seem to be copied hundreds of times but are kept in different types of packages. A DNA body like the one in *T. oleracea* is found in other species of Diptera and in the Coleoptera. There is no indication, from the present investigation, that the DNA body is in any way associated with a virus. (Auth.)

- 811 Lockshin, R.A. INSECT EMBRYOGENESIS; MACROMOLECULAR SYNTHESIS DURING EARLY DEVELOPMENT. *Science*, N.Y. 154 (1966) 775-776.

A new technique permits the injection of aqueous solutions into the eggs of certain Coleopteran insects (*Leptinotarsa decemlineata*, *Tenebrio molitor* and *Dermestes maculata*). 0.001 or 0.01  $\mu\text{Ci/ml}$  of  $^3\text{H}$ -thymidine, -uridine, -leucine, or -phenylalanine (1-10 ml of solutions containing 1 or 10  $\mu\text{Ci/ml}$ ) were injected into eggs of *Leptinotarsa* and *Dermestes*. DNA and protein are synthesised from the onset of development, but the synthesis of RNA is not detectable until the migrating cleavage nuclei arrive at the cortex of the egg.

- 812 Mattingly, E., Whitfield, V.B. VARIATIONS IN METABOLIC ACTIVITY DURING THE LARVAL DEVELOPMENT OF *Rhynchosciara*. p. 50 of "Biology Division Semiannual Progress Report for Period Ending July 31, 1966". ORNL-3999, Oak Ridge National Lab., Tenn. 1966, 217p.

DNA synthesis in the salivary gland remains extremely vigorous throughout early development. Other tissues with polytene chromosomes (gastric caeca, intestine, Malpighian tubules) show progressive diminution of DNA synthesis. Cells that are most active in synthesising DNA also show the highest degree of incorporation of  $^3\text{H}$ -uridine and -leucine. The label resulting from the incorporation of amino acid is associated most strongly with the DNA-positive bands of the chromosomes. After several hours' exposure to the isotope the puffs become heavily labelled.

- 313 Mattingly, E.M. VARIATIONS IN METABOLIC ACTIVITY DURING THE LARVAL DEVELOPMENT OF *Rhynchosciara*. *J. Cell Biol.* **31**, 2 (1966) 74A. Abstracts of Papers Presented at the "6th Annual Meeting of the American Society for Cell Biology, Houston, Tex., USA, 17-19 Nov. 1966". Abstr. 148.

The habit of synchronous development seen in the insect *R. angelae* and related species has been used for the study of metabolic events in relation to larval development and to chromosomal phenomena such as the formation of puffs. DNA synthesis in the salivary gland, as demonstrated by radioautography, remains extremely vigorous throughout early development. Other tissues with polytene chromosomes, such as gastric caeca, intestine, and Malpighian tubules, show progressive diminution of DNA synthesis, so that at the stage of formation of the previously described DNA puffs on the salivary gland chromosomes there is very little evidence of DNA synthesis in the other tissues. The cells that are most actively synthesising DNA also show the highest degree of incorporation of  $^3\text{H}$ -uridine and  $^3\text{H}$ -leucine. Particular attention has been given to the labelling of polytene chromosomes with  $^3\text{H}$ -leucine at different stages of development. The label resulting from incorporation of the radioactive amino acid is associated most strongly with the DNA-positive bands of the chromosomes. After several hours' exposure to the isotope, the chromosome puffs also become heavily labelled. The most rapidly labelled nuclear components are the many micronucleoli associated with certain bands of the chromosomes. Fixation with neutral formalin shows that much of the label that is removed by acid fixation is concentrated in these micronucleoli. (Abstr.)

- 314 Morris, O.N. INCORPORATION OF RADIOACTIVE URIDINE INTO THE RNA OF THE LEPIDOPTERAN, *Barathra brassicae*. *J. invertebrate Path.* **8** (1966) 259-261.

The incorporation of uridine- $^3\text{H}$  into RNA of the tissues of *B. brassicae* was followed by autoradiographic techniques. Autoradiographs were prepared 60 min to 24 h after interhaemocoelic injection. At 70 min, nuclear RNA was heavily labelled but cytoplasmic RNA only lightly. At 100 min all organs were heavily labelled but fat body relatively less than gut and other ectodermal tissues. Except in fat body and neurolemma, the amount of labelling appeared to decrease after 3 h. (CA 66: 1966, 9404 h)

- 315 Muckenthaler, F.A., Mahowald, A.P. DNA SYNTHESIS IN THE OOPLASM OF *Drosophila melanogaster*. *J. Cell Biol.* **28**, 2 (1966) 199-208.

$^3\text{H}$ -thymidine was injected into 2-d-old *D. melanogaster* females, and tissue sections were prepared from the ovary for radioautography with both the light and electron microscopes. Thymidine- $^3\text{H}$  was incorporated into nuclei of nurse cells, follicle cells, and into ooplasmic DNA. The highest level of incorporation occurred at stage 12. The 15 nurse cell nuclei also incorporated thymidine at this stage even though they were degenerating. The label in the ooplasm was removed by extraction with DNase only if the extraction was preceded by a treatment with protease to remove the proteins from the sections. Electron microscopic radioautography revealed that 36% of the Ag grains resulting from decay of thymidine- $^3\text{H}$  were found over mitochondria, 28% were located within 0.25  $\mu$  of these organelles, and 36% probably represented synthesis in the cytoplasm by the "storage DNA" characteristic of many eggs. It was suggested that one mechanism acting throughout the egg chamber is responsible for the synchronous synthesis of DNA in the degenerating nurse cells, in the mitochondria of the egg, and in the "storage DNA" of the ooplasm. (CA 64: 1966, 16342 de)

- 316 Muhammed, A., Gonçalves, J.M., Trosko, J.E. STUDY OF DNase AND DNA POLYMERASE ACTIVITY DURING *Drosophila* DEVELOPMENT. p.41-42 of "Biology Division Semiannual Progress Report for Period Ending January 31, 1966". ORNL-3922, Oak Ridge National Lab., Tenn. May 1966, 207p.

The study was undertaken to delineate the levels of some of the synthetic and degradative enzymes related to DNA during insect embryogenesis and histolysis-histogenesis. Sterile extracts of *Drosophila* were collected throughout the developmental cycle (fertilized egg to adult). DNA polymerase activity was determined by the incorporation of  $^3\text{H}$ -ATP into an acid-insoluble DNA product. The pattern of DNase activity during development is shown graphically, but proved almost impossible to characterize since the DNases in the crude extract interfered with the assay. DNA polymerase activity in egg extracts indicates, however, that the enzyme could utilise either native or denatured DNA as a primer for DNA synthesis.

- 317 Muhammed, A., Gonçalves, J.M., Trosko, J.E. DEOXYRIBONUCLEASE AND DEOXYRIBONUCLEIC ACID POLYMERASE ACTIVITY DURING *Drosophila* DEVELOPMENT. *Devl Biol.* 15 (1967) 23-32.

