

VI.

RADIATION STUDIES: SPECIFIC EFFECTS

EFFECTS OF RADIATION ON GERM CELLS OF INSECTS: DOMINANT LETHALS, GAMETE INACTIVATION AND GONIAL-CELL KILLING

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Abstract — Résumé — Аннотация — Resúmen

EFFECTS OF RADIATION ON GERM CELLS OF INSECTS: DOMINANT LETHALS, GAMETE INACTIVATION AND GONIAL-CELL KILLING. Radiations and chemical mutagens kill cells in numerous ways: by one of several kinds of induced dominant lethality, by a direct inactivation of function as with sperm, and by genetically undefinable types of death which may or may not be related to dominant lethality *per se*. Also, chemical mutagens appear to exert a curious enhancement of the fertilizing capacity of sperm.

The different stages of oögenesis and spermatogenesis respond with unequal sensitivity to radiation, and individual cells pass through stages conferring as much as a 50-fold difference in sensitivity.

Where species of Diptera, Hymenoptera and Coleoptera can be compared, a striking similarity of response to radiation can be observed, both to stage sensitivity and degree of response with dose. The silkworm, *Bombyx mori* (Lepidoptera), seems to be similar in most respects to representatives of the other orders in response of germ cells to radiation, but differs sharply in types of dominant lethality induced. Species having atypical genetic mechanisms (e.g., the lecanoid system of *Planococcus citri* (Hemipt.: Coccidae) are special cases, and their responses to radiation are considerably modified from those of other species.

For insect population control by the irradiation-of-male method, dominant lethality is as advantageous in species where matings are multiple as in species where mating occurs once. Sperm inactivation and gonial killing can be regarded as instances of true sterility and are maximally effective only in species where mating occurs once. For most efficient control, doses should be chosen which would induce maximum dominant lethality, minimum sperm inactivation and complete killing of gonial cells. These parameters are simple to determine by gamete viability measurements, irradiated and unirradiated population competition experiments and histological examination of gonidia.

EFFETS DES RAYONNEMENTS SUR LES CELLULES GERMINALES DES INSECTES: LÉTALITÉ DOMINANTE, INACTIVATION DES GAMÈTES ET DESTRUCTION DES CELLULES DES GONADES. Les rayonnements et les agents chimiques de mutation détruisent les cellules de nombreuses façons: par l'un des modes d'induction d'une létalité dominante, par l'inactivation directe d'une fonction et par des manières génétiquement indéfinissable, liées ou non à la létalité dominante. Les agents chimiques de mutation paraissent également provoquer un accroissement curieux du pouvoir fertilisant du sperme.

La radiosensibilité varie avec les divers stades de l'oogenèse et de la spermatogenèse; certaines cellules passent par des stades au cours desquels leur radiosensibilité peut varier dans un rapport de 1 à 50.

Des comparaisons entre des diptères, des hyménoptères et des coléoptères ont révélé une similitude frappante dans la manière dont ces espèces réagissent aux rayonnements, tant en ce qui concerne la sensibilité aux divers stades de développement que l'intensité de la réaction en fonction de la dose. Le ver à soie *Bombyx mori* (lépidoptère) semble avoir beaucoup de points communs avec les représentants des autres ordres en ce qui concerne l'effet des rayonnements sur les cellules germinales, mais il en diffère nettement pour ce qui est des facteurs létaux dominants induits. Les espèces présentant des mécanismes génétiques anormaux (par exemple, le *Planococcus citri*, coccidé hémiptère) constituent des cas particuliers et ils réagissent aux rayonnements de façon très différente des autres espèces.

Dans la lutte contre les insectes par la méthode de l'irradiation des mâles, la létalité dominante est aussi efficace pour les espèces où l'accouplement a lieu plusieurs fois que pour celles où il a lieu une seule fois. On peut considérer l'inactivation du sperme et la destruction des cellules des gonades comme des facteurs

de stérilité réelle; leur efficacité est maximum chez les espèces où l'accouplement n'a lieu qu'une seule fois. Pour le contrôle le plus efficace, il faudrait avoir recours à des doses provoquant la létalité dominante maximum, l'inactivation minimum du sperme et la destruction totale des cellules des gonades. Il est facile de déterminer ces paramètres en calculant la viabilité des gamètes, au moyen d'expériences comparatives avec des populations non irradiées ainsi que par l'examen histologique des gonades.

ВОЗДЕЙСТВИЕ РАДИАЦИИ НА ПОЛОВЫЕ КЛЕТКИ НАСЕКОМЫХ: ДОМИНАНТНЫЕ ЛЕТАЛИ, ИНАКТИВАЦИЯ ГАМЕТ И УМЕРЩВЛЕНИЕ ПОЛОВЫХ КЛЕТОК. Излучения и химические мутагенные вещества умерщвляют клетки различными путями: одним из видов образования доминантных леталей, непосредственной инактивацией функции, как, например, в отношении спермы, и генетически неопределимыми видами смерти, которые могут быть связаны или не связаны с доминантной летальностью. Химические мутагены производят, по-видимому, необычайное усиление оплодотворяющей способности спермы.

Различные стадии оогенеза и сперматогенеза реагируют с неодинаковой чувствительностью на облучение, и разница в чувствительности отдельных клеток на некоторых стадиях развития достигает 50 раз.

Там, где представляется возможным сравнивать двукрылых, перепончатокрылых и жесткокрылых насекомых, можно наблюдать замечательное единообразие реакции на облучение как по уровню радиочувствительности на определенной стадии, так и по зависимости степени реакции от дозы. Шелкопряд, *Bombyx mori* (отряд чешуекрылых), представляется похожим в большинстве отношений на представитель других отрядов по реакции половых клеток на радиацию, но резко отличается по типам индуцированных доминантных леталей. Виды с атипичным генетическим механизмом (например, леканоидная система *Planococcus citri* (отряд настоящих полужесткокрылых *Coccidae*)) представляет собой особые случаи, и их реакция на облучение значительно отличается от реакции насекомых других видов.

При истреблении популяций насекомых облучением самцов доминантная летальность представляется столь же перспективной для видов с однократным спариванием, как и для видов с многократным спариванием. Инактивацию спермы и умерщвление половых клеток можно считать примерами получения истинной стерильности, наиболее эффективной для видов, где спаривание происходит однократно. Для наиболее эффективного истребления должны выбираться такие дозы, которые ведут к получению максимальной доминантной летальности, производят минимальную инактивацию спермы и вызывают полное умерщвление половых клеток. Эти параметры легко поддаются определению посредством измерения жизнеспособности гамет, экспериментами с конкуренцией между облученными и необлученными популяциями, а также гистологическим исследованием гонад.

EFFECTOS DE LAS RADIACIONES SOBRE LAS CÉLULAS GERMINALES DE LOS INSECTOS: ELEMENTOS LETALES DOMINANTES, INACTIVACIÓN DE LOS GAMETOS Y EXTERMINACIÓN DE LAS CÉLULAS GONÁDICAS. Las radiaciones y los productos químicos mutágenos pueden matar las células de modos diversos, a saber: mediante la inducción de uno o varios tipos de elementos letales dominantes, por inactivación directa de una función, como la producción de esperma, y por procedimientos genéticamente indefinibles de exterminación que pueden o no estar relacionados con la letalidad dominante intrínseca. Por otra parte, los productos químicos mutágenos parecen provocar un curioso aumento de la capacidad fertilizante de la esperma.

La radiosensibilidad varía según las diferentes etapas de la oógenesis y de la espermatogénesis, y las células pasan por fases en que dicha sensibilidad llega a ser 50 veces superior o inferior que en otras.

En la medida en que distintas especies de dípteros, himenópteros y coleópteros son comparables, puede observarse una sorprendente analogía en la radiosensibilidad, tanto según las etapas como según las dosis. La radiosensibilidad de las células germinales del gusano de seda *Bombyx mori* (Lepidópt.) parece similar en muchos aspectos a la de los insectos de otros órdenes, pero en cambio hay grandes diferencias en cuanto a los tipos de elementos letales dominantes que en él pueden inducirse. Las especies con mecanismos genéticos atípicos (por ejemplo, el sistema lecanioideo del *Planococcus citri* (Hemípt.; *Coccidae*) constituyen casos especiales y su radiosensibilidad varía considerablemente en relación con la de otras especies.

Para la lucha contra los insectos por el método de irradiación de los machos, la creación de un elemento letal dominante ofrece en las especies donde las cópulas son múltiples las mismas ventajas que en las especies que copulan sólo una vez. La inactivación de la esperma y la exterminación de las células gonádicas pueden

considerarse como casos de auténtica esterilización que alcanzan su máxima eficacia sólo en las especies que copulan una vez. Para que la lucha contra los insectos sea eficaz hay que aplicar dosis que produzcan un factor letal dominante máximo, una inactivación mínima de la esperma y una exterminación completa de las células gonádicas. Estos parámetros se pueden determinar fácilmente con la medición de la viabilidad de los gametos, los experimentos con poblaciones irradiadas y no irradiadas y el examen histológico de las gonias.

INTRODUCTION

Dominant lethality, sperm inactivation, and gonial and gametocyte killing have been studied more in insects than in any other group of animals. These types of damage can be measured by counting the proportion of viable offspring from laid eggs, or by studying changes in sex ratio in parthenogenetic species. Cytological and genetic investigations can be used to augment simple dose-survival studies.

TYPES OF RADIATION DAMAGE

Through cytogenetic analyses, the different known types of dominant lethal, inactivation, and killing events during gametogenesis have been classified; these are summarized in Table I. Discussions of each of these types of radiation damage can be found in the references cited in the Table. There are two broad categories of lethal damage to gametes, induced dominant lethality and induced sterility. Dominant lethality is an effect on a sperm or egg that will kill the zygote some time during development. Sterility derives from the killing or inactivation of a gamete, or a cell that will become a gamete, so that it will no longer participate in formation of a zygote. As will be seen, the most common type of dominant-lethal effect is the Type I damage, that causing death of the embryo very early in development. This particular type is undoubtedly an expression of numerous kinds of damage, and these seem to vary in proportion from one mutagenic agent to the other. Probably cell killing itself, which causes depopulation of the gonial region of the testes, is mainly brought about by the same type of events that cause death early in development [1]. The Type IV damage, which results in immediate death, appears to be manifested only in certain kinds of cells. Cells such as lymphocytes, neuroblasts, young primary gametocytes, and late or secondary gonial cells respond by becoming pyknotic and dying relatively soon after irradiation without completing a mitotic division.

Recognizable sperm inactivation seems to occur in insects at doses beyond that dose needed to induce 99% dominant lethality. In the parthenogenetic Hymenoptera, sperm inactivation by radiation or exposure to chemical mutagens can be detected easily (Fig. 1). At low doses, hatchability is first reduced through induction of dominant lethality; thus fertilized eggs are killed. As the dose increases, hatchability returns to a higher level. The degree of return signifies the extent of sperm inactivation, because now the eggs are producing only haploid males. In species where fertilization is obligate, sperm inactivation is difficult to detect. The relative degree

TABLE I

TYPES OF DOMINANT LETHAL, CELL KILLING AND SPERM INACTIVATION EVENTS INDUCED BY RADIATION OR CHEMICAL MUTAGENS IN MATURE GAMETES AND DURING GAMETOGENESIS

Type	Effect	Probable basis	References
Ia	Death very early in development	Mitotic inhibition through chromosome bridge formation	[1]
Ib	Death very early in development	Mitotic inhibition induced by ultraviolet and repairable by photoreactivating light	[49]
Ic	Death very early in development	Mitotic inhibition repairable by presence of another nucleus (genetically non-transmissible damage, not dominant lethality <u>sensu stricto</u>)	[9, 50-52]
Id	Death very early in development	Mitotic inhibition, non-repairable, but cause unknown	[51]
II	Late embryonic death before hatching in unfertilized eggs of <u>Habrobracon</u> and after hatching in fertilized eggs	Genetic death, through loss of chromosome parts needed for normal development	[21, 50]
III	Late embryonic death before hatching in both unfertilized and fertilized eggs of <u>Habrobracon</u>	Genetic death, through loss of chromosomes or drastic loss of chromosome parts	[21, 50]
IVa	Causes sterile period. Cells die before going through division. Death at G ₂ to early prophase.	Unknown	[50, 53, 54]
IVb	Causes sterile period. Cells die just after going through division. Death at anaphase or early telophase.	Unknown	[54]
V	Dominant lethality induced in gonial cells	Possibly destruction of gene function or enhancement of normal aging processes	[9, 12, 50, 55]
VI	Sperm inactivation. Causes male sterility.	Unknown	[2-4, 36-38, 56, 57]

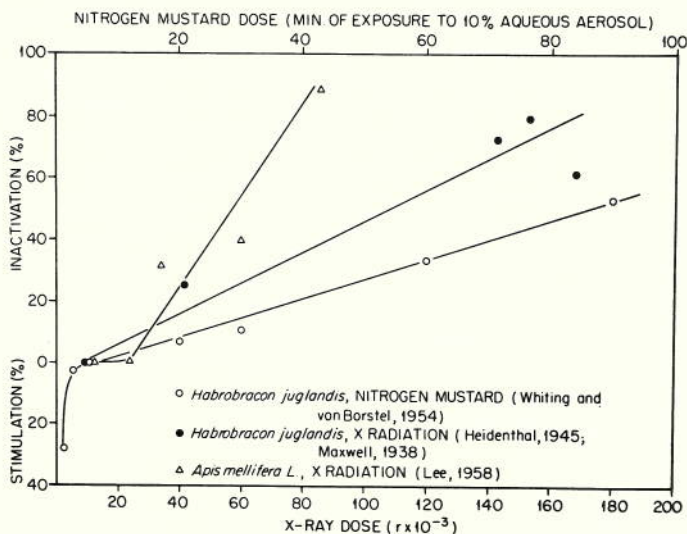


Fig. 1

Dose-inactivation curves of Hymenopteran sperm [36, 38, 56, 57]

Inactivation is measured as an increase in frequency of parthenogenetic embryo development to hatching, or maturity. At low doses, nitrogen mustard enhances the fertilizing capacity of the sperm.

of successful inseminations [2], and sperm competition experiments [3] indicate that sperm of the fruit fly, *Drosophila melanogaster*, are inactivated by radiation. These experiments should be repeated at higher doses. It has been shown in the white pine weevil, *Pissodes strobi*, [4] that virgin females mated with males irradiated at 10 000 and 20 000 r produced no viable offspring, whereas females previously mated with unirradiated males and again mated with males receiving the same doses produced more viable offspring at the higher dose, indicating inactivation of the irradiated sperm. Thus there was inefficient sperm competition by the sperm irradiated at 20 000 r.