Relative DNase activity has been found to increase in extracts of different developmental stages of *Drosophila* in the following manner: eggs, 2-d-old pupae, 1-d-old pupae, adults, and 3-, 5- and 7-d-old larvae. Some of the activity might be of the endonuclease type. DNA polymerase activity was measured in the egg, but not in extracts of other development stages due to interference from DNases. Incorporation assays for DNA polymerase (replicative deoxyribonucleotidyl-transferase) contained the deoxyribonucleoside triphosphates of adenine, guanine, cytosine, and thymine, one of which was labelled with  $^3\text{H}$  (dATP); calf thymus DNA (native or heat-denatured);  $\text{Mg}^{++}$ ; extract; and Tris buffer (pH 7.0). A blank without DNA was used as a control of the incorporation of  $^3\text{H}$ -dATP. The DNA polymerase seems to utilize either native or denatured DNA as primer.

- 318 Mukherjee, A.B. A COMPARATIVE STUDY OF THE KARYOTYPES AND PATTERN OF DNA SYNTHESIS IN MOSQUITOES. *Diss. Abstr.* 27, 4 (1966) 1039-B-1040-B.

This study was undertaken in an attempt to obtain additional information pertaining to the cytogenetics of mosquitoes. Five genera and 30 species of mosquitoes were included in the study of the karyotypes, and four genera and five species were considered for the study of the pattern of DNA synthesis. Chromosome preparations were made from the brain tissues of field collected 4th-instar mosquito larvae (prepupae). The standard procedure of squash technique was followed in preparing this material. For the autoradiographic study  $^3\text{H}$ -thymidine (sp. activity 3000 mCi/mM) was used for labelling the newly synthesised DNA. Kodak NTB, nuclear track liquid emulsion was used for tracking the  $\beta$ -particles emitted by thymidine- $^3\text{H}$ . 4th-instar mosquito larvae were exposed to thymidine- $^3\text{H}$  in a range from 1 - 12 h. Chromosome preparations were made from brain cells using aceto-lacto-orcein stain. In the study of each species several slides were prepared by staining with Feulgen reaction until the heterochromatin and euchromatin were distinguishable. To identify the karyotypes, their morphology, the position of the centromere in the chromosomes, and the number and ratio of length of chromosomes  $\frac{\text{I}}{\text{II} + \text{III}}$  were studied. For autoradiographic study, the density of the silver grains over heterochromatin and euchromatin, the percentage of labelled cells in different time periods of exposure to thymidine- $^3\text{H}$  and late replicating arms in chromosome pairs of different genera and species were observed and compared. The following conclusions are based on the results of this study: (1) The diploid chromosome complement of all species studied is six ( $2n = 6$ ). (2) The karyotypes of each of the five genera are distinct and readily recognizable. The karyotypes of the species within each genus are similar and are not easily recognizable. (3) Only in the genus *Anopheles* have sex chromosomes (X and Y type) been identified. This is also in agreement with the reports of previous investigators. (4) Pairing of chromosomes at least in some stages of mitosis is a characteristic phenomenon of all the species studied. (5) Polyploid cells were found in one specimen of *Culiseta inpatiens* (Walker), and chromosomes with abnormal arm lengths were found in the homologous pairs of another specimen of the same species. (6) A satellite, which is a pinched off portion of a chromosome in the distal region of an arm which remains attached to the main body by a tenuous thread of chromatin, was found with the chromosome pair II in *Psorophora signipennis* (Coquillett). (7) The pattern of DNA synthesis, as revealed from the amount of labelling of the interphase nuclei, the silver grain density over euchromatin and heterochromatin, and the percentages of labelled interphase nuclei, is very distinct in the different genera and species of mosquitoes investigated in the present study. (8) A heterochromatic mass was found in the Feulgen stained preparations of all species investigated, and these heterochromatic regions were found to synthesise their DNA at a different time than the euchromatin. (9) A late replicating region was found in one pair of chromosomes in each species. In *Aedes dorsalis* (Meigen), *A. cataphylla* Dyar, and *Culiseta inornata* (Williston) the late replicating region is found in the chromosome pair III. In *P. signipennis* this is present in chromosome pair II which is the satellited pair of chromosomes, and in *Culex tarsalis* Coquillett one arm of the chromosome pair I replicated later than the other arm. (10) As a result of this study it is concluded that although mosquitoes are very similar from the standpoint of chromosome number, they show many distinctive features in their karyotypes and DNA synthetic pattern. (11) White's hypothesis (1949) of the evolution of the mosquito karyotype, other than in the genus *Anopheles*, from the tipuloid type of Diptera by incorporation of sex chromosomes with the autosomes is supported by the results of this study. (From DA)

- 319 Nigon, V., Legay, J.M., Nonnenmacher, J. L'INCORPORATION DE THYMIDINE TRITIEE DANS LES GLANDES SERICIGENES DE *Bombyx mori*. *Bull. biol. Fr. Belg.* 95 (1961) 128-133.

De la thymidine tritiée a été injectée à des chenilles de *Bombyx mori* au 5<sup>e</sup> âge. Les glandes séricigènes ont été prélevées de 15 minutes à 5 jours après l'injection et l'incorporation de la thymidine a été étudiée par autoradiographie. La thymidine paraît se fixer exclusivement dans l'ADN. Le maximum d'incorporation est observé environ une heure après l'injection. Après 5 jours, on constate une dilution très forte qui pourrait indiquer l'existence d'une perte d'ADN marqué. La fixation de la thymidine se fait principalement dans le tube sécrèteur, à l'exclusion des autres parties de la glande. On peut mettre ces différences en parallèle avec l'activité différente, qualitativement et quantitativement, des deux régions considérées. Dans certains noyaux, le marquage ne se produit que sur une partie du territoire nucléaire, attestant l'existence d'un asynchronisme fonctionnel entre les diverses régions d'un même noyau. La permanence de cette hétérogénéité conduit à diverses hypothèses au sujet de la structure nucléaire. (Aut.)

- 320 Oak Ridge National Lab., Tenn. CYTOLOGY AND GENETICS. p. 41-42 of "Biology Division Semiannual Progress Report for Period Ending January 31, 1968". ORNL-3922. May 1966, 207p.

DNA polymerase activity was determined by means of <sup>3</sup>H-ATP incorporation. The enzyme could utilize either native or denatured DNA as a primer for DNA synthesis. Work was also carried out on *Drosophila*.

- 321 Oberlander, H. Schneiderman, H.A. JUVENILE HORMONE AND RNA SYNTHESIS IN PUPAL TISSUES OF SATURNIID MOTHS. *J. Insect Physiol.* 12 (1966) 37-41.