The major difficulty in interpreting effects that can be ascribed to inactivation of sperm is that it is simulated by lack of sexual vigour of males, a response that could possibly be elicited by radiation. The problem is whether the competition is among males or among sperm. In parthenogenetic Hymenoptera the answer is clearly at the level of the sperm.

THE STIMULATING EFFECT OF ALKYLATING AGENTS ON SPERM

The same problem of competition among sperm or males exists also when alkylating agents, such as nitrogen mustard or some of the "chemosterilants" are used (Fig. 1). An interesting effect occurs at low doses of nitrogen mustard, "low" still being a dose that gives over 99% dominant lethality. This effect is one of apparent stimulation of the treated sperm to fertilize more eggs than normally would be fertilized. A similar effect ascribed to increased sexual vigour of males was found among house-flies,

Musca domestica (L.), treated with alkylating agents [5]. In any event, this effect, which enhances the fertilizing capacity of sperm in *Habrobracon* and the fertilizing capacity of sperm or mating competitiveness of males in house-flies, occurs beyond the 99% induced dominant lethal dose to sperm. For population control, this effect, properly employed, could be a powerful weapon.

DIFFERENTIAL SENSITIVITY OF MEIOTIC STAGES

This topic has been reviewed by WHITING [6] and has been the subject of a recent symposium [7, see especially 8]. The reasons for differential sensitivity are unknown, but action of repair mechanisms or presence of radical trapping agents at some stages and not at others seem to be among the most likely suggestions [8, 9].

Oogenesis

The differential sensitivity of the first meiotic metaphase and prophase of *Habrobracon juglandis* oocytes to two different alkylating agents and to X-radiation are shown in Fig. 2. It can be seen that in each instance metaphase-I oocytes are considerably more sensitive than are prophase-I oocytes. This shows that the phenomenon of differential sensitivity may well be of a general nature; i. e. the relative sensitivity of a given type of meiotic cell to certain mutagenic agents (particularly those other than purine and pyrimidine analogues) may be independent of the mutagen but dependent on the stage of the chromosome cycle [cf. 10].

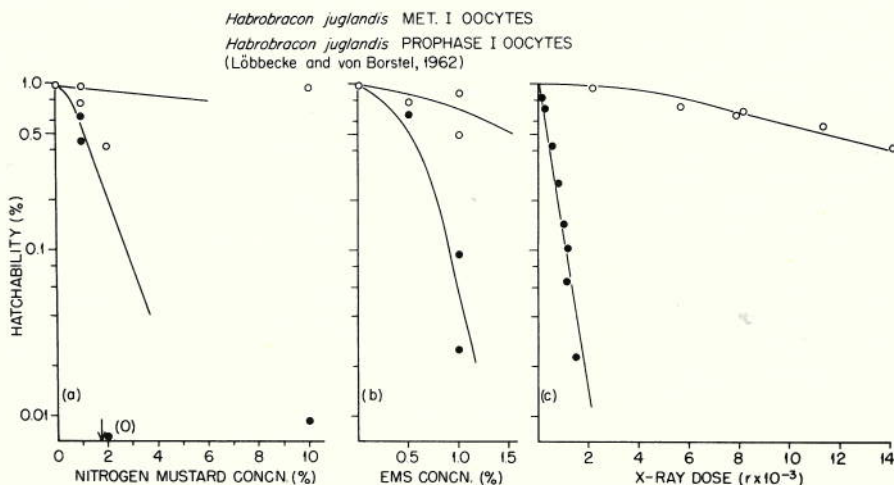


Fig. 2

Dose-hatchability curves for *Habrobracon juglandis* metaphase I and prophase I oocytes treated with (a) nitrogen mustard, (b) ethyl methanesulphonate (EMS), and (c) X-radiation [51].

●, metaphase I
○, prophase I

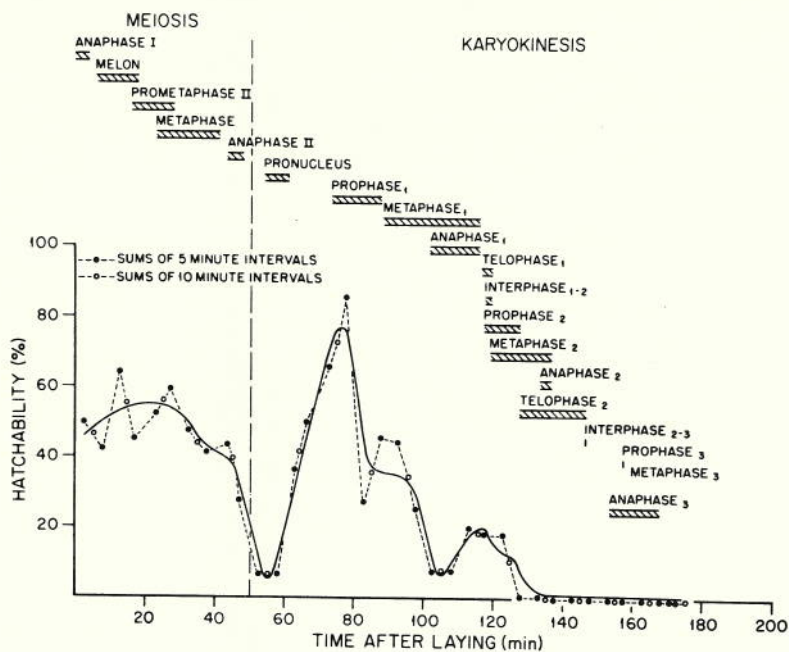


Fig. 3

Hatchability of *H. juglandis* embryos irradiated with 500 r at different times after laying [9]

Horizontal bars represent stages of meiosis and karyokinesis observed in embryos fixed at different times after laying.