Pupae and developing adults received injections of <sup>3</sup>H-uridine (10 µCi/g, 0.37 Ci/mM). (RNase digestion was used in controls and removed >70% of the label). It was found that when intact pupae of saturniid moths developed into "2nd pupae" under the influence of a juvenile hormone extract, their fat body, blood cells, and tracheal epithelium synthesised RNA at a higher rate than during normal development. In contrast, juvenile hormone extract had no effect on the rate of RNA synthesis of tissues of isolated pupal abdomens which lack prothoracic glands. It is suggested that juvenile hormone extract has no direct metabolic effects on any pupal tissues in saturniid moths except for the prothoracic glands.

- 322 Orlando, E. THYMIDINE H<sup>3</sup> INCORPORATION IN THE NURSE CELLS OF AMPHIGONIC AND PARTHENOGENETIC OVARIES OF *Megoura viciae* (HOM. APH.). *Experientia* 22, 10 (1966) 686-687. (In English, with Italian summary)

Both amphigonetic and parthenogenetic female individuals are present in aphids, the former carrying ovaries with large polyploid nurse cells while the latter are viviparous and carry ovaries with very small, usually diploid nurse cells. Amphigonetic and parthenogenetic females of *M. viciae* at various stages of development were injected in the abdomen with 0.1 µCi of <sup>3</sup>H-thymidine. The animals were fixed and embedded at intervals of from 1 h to 4 d. In the amphigonetic female an active nuclear incorporation of <sup>3</sup>H-thymidine occurs during growth. However, even when the nurse cells are functioning fully, and when the nuclei appear to have achieved max. development, incorporation continues, without affecting all the nuclei. Results indicate that the active synthesis occurring in the nuclei concerns metabolic but not genetically stable DNA. Metabolic DNA may be synthesised in the nurse cells of amphigonetic insects.

- 323 Pavan, C., Basile, R. CHROMOSOME CHANGES INDUCED BY INFECTIONS IN TISSUES OF *Rhynchosciara angelae*. *Science*, N.Y. 151 (1966) 1556-1558.

The main effects of two infections, one by a protozoan and the other by a virus, in cells of *R. angelae* (Diptera, Scleridae) are an increase in cell size and changes in the size, shape, and behaviour of the chromosomes. The X-chromosome of some cells reacts differently from the autosome to the protozoan infection. In cells heavily infected with the protozoan (presumably a microsporidian), the surface of the chromosomes frequently had a coat of RNA, which showed massive incorporation of <sup>3</sup>H-uridine from 20-40 min after injection of the larva. Some chromosomes show specific, easily traceable points after infection by the virus. Some of the effects of these infections may be similar to the effects of infective agents in other organisms.

- 324 Pelling, C. A REPLICATIVE AND SYNTHETIC CHROMOSOMAL UNIT: THE MODERN CONCEPT OF THE CHROMOMERE. *Proc. R. Soc., Ser. B* 164 (1966) 279-289.

New approaches to studying the chromomere organization of the chromosome have recently been made by the combined use of cytological, autoradiographic, and photometric methods. The evidence thus obtained is reviewed. Giant Dipteran chromosomes (e.g. *Chironomus tentans*, *thummi thummi*, *th. piger*) permit a study of the topography of DNA replication in great detail. Each chromomere appears to replicate its DNA independently of other chromomeres. Autoradiographic studies suggest a correlation between the length of the replication period of a chromomere and the amount of DNA present in it. The chromomere may be regarded as an essential part of chromosome structure, characterized by its own DNA synthesis. Microspectrophotometric findings together with autoradiographic results point to the chromomere as a molecular unit of replication. A study of the puffing phenomenon reveals, moreover, that the gradual loosening of the chromosomal material, mainly DNA, tightly packed within the band, goes hand in hand with a gradual increase of the RNA production of that band. The regulation of RNA synthesis at the chromomere level is discussed. The functional significance of the chromomere is linked with the specificity of the unfolding mechanism, which affects each chromomere independently. It is not possible yet to clearly separate the mechanisms involved in the unfolding of the chromomeric DNA from the factors which guarantee that these mechanisms start their work and always act at defined places.

- 325 Pettit, B.J., Rasch, R.W., Rasch, E.M. DNA SYNTHESIS IN THE GIANT SALIVARY CHROMOSOMES OF *Drosophila virilis* PRIOR TO PUPATION. *J. Cell Physiol.* **69**, 3 (1967) 273-279.

There was no consistent increase in DNA in late larval or prepupal stages. Three classes of DNA amounts from the salivary gland were compared with the DNA amounts from haemocyte nuclei of the same larva. The salivary gland nuclei were geometric multiples of the haemocyte DNA amounts. Female prepupa had a larger amount of nuclei in higher polytene classes than in the male of the same age. The percentage of salivary nuclei incorporation of thymidine-<sup>3</sup>H (*I*) markedly decreased shortly after spiracle pigmentation in both sexes. Female larvae had slightly increased *I* incorporations ~4 h after pigmentation. *I* was incorporated into chromosomes with a continuous or discontinuous type of pattern. All labelled nuclei were labelled to the same extent in the heterochromatin. (CA 67:1967, 88659 p)

- 326 Platova, T.P. THE ROLE OF NURSE CELL NUCLEI IN THE FORMATION OF OOCYTE CYTOPLASMIC DNA IN *Drosophila*. *Tsitologiya* **9**, 7 (1967) 834-839.

Females of *D. virilis*, 5 d old, were treated with thymidine-<sup>3</sup>H (specific activity 4.4 Ci/mM, 0.2 µl/animal) at 10 or 22°C. Incorporation of the labelled material was studied autoradiographically in thin slices of ovarian tissue. At the period of yolk formation when reduplication of DNA took place in nurse cell nuclei (stage 9 - 10 of the oocyte development), quick incorporation of thymidine-<sup>3</sup>H proceeded in nuclei of these cells that was observable 5 min after the application of the labelled material (at 22°C). Incorporation of thymidine-<sup>3</sup>H in cytoplasm was found at least 20 min after the injection. At 10°C, a pronounced retardation of thymidine incorporation in cytoplasm was observed: as late as 2 h after thymidine injection the ooplasm was labelled slightly, if at all; the nurse cell nuclei were labelled intensively. Nurse cell nuclei took part in the ooplasmic DNA formation. A part of cytoplasmic DNA was synthesised in cell nuclei and passed into the ripening oocyte. (CA 67:1967, 106357 v)

- 327 Plaut, W., Nash, D., Fanning, T. ORDERED REPLICATION OF DNA IN POLYTENE CHROMOSOMES OF *Drosophila melanogaster*. *J. molec. Biol.* **16**, 1 (1966) 85-93.

Sites of DNA synthesis on the polytene chromosomes of *Drosophila* can be identified autoradiographically after incubating excised salivary glands for 10-15 min in a medium containing <sup>3</sup>H-thymidine. Using this technique the patterns of DNA synthesis have been mapped relative to the cytologic banding pattern and the frequency measured with which specific chromosome regions appear labelled in a population of nuclei. This frequency is found to be a specific function of the region, reinforcing the previous suggestion that the chromosome contains a longitudinal array of DNA replicative units. Following Jacob and Brenner (1963), these units were denoted as 'replicons'. The precision with which any single replicon can be localized is limited by autoradiographic resolvability as well as the necessity for its temporal differential replicative activity relative to its neighbours. At least 30 replicons have been identified in the less than 15% of the cumulative lengths of the *D. melanogaster* chromosomes studied. While the identification of a replicon requires spatial discreteness and differential behaviour, and thus implies a degree of independence, its autonomy is demonstrably incomplete. The data fit most simply the notion that all replicons are active together at some time in the overall replicative cycle, but that some remain active over longer periods than others. (Auth.)

- 328 Pollister, A.W., Arnold, G. AN AUTORADIOGRAPHIC STUDY OF RNA SYNTHESIS IN NUCLEI OF ISOLATED SALIVARY GLANDS OF *Drosophila hydei*. *J. Cell Biol.* 23, 2 (1964) 75A. Abstr. 153. "4th Annual Meeting of the American Society for Cell Biology, Cleveland, Ohio, USA, 11-13 Nov. 1964".

Isolated salivary glands were incubated in a Ringer type solution containing cytidine- $^3\text{H}$  or uridine- $^3\text{H}$  for periods from 3 - 60 min. Autoradiographs were made on 4- $\mu$  sections of frozen-substituted glands. Relative RNA concentrations were estimated photometrically as azure B stain. Differences in relative amounts of incorporation of the two labelled precursors suggest the presence of two RNA fractions in the nucleolus: (a) characterized by a high proportion of uridine, and (b) with less uridine and a longer association with the nucleolus. Specific turnover rates of RNA in nucleolus and chromatin appear to be approximately equal, as found by previous workers in various materials. Determinations of dry weight concentrations were made by interferometry, in order to apply a correction for self absorption, as given by Maurer and Primbsch (*Exp. Cell Research*, 33; 1964, 8). The concentrations (mg/cm $^2$ ) were as follows (4- $\mu$  sections): nucleolus, 0.240; cytoplasm, 0.192; chromatin, 0.108 (means of 55 cells on 3 glands). These values lead to the following correction factors: nucleolus,  $\times 11$ ; cytoplasm,  $\times 8.8$ ; chromatin,  $\times 5.0$ . Grain counts corrected by these factors show the specific nucleolar rate of turnover to be approx. 2.50 times that of the chromatin for uridine and approx. 4.25 times that of chromatin for cytidine. (Abstr.)

- 329 Prudhomme, J.C., Gillot, S., Daillie, J. EFFETS DE L'ACTINOMYCINE D SUR LA SYNTHÈSE DE L'ADN ET DE L'ARN DANS LA GLANDE SERICIGÈNE DE *Bombyx mori* L. *Expl Cell Res.* 48, 1 (1967) 186-189.

Les auteurs ont employé thymidine-méthyl- $^3\text{H}$  (6.5 et 8.5 Ci/mM) et uridine- $^3\text{H}$  (18 Ci/mM). Même à la plus forte dose d'antibiotique, la phosphorylation de la thymidine n'est pas réduite de manière significative. L'arrêt de la synthèse de l'ADN, en présence de l'antibiotique, ne peut être attribué à un défaut de phosphorylation de la thymidine. Il semble probable que l'actinomycine D qui se fixe sur l'ADN empêche ainsi sa réplication. On peut définir la concentration d'actinomycine susceptible de déterminer la réduction de la synthèse d'ARN à 50% de sa valeur (DI 50 ARN) et sur le même matériel le paramètre correspondant pour ce qui concerne la synthèse d'ADN (DI 50 ADN). Les résultats donnent un rapport  $\Delta = \text{DI 50 ADN} / \text{DI 50 ARN} = 51$ .

- 330 Przelecka, A. NUCLEIC ACID METABOLISM AND CELL INTERACTION IN THE OVARIOL OF *Galleria mellonella*. *Folia Histochem. Cytochem.* 4, 3 (1966) 223-236.

Nucleic acid metabolism was studied in the ovariole of a lepidopteran, *G. mellonella*. In the cells composing the follicular vesicle, differences in localization of RNA during vesicle differentiation were visible. Isotope experiments showed that nuclei of the trophic cells were sites of intense synthesis of RNA. In young vesicles the oocyte nucleus was equally active in the synthesis of RNA, while at a later stage a remarkable preponderance of the polyploid trophocyte nuclei in this process was observable. The synthesis of RNA in the follicular epithelium was highest after the autolysis of trophocytes. The structural disintegration of trophocyte nuclei was accompanied by loss of their synthetic activity. Both the cytochemical staining and the results of isotope experiments showed migration of RNA from the trophocyte chamber into the oocyte. The activity of glucose-6 phosphate dehydrogenase indicating the cell ability for the synthesis of nucleosides, was either localized in basophilic regions of the oocyte or extended in the direction of the contiguous cytoplasm of the neighbouring cells. It is suggested that physiologic contact between the oocyte and the trophocyte cells may have a reciprocal character. The endoplasmic reticulum appeared to be a structure that makes contact possible between the oocyte and the trophocyte chamber. (Nuclear Medicine)

- 331 Rasch, E.M., Pettit, B.J. NUCLEOPROTEIN METABOLISM IN SALIVARY GLAND CHROMOSOMES OF *Sciara* DURING PUPATION. *J. Cell Biol.* 35, 2, Pt.2 (1967) 110A-111A. Abstr. 228. "7th Annual Meeting of the American Society for Cell Biology, Denver, Colo., USA, 13-15 Nov. 1967".

Differences in relative rates of nucleic acid and protein synthesis during periods of maximal puffing activity by the giant salivary chromosomes of *S. coprophila* were studied by radioautography. Pairs of glands from carefully staged female larvae were pulse labelled by incubation for 15, 30, or 60 min in insect saline enriched with 25  $\mu\text{Ci/ml}$  of tritiated precursors. Heavy, differential labelling by DNA puffs and maximal labelling above non-puffing chromosomal regions occurred with  $^3\text{H}$ -thymidine in mid 4th instar, followed by a sharp drop in percentage of labelled nuclei in glands from prepupae

and virtual cessation of DNA synthesis all along the chromosome by the time of pupal moult and throughout the period preceding gland lysis in 72-h-old pupae. During these stages, salivary chromosomes continued synthesis of RNA, showing maximal incorporation of  $^3\text{H}$ -uridine or  $^3\text{H}$ -cytidine about 72 h before pupation, and again just before pupal moult. Vigorous incorporation of  $^3\text{H}$ -proline or  $^3\text{H}$ -histidine was found in both the nucleus and the cytoplasm throughout 4th larval instar, with moderate labelling of chromosomes occurring in glands from prepupae and 24- or 48- h-old pupae. With  $^3\text{H}$ -arginine,  $^3\text{H}$ -lysine, or  $^3\text{H}$ -tyrosine there were no clearly marked cyclic fluctuations in percentage of labelled nuclei or in distinctive labelling patterns of specific chromosome regions to mark formation of DNA puffs. By feeding larvae with  $^3\text{H}$ -thymidine at mid 4th instar and then sacrificing at subsequent stages of prepupal or pupal development, fate of the extra DNA synthesised at specific puff sites of chromosome 2 was followed over an 8-d-period of growth on unlabelled medium. Grain counts showed no significant diminution of labelling above DNA puffs, nor was there displacement of label to suggest dispersion of DNA from these chromosomal sites to other cellular organelle systems. The DNA produced by heterochromatic puff loci thus shows metabolic stability as expected for genetic or template DNA. (Abstr.)