---●---, sums of 5-min intervals

---○---, sums of 10-min intervals

The large magnitude of changes in radiation sensitivity with the meiotic stage in *Habrobracon* can be adjudged by comparing the resistance of the first meiotic prophase, shown in Fig. 2c, with the extreme sensitivity of the second meiotic anaphase and first mitotic telophase in Fig. 3. There is about a 60-fold difference in sensitivity between these stages but complete dose-action data are needed before an accurate quantitative difference in sensitivity can be assigned. Unmated females only were utilized in the *Habrobracon* experiments reported here, so the differences in sensitivity are a reflection not only of the different types of dominant lethality, but also of recessive lethality and genetically non-transmissible damage as well. Dominant lethality is known to account for at least 80% of the killing at all stages in *Habrobracon*.

That the sensitivity difference between metaphase I and prophase I occurs elsewhere in insects than in *Habrobracon* can be seen in Fig. 4. There is a striking similarity between the fruit fly and the screwworm in responses of metaphase I and prophase I oocytes [11, 12] to that of *Habrobracon* (Fig. 2c). In *Drosophila* literature and in the Figure these are referred to as stages 14 and 7 [13]. Cytological studies have not yet been thorough enough to determine if stage 14 is explicitly in the first meiotic metaphase, although it appears certain that stage-7 oocytes are equivalent to the prophase-I

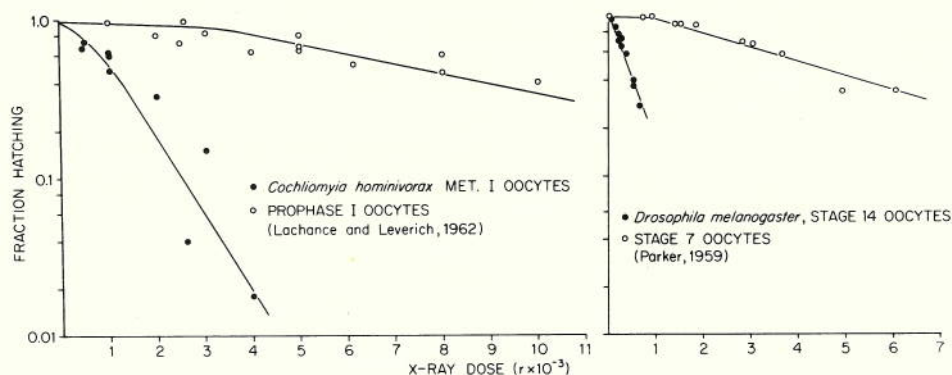


Fig. 4

Dose-hatchability curves for Dipteran oocytes irradiated in the first meiotic metaphase and prophase
[Redrawn from [11, 12]]

Note similarity of stage sensitivity responses to *H. juglandis* (Fig. 2c).

oocytes listed for the other species. For stage 14, it is known that the nuclear membrane has disappeared, leaving a "karyosphere," a feulgen-positive body about the same diameter ($2\mu\text{m}$) as the entire group of metaphase-I chromosomes ($2.8\mu\text{m}$) in *Habrobracon* [27]. The striking similarity in the stage response to other species indicates indirectly that this stage may be the equivalent of metaphase I.

In *Cochliomyia*, data are also available for the dose-response of the first meiotic anaphase [12]. This stage has essentially the same sensitivity as the first meiotic metaphase, which is similar to the findings in *Habrobracon* (Fig. 3).

Apparently, similar oocyte stage responses also exist in the fungus gnat, *Sciara coprophila* [15], and the chalcid wasp, *Mormoniella vitripennis* [16, 17], but the data are limited and dose-survival curves cannot be plotted from the available data.

The dose-response of mature oocytes of the silkworm, *Bombyx mori* L. [18], presents certain difficulties for interpretation. The dominant lethal curve is convex upward to a marked degree (Fig. 5). Also the dose-hatchability data indicate, over the first part of the curve at least, that *Bombyx* metaphase I oocytes are more resistant than metaphase I oocytes of other insect species. One possible reason for this may be that the contracted chromosomes are not yet in a completely defined metaphase stage, since even though the chromosomes are discrete, the spindle has not yet formed [19, Figs. 133, 134]. A more likely reason for the different stage response lies in the observation of TAZIMA [20] that most dominant lethals induced in *Bombyx* cause death late in embryonic development. This is quite the reverse of that found in other insect species, and may set the Lepidoptera apart from other insect orders. A comparative radiobiological survey of the time of action of induced dominant lethals is needed.

Fig. 6 shows that the preponderant frequency of dominant lethality induced in oocytes of two different wasps, a braconid and an ichneumonid, results in death early in development [21]. The upper curves in each case represent 1.0 minus the proportion dying during early development. In

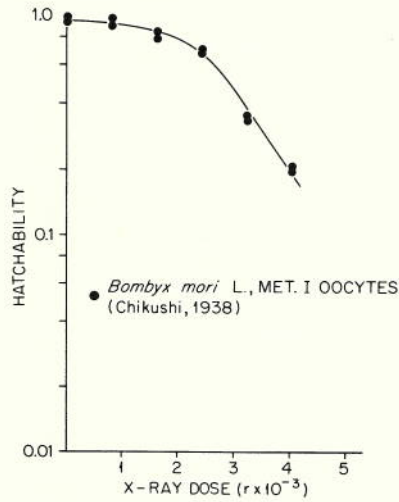


Fig. 5

Dose-hatchability curves for silkworm oocytes irradiated in the first meiotic metaphase [18]

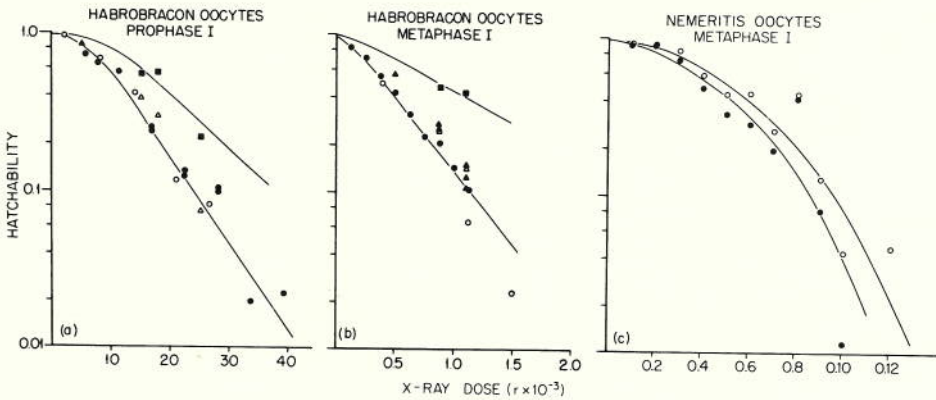


Fig. 6

Dose-hatchability curves for Hymenopteran oocytes

The upper curve in each case represents the fraction of induced dominant lethals that act early in development.