- 332 Reddy, S.R.R., Wyatt, G.R. INCORPORATION OF URIDINE AND LEUCINE IN VITRO BY *Cecropia* SILKMOTH WING EPIDERMIS DURING DIAPAUSE AND DEVELOPMENT. *J. Insect Physiol.* **13**, 7 (1967) 981-994.

A preparation consisting of fragments of wing epidermis from pupae of the silkmoth, *Hyalophora cecropia*, has been incubated in a synthetic medium, and the effects of some conditions on incorporation of  $2\text{-}^{14}\text{C}$ -uridine and  $^3\text{H}$ -leucine into RNA and protein, respectively, have been studied. Under standard conditions, the rates of incorporation remained unchanged when the precursors were added to tissue that had been preincubated for different times up to 20 h. Tissue from insects commencing adult development after diapause, through the action of either endogenous or injected ecdysone, showed increased biosynthetic rates. Stimulation was evident 10 h after injection of the hormone. Quantitatively, however, increases were less than those found when incorporation was measured in vivo. No effect was detected of pure ecdysone added in vitro to tissue from diapause pupae, which suggests that the tissue had lost sensitivity to the hormone. (Auth.)

- 333 Ritossa, F. A NEW PUFFING PATTERN INDUCED BY TEMPERATURE SHOCK AND DNP IN *Drosophila*. *Experientia* **18**, 12 (1962) 571-573.

The same effects on the puffing pattern are obtained whether temperature shocks are given to the whole larvae or to salivary glands extracted and incubated in Ringer solution. The induced structural modifications can be shown to correspond to actual changes in the synthetic activity of the chromosome bands concerned.  $^3\text{H}$ -cytidine was administered to salivary glands incubated in vitro and heated. The presence of rather large quantities of tracer in the puffs was apparent already after 3-4 min and reached high values after 10 min. Radioactivity was removed by RNase. This proves that as previously shown for normal spontaneous puffs, a rather high rate of RNA synthesis occurred also in temperature-induced puffs (2L 14, 2L 15, 2L 20).

- 334 Ritossa, F.M., Pulitzer, J.F. ASPECTS OF STRUCTURE OF POLYTENE CHROMOSOME PUFFS OF *Drosophila busckii* DERIVED FROM EXPERIMENTS WITH ANTIBIOTICS. *J. Cell Biol.* **19**, 2 (1963) 60A. Abstr. 143.

By autoradiographic methods it can be shown that DNA is present all along the structure of a puff. Since puff formation does not require new DNA synthesis, this DNA is apparently the same as that of the bands from which the puffs originate. Preferential amino acid incorporation (leucine, tryptophan, and lysine) at the puff level is not found either when the puff is functioning or when the puff is forming. This suggests that enhanced protein turnover does not occur in the puff and that new protein synthesis is not necessary for puff appearance. The latter conclusion is also supported by experiments utilising puromycin. Although puromycin inhibits amino acid incorporation into protein in salivary gland cells, it does not inhibit puff formation. On the other hand, RNA synthesis appears to be specifically related to puff structure in the sense that an intense RNA synthesis is found in puffs. Actinomycin C and actinomycin D completely block RNA synthesis in the salivary gland cells without apparent interference with protein synthesis. Also actinomycin C and D completely inhibit puff formation. This indicates that the only synthesis required for a puff to appear is that of RNA. (From abstr.)

- 335 Ritossa, F.M., Atwood, K.C., Spiegelman, S. ON THE REDUNDANCY OF DNA COMPLEMENTARY TO AMINO ACID TRANSFER RNA AND ITS ABSENCE FROM THE NUCLEOLAR ORGANIZER REGION OF Drosophila melanogaster. Genetics 54 (1966) 663-676.

Experiments are described which establish that approx. 0.015% of the DNA of D. melanogaster is complementary to the amino acid transfer RNA (t-RNA). This number leads to about a 13-fold redundancy for each of the approx. 60 t-RNA species. Hybridizations with DNA from stocks carrying one, two, three, and four doses of the nucleolus organizer region established that the t-DNA cannot be detected in the region of the genome which has been shown to contain the complete cluster of DNA complementary to the two ribosomal RNA components. For isotopic labelling the standard medium of Ritossa and Spiegelman was modified to contain 0.5 g of yeast/10 ml. To each 10 ml were added either 7 mCi of  $^3\text{H}$ -uridine (21 Ci/mM) or 5-10 mCi of  $^{32}\text{P}$  after hydrolysis to remove pyrophosphate, and neutralization. The usual RNA employed in these studies had a specific activity > 50 000 cpm/ $\mu\text{g}$ . Details are given of the procedures used for extracting and purifying RNA, preparing DNA, and for determining the base composition of RNA.

- 336 Ritossa, F.M., Atwood, K.C. UNEQUAL PROPORTIONS OF DNA COMPLEMENTARY TO RIBOSOMAL RNA IN MALES AND FEMALES OF Drosophila simulans. Proc. Natn. Acad. Sci. U.S.A. 56, 2 (1966) 496-499.

Drosophila DNA and RNA were labelled, isolated, and molecular hybridization procedures performed. In D. simulans the X-chromosome contained 3 times as much rDNA as the Y, and 1.5 times as much as either X or Y of D. melanogaster. The differences were correlated with the absence of a wild-type allele of bobbed on the D. simulans Y-chromosome. (CA 65: 1966, 20568 c)

- 337 Rodman, T.C. FACTORS INFLUENCING INITIATION OF POLYTENIC REPLICATION. J. Cell Biol. 35, 2, Pt. 2 (1967) 115A-116A. Abstr. 240. "7th Annual Meeting of the American Society for Cell Biology. Denver, Colo., USA. 13-15 Nov. 1967".

Earlier work had indicated that initiation of the DNA synthesis of a polytenic replication cycle may take place as long as the larval state prevails, and the DNA synthesis occurring in the polytene nuclei of prepupae is that of propagation of the final cycle. That conclusion is supported by the present study, in which radioautographs interpreted as characteristic of the initial phase of the DNA synthetic cycle are obtained until shortly after formation of the white prepupa, then abruptly cease to be produced. On the other hand, thymidine incorporation in radioautograph patterns believed to represent the latter part of the cycle is observed throughout the larval and prepupal periods, suggesting that a condition essential for initiation of polytenic replication is not obligatory for propagation. This report includes a description of the bases for assigning the various radioautograph patterns produced in the nuclei of a salivary gland to an ordered sequence in the replication cycle, and an analysis of the distribution of those patterns at successive larval and prepupal stages. The data are compatible with two possible interpretations: (1) The information for a component of a milieu supporting initiation of polytenic replication is transcribed only in the larval state; (2) the information for an inhibitor of initiation is transcribed in the prepupal state. (From abstr.)

- 338 Roels, H. "METABOLIC" DNA: A CYTOCHEMICAL STUDY. Int. Rev. Cytol. 19 (1966) 1-34.

Review article drawing on examples taken from studies which include plants and insects. The review is divided into sections on cell growth and nuclear DNA (embryonic development, regeneration and compensatory hypertrophy, tumour growth); cell function and nuclear DNA (plant cell nucleus, giant chromosomes of insects, nutrient cells, liver cell nuclei, exocrine glands, endocrine glands, target organs of the sex hormones); sites of gene activity in the interphase nucleus (nucleolus-associated chromatin, heterochromatin). Insect data are cited on p.13-14, 22-24, and 26.

- 339 Rudkin, G.T. NUCLEIC ACID METABOLISM IN GIANT CHROMOSOMES OF Drosophila melanogaster. Ann. Histochim., Suppl. 2 (1962) 77-84.

The total absorbancy of monochromatic u.v. radiation and the incorporation of  $^3\text{H}$ -cytidine and -thymidine were measured in short sections of giant chromosomes of salivary glands at two stages in development, with and without treatment with RNase. The ratio of RNA:DNA was found to vary from region to region; the rate of incorporation of cytidine was not correlated with the amount of RNA or DNA present in a region. The rate of RNA synthesis was found to change very rapidly with

time in a single region; a high rate of synthesis was correlated with the process of swelling in the formation of a puff. — The tritiated compound was either injected into the full grown larva (500  $\mu\text{Ci}/\text{ml}$ , specific activity 380  $\text{mCi}/\text{mM}$  or 1000  $\text{mCi}/\text{mM}$ ) or, in vitro, dissolved in a salt solution in which the salivary gland was placed (5  $\mu\text{Ci}/\text{ml}$ ).

- 340 Sevastyanova, G.A., Smolin, A.N. THE FREE NUCLEOTIDES AND THEIR DERIVATIVES IN INSECTS. Prog. mod. Biol. 61, 3 (1966) 321-337 (In Russian)

A review paper with 82 references. It deals with free nucleotides and nucleic acids in insects, free nucleotides and their derivatives as co-enzymes, and the luminescence of insects.

- 341 Simoes, L.C.G., Pavan, C. CHROMOSOME ACTIVITY IN THE SALIVARY GLAND OF Rhynchosciara MAINTAINED IN VITRO. p. 51 of "Biology Division Semiannual Progress Report for Period Ending July 31, 1966". ORNL-3999, Oak Ridge National Lab., Tenn. 1966, 217p.

Preliminary data suggest that in these chromosomes the synthesis of DNA starts independently in different bands, some of which are heterochromatic.

- 342 Sirlin, J.C.\* Nature, Lond. 211 (1966) 1347.

" - reported labelling with  $^{14}\text{C}$ -methionine of RNA components in Smittia larvae. He found that only the 4S peak of nucleolar RNA became labelled. This evidence, he suggested, supports the concept that the nucleolus synthesises 4S RNA which has a transfer-RNA function." (From a report by H. Busch on "The Cell Nucleus: Metabolism and Radiosensitivity", a meeting held at the Radiobiological Institute of the Organization for Health Research TNO in Rijswijk, Holland, May 9-11, 1966.)

\* This author is presumably identical with J.L. Sirlin, the "C" being the result of a misprint.

- 343 Sirlin, J.L., Jacob, J., Bimstiel, M.L. SYNTHESIS OF DIFFERENT SPECIES OF NUCLEOLAR RIBONUCLEIC ACID. Biochim. biophys. Acta 108 (1965) 718-718.

The different species of RNA newly synthesised in the nucleolus of fully-grown larval salivary glands of Smittia parthenogenetica are described. Salivary glands were incubated in vitro, with appropriate radioactive precursors. The sucrose-gradient sedimentation profile of the RNA shows typically a pattern of radioactive peaks ranging from 4 to > 28 S, while the heights of the intermediate peaks differ somewhat with the experiment. In glands treated with DRB and TRB (5,6-dichloro- and 4,5,6,-trichloro-1-(8-D-ribofuranosyl)-benzimidazole, respectively), the radioactivity is largely confined to the 4-S and > 28-S peaks, the relative height of which is variable. These two molecular species of newly synthesised RNA are therefore nucleolar, and represent a true nucleolar synthesis.

- 344 Sirlin, J.L. SYNTHESIS OF 4 S RNA IN THE NUCLEOLUS OF Smittia. p. 87-96 of "International Symposium on the Cell Nucleus: Metabolism and Radiosensitivity. Rijswijk (Z.H.), The Netherlands, 9-12 May 1966". London, Taylor & Francis Ltd, 1966, 345p.

The synthesis of nucleolar RNA in the larval salivary glands of a chironomid was isolated within the intact gland by means of specific inhibition. Quantitative autoradiography of RNA synthesis was carried out using  $^3\text{H}$ -uridine (91  $\mu\text{Ci}/\text{ml}$ ), either with or without chlorine-substituted benzimidazoles (DRB and TRB) present throughout. In autoradiography of RNA methylation, glands were first incubated with puromycin, then with methionine (methyl- $^{14}\text{C}$ ) (50  $\mu\text{Ci}/\text{ml}$ ) and puromycin. The picture of nucleolar synthesis proved to be identical with autoradiography of squashes or sections. Radioactive RNA extracted from glands in which nucleolar synthesis or methylation has been established autoradiographically is assigned to the nucleolus. Methylation of nucleolar RNA depends closely on synthesis. Parallel biochemical work indicates that part of the newly synthesised RNA is of high mol. weight, and part is of 4 S values with characteristics of transfer-RNA. Work on the latter RNA is discussed.

- 345 Sirlin, J.L., Jacob, J., Bimstiel, M.L. SYNTHESIS OF TRANSFER RNA IN THE NUCLEOLUS OF Smittia. Natn. Cancer Inst. Monogr. No. 23 (1966) 255-266 (discussion 266-270 (?) )

Larval salivary gland cells of the chironomid, S. parthenogenetica, were incubated with radioactive precursors of RNA. Sucrose gradient sedimentation patterns of newly synthesised RNA showed rRNA, ribosomal RNA, and RNA larger than 28S. That a high proportion of RNA synthesised in the larvae was in the nucleolus and that it was 4S RNA was concluded from several factors: (a) a high pro-

portion of RNA was synthesised when incubation was carried out in the presence of DRB and TRB (5, 6-dichloro- and trichloro-1- $\beta$ -D-ribofuranosylbenzimidazole, respectively), reagents which are known to allow only nucleolar synthesis; (b) when methylation was carried out, only 4S RNA was methylated and the nucleolus is known to be the predominant cell site of RNA methylation; (c) RNA synthesis was suppressed when actinomycin was used, and actinomycin suppresses nucleolar RNA synthesis. (CA 66:1967, 113432 g)

- 346 Spencer, J.B. NUCLEIC ACID AND PROTEIN SYNTHESIS IN SATURNIID PUPAE. Diss. Abstr. 27, 1 (1967) 40-B - 41-B.