Hatchability data for (a) and (b) are from refs: [22] ●; [23] ○; [24] ▲; [21] △

■ 1.0 minus the proportion dying before blastoderm formation [21]

Hatchability data for (c) are from:

● Speicher and Speicher, unpublished data

○ 1.0 minus the proportion dying before blastoderm formation

Habrobracon irradiated at metaphase I, about half of the induced dominant lethality is expressed late in development. This group is composed of a mixture of dominant and recessive lethals (since the hatchability curve is obtained from irradiated virgins) [21-24], whereas the early death group contains all dominant lethals. Embryo recessive lethal mutations act only

after blastula formation and the early death group never reach the blastula stage. In *Nemeritis*, all deaths are from induced dominant lethality and almost all cause death early in development. *Nemeritis canescens* is arrhenotokous, virgin females producing only virgin female offspring.

Spermatogenesis

Many different facets of stage response during spermatogenesis have been examined in detail in *Drosophila melanogaster* [7]. Many of these are summarized in Fig. 7. All of the induced events have peaks that occur during spermatogenesis (as shown by successive matings of males on days following X-irradiation). Of interest to us is that dominant lethality is the broadest peak, comprising all the other types of analysable damage. This also indicates the possible heterogeneity of origin of induced dominant lethality, coming from a variety of phenomena associated with chromosomal breakage events as listed in Table I. This peak covers not only the spermatocyte divisions but the spermatid stage as well.

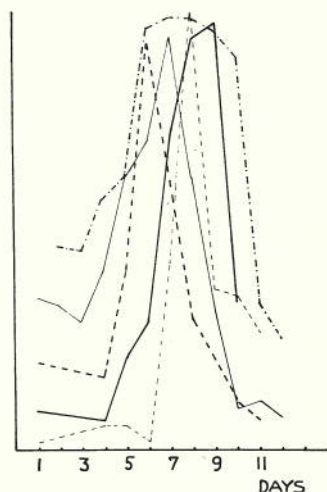


Fig. 7

Sensitivity patterns for different types of radiation effects at different stages of spermatogenesis in *Drosophila melanogaster* [29]

- Dominant lethals [25]
- XXO females [26]
- Recessive lethals [27]
- Translocations [26]
- Non-disjunction females [28]

Stage sensitivity during spermatogenesis for dominant lethality has also been observed in *Tribolium* (Fig. 8) by McDONALD [30]. He compares the dominant lethal peaks in *Tribolium confusum* at two different doses with the dominant-lethal peak observed in *Drosophila virilis* [31]. It is not known whether the same stages are the most sensitive, but the peak is large enough, as in Fig. 7, to encompass a considerable portion of spermatogenesis.

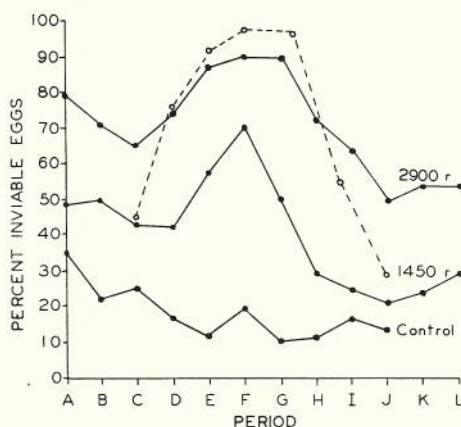


Fig. 8

A comparison of the radiation damage in the male germ cells of *Tribolium confusum* (solid lines) and *Drosophila virilis* (broken line) [30]. (The *Drosophila* data were taken from [31])

When males are irradiated, after the cells going through spermatogenesis are exhausted, a sterile period sets in. The sterile period indicates that early spermatocytes and secondary spermatogonia have been killed, as seen in Table II [from 44]. At lower doses, the spermatogonial region is not excessively depopulated and spermatogenesis is restored with consequent full fertility of the males. At higher doses (about 10 000 r in *D. melanogaster*), all cells in the gonial region are destroyed, and the male becomes completely sterile after the sperm supply is exhausted.

Sterile periods during spermatogenesis have also been observed in *Bombyx* by SADO [35]. Here the slow and fairly well synchronized development of gametic cells makes possible a separation of stages close to one another. He notes the good agreement in time of occurrence of cell killing during gametogenesis among species of Diptera, Lepidoptera, and Orthoptera.

Sperm

Induced dominant lethality has been investigated more in sperm than in any other cell type. Certainly sperm represent an extremely uniform population of cells that can be easily assayed.

In species of Diptera and Hymenoptera [21, 36] that have been studied by hatchability techniques there is a striking similarity of sensitivity to the X-radiation (Fig. 9). Here also it can be seen that the type of dominant lethality most often induced is that which results in death of the embryo early in development.

In some Hymenopteran species where it is difficult to record hatchability (such as those species where eggs are laid inside the host), accurate dose-survival curves can be formulated by observing the shift in sex ratio among progeny of the irradiated wasps. Curves plotted from data collected on two different chalcid wasps [37, 37a] and on *Habrobracon* [38] are shown in Fig. 10.

TABLE II

HISTOLOGICAL CONDITION OF DROSOPHILA TESTIS AFTER ADMINISTRATION OF 4000 r OF X-RAYS TO ADULT MALES*

Days after irradiation	Gonial region	Primary spermatocytes	Spermiogenic cysts
2½	+	++	+++
3	++	+	+++
4	+++	0	+++
5	+++	++	+
6	+++	+++	0
7	+++	++++	0
8	+++	++++	0
9	+++	+++	0
10	+++	+++	+
11	+++	+++	+
12	+++	+++	+
13	+++	+++	++
14	+++	+++	++
15	+++	+++	+++

* The normal condition of a testis is designated by +++ [From 32].

Most dominant lethality is expressed in the embryo; this accounts for the similarity of slopes in Figs. 9 and 10 even though different parameters – on different species – are measured.

The silkworm Bombyx exhibits a dose-response for sperm (Fig. 11) that is strongly convex upward [39, 40]. This resembles the metaphase-I oocytes with respect to shape of the curve and also with respect to a general increase in resistance when compared with species in other insect orders. Again, few of the dominant lethals are expressed early in development [20].

A study of induced "sterility" has been performed on the codling moth, Carpocapsa pomonella L. [33]. Although the data as presented are difficult to assess, one gains the impression that the codling moth is like Bombyx in that the sperm are comparatively resistant to ionizing radiation. Similarly, the sperm of the European corn borer, Ostrinia nubilalis Hbn., are extremely resistant to radiation compared to wasps and flies [34]. Except possibly for the lack of early deaths in embryos carrying a dominant-lethal event, the basis of the sensitivity difference is not known. However, among insects, it appears that the Lepidoptera can be set apart from the other orders.