In the present study the pattern of incorporation of  $^3\text{H}$ -labelled uridine and thymidine into the nucleic acids of a number of tissues of the giant silkworms Hyalophora cecropia and Samia cynthia was examined by autoradiographic procedures. The patterns of incorporation were recorded for diapausing pupae, injured diapausing pupae, and at different stages of adult development. Changes in the protein patterns were studied by electrophoretic separation of the blood serum at the same stages of the life cycle. Routine histological observations were also recorded for these animals. Autoradiographic analysis of DNA synthesis revealed that it occurs during adult development, but is suspended in all tissues except the blood cells and gonads during the diapause period. Injury which activates a number of biochemical systems held in abeyance during diapause, does not stimulate DNA synthesis in any tissues except the blood cells and those epidermal cells directly involved in the closure of the wound. Pulse-labelling experiments showed that resumption of DNA synthesis occurred in the wing epithelium at least 5 d before any visible morphological signs of development could be detected. Evidence which indicates that the resumption of DNA synthesis may be closely tied to ecdysone activation is discussed. It was shown that the synthesis of DNA does not resume synchronously in all tissues of the developing adult, but that each tissue resumes synthesis at a characteristic period. Not all tissues of the developing adult exhibit DNA synthesis, indicating that the hormonal environment at each moult may determine whether or not new DNA molecules are produced. The synthesis of RNA never seems to be suspended in any tissue examined, but injury and the onset of development cause an increase in the rate of RNA synthesis. Two distinct types of injury-stimulated response in the rate of RNA synthesis were detected. Incorporation patterns observed in tissues exposed to labelled uridine for periods longer than 2 h are consistent with present theories concerning the nuclear synthesis and subsequent transfer to the cytoplasm of RNA molecules. The incorporation of labelled uridine into the fat body tissues adjacent to the ovary was higher than for the fat body in other regions and this suggests that the ovary influences the metabolism of the surrounding fat body. The antibiotic actinomycin D when administered at the level of 0.5  $\mu\text{g/g}$  reduced the rate of RNA synthesis in the tissues of injured diapausing pupae, but was not lethal. The synthesis of a blood protein elicited by injury was, however, inhibited by the administration of actinomycin concurrently with the injury. Inspection of the zymograms of blood proteins and examination of the incorporation of labelled amino acids into other proteins revealed that actinomycin at this concentration did not prevent the injury-stimulated increase in the amount of other proteins which are normal constituents of the blood of diapausing pupae. This differential action of actinomycin suggests that gene loci already employed as templates for the synthesis of messenger RNA are protected by this activity whereas nonfunctional loci are not. Preliminary experiments indicated that the blood cells are one source of the injury-elicited protein, and that this protein is a characteristic component of the blood of the developing adult. Crystalline inclusions in the Malpighian tubules and subepidermal glands of developing adults are described. Neither type of crystal was chemically characterized, but those in the Malpighian tubules exhibited label after the animal had been injected with either labelled thymidine or uridine. (From DA)

- 347 Swift, H. NUCLEIC ACIDS AND CELL MORPHOLOGY IN DIPTERAN SALIVARY GLANDS. p. 73 of "The Molecular Control of Cellular Activity". Allen, J.M., Ed. New York, McGraw-Hill Book Company, 1962.

Review article. Work is cited on Drosophila, Sciara coprophila, Rhynchosciara, Chironomus, Glyptotendipes, grasshopper, etc. The DNA content of the chromosome, DNA and chromosome structure, proteins and chromosome structure, RNA-containing components, cytoplasmic protein synthesis, and implications of the work described are discussed. Radioisotopes were employed in numerous of the studies cited.

- 348 Takahashi, S. STUDIES ON RIBONUCLEIC ACID IN THE FAT BODY OF *Philosamia cynthia ricini* Donovan (LEPIDOPTERA) DURING DEVELOPMENT. *J. Insect Physiol.* **13** (1966) 789-801.

The fluctuation of RNA was determined using the fat body cells of *P. cynthia ricini* during the course of development from the 5th larval instar to the early pupal stage. The ratio of total RNA to DNA was high in the early 5th instar, but it decreased afterwards to reach a min. at pupation. This change was mainly accounted for by the reduction of RNA contained in the microsomal fraction. For the purpose of analysing further such changes in RNA, nucleic acids were isolated from the fat body cells by a slightly modified SDS-phenol method after injection of  $^{32}\text{P}$  into the body cavity. The sRNA\*, rRNA and DNA were fractionated by methylated serum albumin column chromatography. The ratio of sRNA to DNA was rather constant, while the ratio of rRNA to DNA decreased gradually during the progress of development. Incorporation of  $^{32}\text{P}$  into sRNA and rRNA took place only in the early stage of the 5th instar. It is concluded that the RNA which exists in the fat body cells in the later stages of the 5th instar and pupal stage was synthesised during the first few days in the 5th larval instar. (Auth.)

\* sRNA - soluble (transfer) RNA.  
rRNA - ribosomal RNA.

- 349 Thomas, K.K., Nation, J.L. RNA, PROTEIN, AND URIC ACID CONTENT OF BODY TISSUES OF *Periplaneta americana* (L.) AS INFLUENCED BY CORPORA ALLATA DURING OVARIAN DEVELOPMENT. *Biol. Bull.* **130** (1966) 442-449.

The concentrations of RNA and proteins were substantially lower in allatectomized females when compared with sham-operated females. Uric acid concentration remained approximately constant in allatectomized females, while in the controls it increased to more than double the concentration observed. The rate of incorporation of  $^3\text{H}$ -labelled amino acids into the fat body of allatectomized females was slow compared to that of the sham-operated roaches. A very slow incorporation into ovarian tissue was observed in allatectomized females, as against a rapid rate of incorporation in controls. Midgut tissue exhibited a significantly greater incorporation of the isotope than that of sham-operated females. The probable relation between corpora allata and the synthesis of RNA, proteins, and uric acid during ovarian development is discussed. (From auth. summary)

- 350 Vermeulen, C.W., Atwood, K.C. THE PROPORTION OF DNA COMPLEMENTARY TO RIBOSOMAL RNA IN *Drosophila melanogaster*. *Biochem. biophys. Res. Commun.* **19** (1965) 221-226.