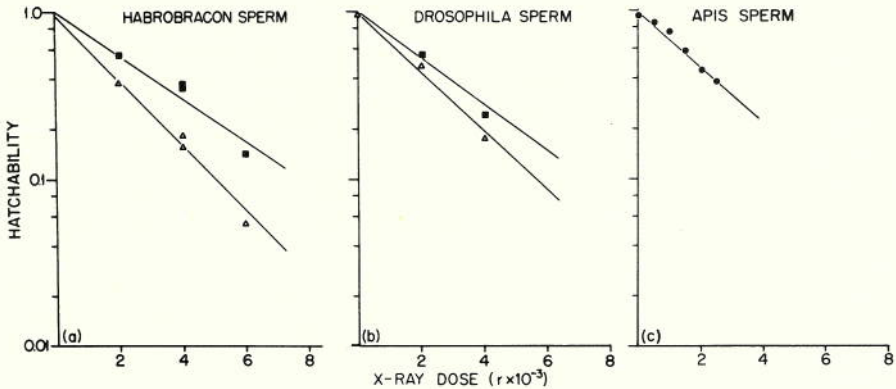


Fig. 9

Dose-hatchability curves for irradiated sperm of the wasp, Habrobracon; the fruit fly, Drosophila melanogaster; and the honeybee, Apis. The upper curve in two of the figures represents the fraction of induced dominant lethals that act early in development. Hatchability data for (a) and (b) are from [21] Δ , for (c) from [36] \bullet . \blacksquare 1.0 minus the proportion dying before blastoderm formation

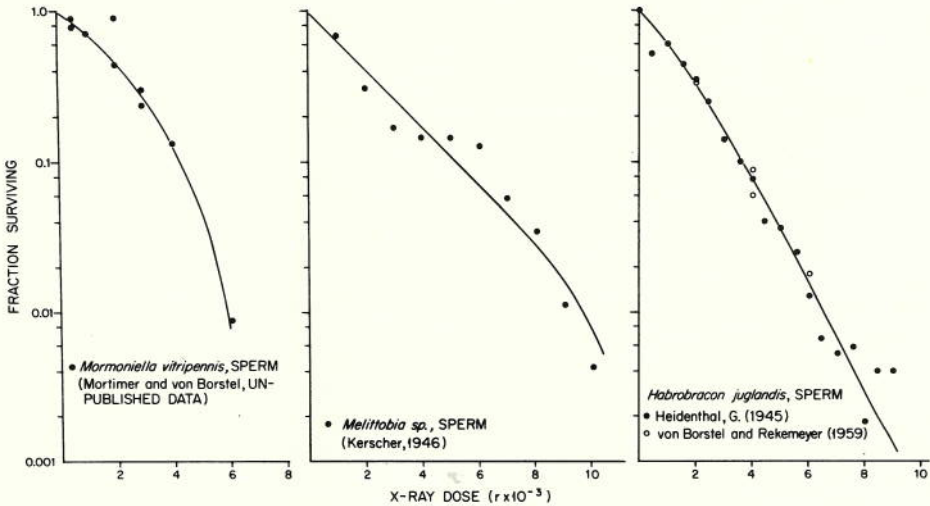


Fig. 10

Dose-survival curves for irradiated sperm of two chalcid wasps and one braconid wasp, computed from adult survival data where a sex-ratio shift was the criterion of induced dominant lethality [37, 37a, 38]

A considerable number of radiation experiments have been done on the Coleopteran Tribolium [30, 41-43]. There seems to be much variation in dose-response relations as measured by the different investigators for the two different species. It is unfortunate that the primary data are published only in one of the papers [34], so that inherent differences in methodology

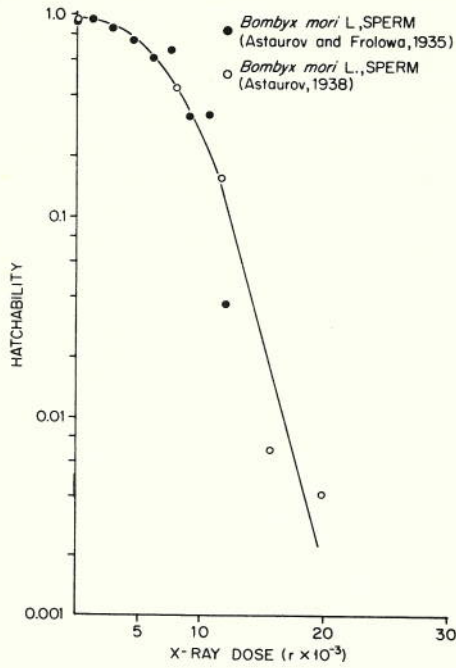


Fig. 11

Dose-hatchability curve for irradiated sperm of the silkworm, Bombyx [39, 40]

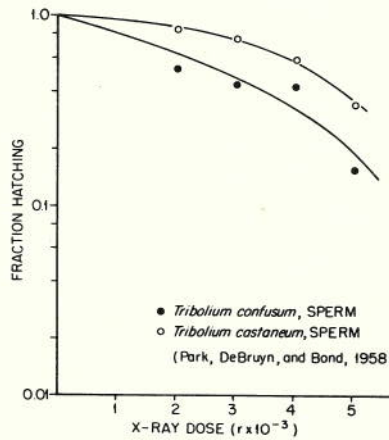


Fig. 12

Dose-hatchability curves for irradiated sperm of two different species of Tribolium [41]

and Tribolium strains cannot be properly gauged. The data in Fig. 12 indicate that a difference between two species of Tribolium may exist. The dose-action data do not differ markedly from that of Drosophila and the wasps, but the shape of the curve seems to differ somewhat.

An intensive investigation of the cytogenetics of Homoptera has been undertaken by Brown and his collaborators at the University of California. In a number of families in this order, one set of chromosomes in the male embryos becomes heterochromatic and is excluded from the genetic continuum during spermatogenesis. These chromosomes have been shown to derive from the male parent [44]. The results of irradiation of the sperm of the coccid *Planococcus citri* are shown in Fig. 13. Only the embryos destined to become females are killed at relatively low doses. At higher doses, females survive, and these derive both chromosome sets entirely from female origin. All embryos destined to become males survive up to doses of about 30 000 r. From here on there is a drastic reduction of this class. This indicates that even though the paternal set of chromosomes becomes heterochromatic in the male embryo, the sperm does contribute essential functional material for proper development [cf. 45] that is damaged only by very high doses of radiation.

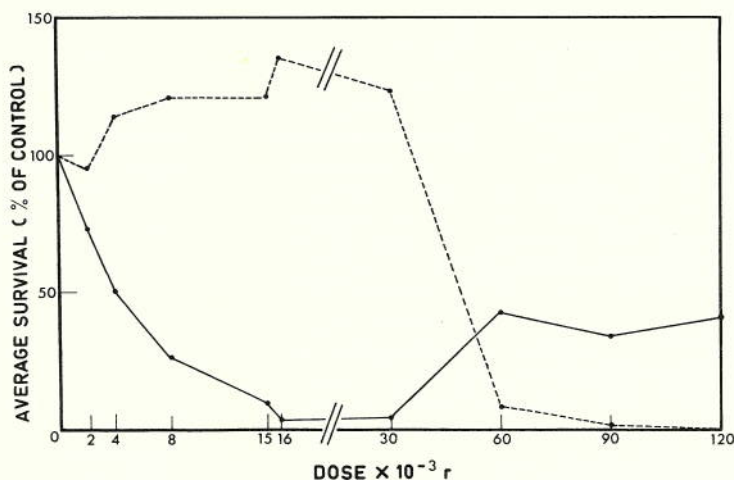


Fig. 13

Dose-survival curves for sperm of *Planococcus citri* indicating the average per-cent survival to maturity of embryos of both sexes [44]

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This lecanoid genetic system that Brown and his colleagues have investigated is restricted to certain families of Hemiptera. Other groups could be expected to respond to radiation in a manner similar to the more conventional responses found in Diptera and Hymenoptera. Variant genetic systems are found throughout the different orders of insects. Some of them could be used to augment population control by the irradiation-of-male method and others, like the lecanoid system, would offer special problems that would require special analysis.