Molecular hybridization experiments have indicated that ~0.3% of the DNA in *E. coli*, *E. megaterium*, and *Pisum sativum* is reserved for the specification of ribosomal RNA. Evidence is presented that a similar proportion of the DNA in *D. melanogaster* can be hybridized with r-RNA. The methods are described for preparing DNA (from Oregon-R wild-type flies) and RNA (when flies were allowed to oviposit on corn meal-molasses medium containing 1 mCi/ml  $^3\text{H}$ -uridine. Details are given. r-RNA-DNA hybridization was carried out under the conditions used by Yankofsky and Spiegelman (Proc. natn. Acad. Sci. U.S.A. **48**; 1962, 1089). The DNA appears to be saturated when ~0.25% of its weight in RNA has been hybridized.

- 351 Watanabe, H. LOCALIZATION OF RIBONUCLEIC ACID SYNTHESIS IN THE MIDGUT CELLS INFECTED WITH CYTOPLASMIC POLYHEDROSIS VIRUS IN THE SILKWORM, *Bombyx mori* L. (LEPIDOPTERA; BOMBYCIDAE). *Appl. Ent. Zool.* **1**, 3 (1966) 154-155.

An autoradiographic technique is described to locate the site of viral RNA synthesis within the midgut cells infected with cytoplasmic-polyhedrosis virus in the silkworm. Fourth-instar larvae of several strains were inoculated perorally with the virus. On the 2nd and 4th day after virus inoculation infected and control larvae were injected with 1 mCi/ml generally labelled  $^3\text{H}$ -uridine; 5-h labelling periods were used. A significant difference was observed in the incorporation of RNA-precursor by healthy and virus-diseased cells. The nucleus appears to be the site of viral RNA synthesis in infected midgut cells. Since the virus particles and the polyhedra are only observable in the cytoplasm with the electron microscope, viral RNA produced in the nucleus is interpreted as being released into the cytoplasm in order to gain its coat of protein.

- 352 Watanabe, H. AUTORADIOGRAPHIC STUDIES ON THE NUCLEIC-ACID SYNTHESIS IN THE FAT BODY AND SOME TISSUES OF THE SILKWORM, *Bombyx mori* L. (LEPIDOPTERA: BOMBYCIDAE), INFECTED WITH NUCLEAR-POLYHEDROSIS VIRUS. *Appl. Ent. Zool.* **2**, 3 (1967) 147-157.

The patterns and changes of nucleic acid synthetic activity during the course of nuclear-polyhedrosis were demonstrated in the fat body and some other tissues of the silkworm larva (*B. mori* L.) by means of autoradiography with  $^3\text{H}$ -thymidine and uridine as nucleic acid precursors. The results indicated that both activities of DNA and RNA syntheses in the infected nucleus of the fat body increased progressively up to a point just prior to the polyhedra development. Beyond this period, there was a sudden breakdown of DNA synthesis, while the activity of RNA synthesis decreased gradually with the polyhedral growth. Some of the newly synthesised RNA in the diseased nuclei seemed to be adsorbed onto polyhedra during their formation. Essentially the same pattern of nucleic-acid synthesis was noted in the other infected tissues such as hypodermis, muscle and tracheal epithelium. (Auth.)

- 353 Watanabe, H. SITE OF VIRAL RNA SYNTHESIS WITHIN THE MIDGUT CELLS OF THE SILKWORM, *Bombyx mori*, INFECTED WITH CYTOPLASMIC-POLYHEDROSIS VIRUS. *J. Invertebrate Path.* **9** (1967) 480-487.

The pattern of nucleic acid synthetic activity within the midgut cells of healthy larvae of the silkworm, *B. mori*, and of larvae infected with the cytoplasmic-polyhedrosis virus was demonstrated by means of autoradiography with labelled nucleic acid precursors. 5 h after injection of uridine- $^3\text{H}$ , the healthy cells generally incorporated the labelled material into cytoplasmic RNA and partly into nucleic RNA, whereas the diseased cells on the 2nd to the 5th day after virus inoculation incorporated much of the labelled uridine into nucleic RNA and some into cytoplasmic RNA. In the nuclei of virus-infected cells, the nucleic label appeared most densely over the nucleoli. Thus the distinct difference in the uptake of the RNA precursor between the healthy and infected cells indicated that the nucleolus of the infected cell may be a site of the viral RNA synthesis. Autoradiograms with thymidine- $^3\text{H}$  revealed no essential difference in the pattern of DNA synthesis between healthy and diseased midguts, and only a few cells incorporated the labelled material into their nuclei. At the late stage of virus infection, however, when some infected midgut cells eventually degenerated, there was a slight increase in the nucleic label in the newly generated cells. (Auth.)

- 354 Wattiaux, J.M., Lamborot, M. INFLUENCE OF AGING ON THE RATE OF INCORPORATION OF TRITIATED THYMIDINE IN THE NURSE CELLS OF *Drosophila melanogaster*. *Curr. Mod. Biol.* **1**, 1 (1967) 5-8.

The rate of incorporation of thymidine- $^3\text{H}$  in polyploid nurse cells was studied in 3-d-old and 8-week-old inbred Nettlebed strain of *D. melanogaster*. The ovaries were labelled by 10 min incubation at  $25^\circ\text{C}$  in a solution containing  $0.17\text{ M NaCl}$ ,  $0.005\text{ M KCl}$ ,  $0.002\text{ M CaCl}_2$ , and  $5\text{ }\mu\text{Ci}$  of thymidine- $^3\text{H}/\text{ml}$ . The speed and probably the duration of DNA synthesis increased with the degree of ploidy in both young and old flies. Old flies were less heavily labelled than young flies; this might be due to an increase of precursor pool size or a reduction of DNA synthesis with age. (CA 67:1967, 30173j)

- 355 Williams, C.M. DNA SYNTHESIS AND HORMONAL CONTROL OF INSECT METAMORPHOSIS. *Science*, N.Y. **148** (1965) 870. Paper presented at the "Annual Meeting of the National Academy of Sciences, Washington, D.C., USA, 26-28 Apr. 1965".

The epidermal tissues of diapausing pupae show no detectable DNA synthesis even after months of exposure to  $^3\text{H}$ -thymidine. By contrast, very rapid synthesis occurs in synchrony with the termination of diapause and the initiation of adult development. The synthesis of DNA is, therefore, one of the most impressive biochemical changes associated with the action of the prothoracic gland hormone (ecdysone) in terminating the pupal diapause. DNA synthesis is known to require formation of the triphosphate derivatives of all four deoxyribonucleosides. Consequently, by virtue of its inhibition of the enzyme thymidylate synthetase, the compound 5'-fluoro-2'-deoxyuridine (FUDR) is recognized as a potent inhibitor of DNA synthesis. Diapausing pupae of the cecropia silkworm show almost unparalleled resistance to FUDR, and are seemingly undisturbed by injection of 5 mg into 5 g pupae. However, the drug singles out and apparently destroys all spindle-shaped haemocytes, one of the few cell types which normally continues to synthesize DNA during diapause: it also blocks the healing of integumentary wounds. When ecdysone is secreted to provoke the termination of diapause and the initiation of adult development, the insect becomes extremely sensitive to FUDR: a single