DOMINANT LETHALITY AND STERILITY IN POPULATION CONTROL

The evidence indicates that there is a good deal of similarity of response to radiation from species to species, and even from order to order (Diptera,

Hymenoptera and Coleoptera) among insects, except for Lepidoptera. There is evidence that species in the Lepidoptera may be more resistant to radiation simply because the most frequent type of dominant lethality induced in other species is not induced here. A special genetic system in the Hemiptera presents special problems, and other variant genetic systems are known to exist in other groups of insects that could also vary responses to radiation [cf. 46].

It is known that induced dominant lethality causes competition to be at the level of the sperm rather than at the level of the male. Therefore the irradiation-of-male (lethal male) method for population control is equally effective whether the female mates once or many times [47, 48]. The essential data needed for any species for control purposes are: (a) the dose needed to achieve 99% or more dominant lethality in sperm; (b) the dose where sperm inactivation or reduced male vigour begins; and (c) the dose required to kill all the cells in the gonial region of the testes.

The rate of dominant lethality induced in sperm can be determined by hatchability measurements, and by extrapolation of the data on a semi-logarithmic plot to the 1% survival level. Sperm inactivation can be measured by simple multiple-mating experiments using irradiated and non-irradiated males. Killing of cells in the gonial region can be determined by histological examination of the gonial region of the testes a few days after irradiation to see if all the gonial cells are dead [32]. These same criteria and tests of male lethality are needed when alkylating agents are used instead of radiation.

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DISCUSSION

C. KRIMBAS: Dr. von Borstel, could you comment on the production of dominant lethals in Homoptera in view of the existence of a diffuse centromere as Schrader has shown?

R.C. von BORSTEL: I suspect that diffuse centromeres would reduce sensitivity where dominant lethality is the criterion. This may in fact explain the increased resistance in Lepidoptera. Do they not have diffuse centromeres?

C. KRIMBAS: The existence of a diffuse centromere has been claimed but not demonstrated in Lepidoptera. It would explain their resistance, and perhaps that of some Coleoptera.

R.C. von BORSTEL: Yes, in general it would tend to reduce the sensitivity, but the precise effect would be different in each case. No experi-

ments have, in point of fact, been done on dominant lethality in species which should be looked into. There have been radiation experiments, of course. What is done is to break up the chromosomes, but then they go to the poles, and no one has studied dominant lethality.

G.T. SCARASCIA: I should like to know if other alkylating agents, such as diethyl sulphate, which are of very little effect in breaking up chromosomes, at any rate in plants, have been successfully used for the induction of dominant lethals. If so, the dominant lethality cannot be strictly correlated with the chromosome aberrations, can it?

R.C. von BORSTEL: Yes, ethyl methanesulphonate, for instance, causes dominant lethality, and again the metaphase and prophase are differentially sensitive. As you say, this indicates that not all dominant lethality need necessarily be a result of chromosome breakage. Apparently it is effected through a slow-down of mitosis, but the actual mechanism behind dominant lethals induced by ethylmethene sulphonate is not known at all.

P. NARDON: Much has been said about dominant lethality, but it should not be thought that the effect of irradiation is exclusively mutagenic. It is very tempting when irradiating sperm to accept dominant-lethal mutations, but when irradiating oocytes, the possible effect of cytoplasm should not be forgotten. Various experiments have shown this. The work of Nakao on the silkworm, in particular, shows the lethality of irradiated cytoplasm to chromosomes, and the work of Duryee shows that isolated nuclei are much resistant.

R.C. von BORSTEL: I am aware of the work of both these investigators. Their results are very interesting, but difficult to interpret on the basis of nuclear injury. It should not be forgotten that heavily irradiated cytoplasm of eggs of *Habrobracon juglandis* Ashmead and *Bombyx mori* L. can still support embryonic development from unirradiated sperm with no effects visible upon the offspring. It has been shown that one alpha particle passing through the nucleus is enough to kill an embryo, but 20 000 000 must strike the cytoplasm before the embryo will die. We were able to show that this is not through any influence back on the nucleus because the types of killing are different. The same kind of experiment has also been done by Astaurov et al. in Russia. He has shown that in the silkworm at doses of approximately 200 000 r, or even higher, you can sometimes get double fertilization in the egg; the nuclei will join and they will develop normally. This effect has also been noticed by Japanese workers and remains unexplained—though I do not think it is of importance here.

K.K. NAIR: You have suggested the plotting of one's data on semi-log paper for calculation of the target number. If one were scoring lethality in an adult insect, would it be possible to calculate the target number in that way?

R.C. von BORSTEL: The question is how many parameters can be computed from two-dimensional data. Some progress has been made on the formal kinetics but the general usefulness is probably limited. One needs to be able to compute: (1) the sensitivity of the target; (2) the total number of targets in the sensitive tissue, for instance; and (3) the number of targets that, when inactivated, will cause the death of the organism. It appears now that all these parameters can be derived from a simple curve but the

problems are enormous. We can go into that in private if you like because all the problems have not yet been solved.

E. HORBER: For our irradiation work on cockchafers we have to decide which type of mobile equipment to acquire, in order to combine the best performance, from the radiobiological point of view, with technical and economical efficiency. Co^{60} or Cs^{137} units have the disadvantage of being rather too bulky and heavy to be used as mobile units, and radiation cannot be stopped when the unit is not in use. Safety measures have to be taken whether the unit is operated or not. These disadvantages are not encountered with X-ray units. Besides the standard X-ray units available in hospitals, new types with a beryllium window are coming into use. They emit a spectrum containing soft radiation. This, being more efficient as expressed in RBE values, is at the same time absorbed to a higher degree in the irradiated material. Therefore one would have to irradiate smaller quantities or thinner layers. The question I am asking the speaker as well as the audience is, what is the best type of mobile unit for our type of field work.

R. C. von BORSTEL: I refer that one to the audience.

B. D. BAINES: In answer to Dr. Horber's question, I would like to suggest that an isotopic source is absolutely dependable, and that an irradiation unit employing such a source can be made sufficiently rugged to operate effectively under field conditions. Of the common sources, Co^{60} and Cs^{137} , the latter is to be preferred as it does in fact permit the construction of the smaller, lighter units which are essential for mobile operation. It would seem probable that such a unit with an irradiation volume of one litre could be constructed with a weight of about one ton, suitable for transport in a van or trailer. I am sure that such a unit, which can never be "switched off", is better than a unit which may fail to operate when switched on.

G. T. SCARASCIA: I would like to ask whether, for induction of the gross chromosomal aberrations with which dominant lethality is generally correlated, densely ionizing radiations such as neutrons have been employed.

R. C. von BORSTEL: Yes, mostly with *Drosophila*. All these things depend upon the three purposes we are aiming at. One is to kill the gonads, another is to kill the sperm and the third is to inactivate the sperm. Gamma radiation seems to be ideal for pest control research in this connection. As far as I know, there have been no experiments at all on what are the different frequencies of the gonial killing and the inactivation cross-sections obtained by these means, although much is known about dominant-lethal cross-sections obtained with neutrons and other kinds of radiation.

THE EFFECTS OF GAMMA RADIATION ON THE OVARIES OF DACUS OLEAE GMEL.

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Abstract — Résumé — Аннотация — Resumen

THE EFFECT OF GAMMA RADIATIONS ON THE OVARIES OF DACUS OLEAE GMEL. The authors have studied by cytological and ultramicroscopic techniques the ovaries of unfertilized adult females of Dacus oleae irradiated in the middle period of the pupal stage with several doses (2-30 kr) of gamma rays. In all cases the treatment inhibits the normal development of the ovary. Nurse cells and egg cells are very small and few in number and show abnormal structure and ultrastructure, particularly as regards the cytoplasmic organelles.

EFFET DES RAYONS GAMMA SUR LES OVAIRES DE LA DACUS OLEAE GMEL. Les auteurs ont fait appel aux techniques cytologiques et ultramicroscopiques pour étudier les ovaires de femelles adultes de Dacus oleae non fécondées, qui avaient été irradiées au milieu du stade pupaire par plusieurs doses (2-30 kr) de rayons gamma. Dans tous les cas, l'irradiation a empêché un développement normal de l'ovaire. Les cellules nourricières et les ovules étaient très petits et rares, avec une structure anormale, surtout pour les organites du cytoplasme.

ВОЗДЕЙСТВИЕ ГАММА-ИЗЛУЧЕНИЯ НА ЯИЧНИКИ DACUS OLEAE. Авторы изучили с помощью цитологических и ультрамикроскопических методов яичники неоплодотворенных женских особей Dacus oleae, облученных в середине фазы окукливания различными дозами (от 2000 до 30 000 р) гамма-лучей. В каждом случае это тормозило нормальное развитие яичников. Питательные и яйцевые клетки были очень маленькими, количество их незначительно, структура и ультраструктура ненормальные, прежде всего в отношении

EFFECTO DE LAS RADIACIONES GAMMA SOBRE LOS OVARIOS DE LA DACUS OLEAE GMEL. Los autores de la memoria han estudiado con técnicas citológicas y ultramicroscópicas los ovarios de hembras adultas, no fertilizadas, de la Dacus oleae irradiadas durante el período medio de la fase ninfal con varias dosis de rayos gamma (de 2 a 30 kr). El tratamiento inhibe el desarrollo normal del ovario. Los oocitos, en todas sus fases, son muy pequeños, aparecen en escaso número y muestran una estructura y ultraestructura anormales, sobre todo por lo que respecta a los orgánulos citoplásmicos.

INTRODUCTION

The modifications that ionizing radiations induce on the ovary have been observed ever since the beginning of radiobiological research: studies in this field were begun simultaneously in 1905 by HALBERSTAEDTER [1] in Germany and by BERGONIE et al. [2] in France.

The investigations were soon extended even to man, and functional studies were added to morphologic ones [3] with the observation of the phenomenon of temporary or permanent sterility.

The many researchers who have dealt with this subject [4, 5, 6, 7] have reached practically the same conclusions on the damage caused on the different ovarian structures of mammals by various types of electromagnetic

and particle radiation from both external and internal sources [6, 8, 9, 10, 11, 12].

In a recent article [13], ERRERA, FORSSBERG, RUGH et al. have made a full and up-to-date synthesis of the morphological and functional alterations of the ovary in the most common laboratory mammals examined by optical microscope. In animals irradiated with a LD50/30 whole-body dose, a rapid disappearance of the egg-cells of the primary follicle, associated with the destruction of the follicular cells of the more mature follicles, is observed. The germinal elements soon disappear. The number of luteal bodies slowly decreases, since they are no longer produced by the follicular cells which have now been destroyed. The interstitial tissue tends towards slow atrophy and in about two months' time the ovary consists of fibrous tissue containing cystic formations.

The egg-cell shows a different sensitivity according to its degree of development; the young or primary follicle is more sensitive than the secondary follicle.

The converse behaviour is exhibited by the cells of the granulosa: in the primary follicle stage they are more resistant than the oocytes, whose residues they eliminate when the oocyte is destroyed, whereas they are much more sensitive than the egg-cell in the mature follicle, and they constitute, in this phase, the most radio-vulnerable units in the ovary.

If the follicular cells are irradiated at the stage when they constitute an element of the luteal body, they are relatively resistant, at least so far as morphological examination by optical microscope reveals, and this fact is true for all mammals [14].

The covering epithelium is also fairly insensitive and may show a flattening of the cellular elements without signs of degeneration. In the interstitial tissue, radiation causes a slow process of atrophy to set in.

The morphological damage caused by radiation in the more sensitive cellular elements is revealed by examination under the optical microscope as consisting in alteration of the nucleus, which shows a thickening of chromatin reaching the extreme stages of pyknosis and karyolysis, and of the cytoplasm, in which vacuolization effects occur.

In insects too, the post-irradiation alterations of the gonads have been accurately studied and fully documented under the optical microscope by a number of authors [15, 16, 17, 18], particularly by ERDMAN [18].

Generally speaking, the doses necessary for the alteration and destruction of ovarian tissue are much higher in insects than in mammals, although they vary in a given animal according to the stage of development of the germinal elements.

The morphological alterations observed by Erdman in Habrobracon juglandis consist in atrophy of the ovarioles, which may be reduced to rudimentary appendages or "horns"; these are most frequently observed after irradiation at sterilizing doses (2500-4500 r) of the final stages of larval life.

Of the various segments of the ovariole, the most damaged is the germarium: its cellular elements appear to be constantly altered and are often reduced to fragments. The oocytes present various degrees of alteration, which range from a sometimes considerable shrinking of the whole cell, with

deformation and wrinkling of the nucleus, to the fragmentation of the cellular elements. Gross changes also appear in the nurse-cells.

In the pupal stage, after a certainly sterilizing dose of irradiation (3900-5700 r), which, in contrast to the situation for larvae, is lower than the lethal dose, one can observe deformation of the ovarioles, whose only well-preserved part seems to be the layer of monostратified epithelial cells that surrounds the ovarioles, whereas the other germinal elements (oogonia, oocytes and nurse-cells) are difficult to identify, being largely reduced to fragments.

Electron microscope study of the fine cellular structures, which has recently been added to the conventional histological examination, permits a more detailed analysis of the post-irradiation alterations of cellular organelles. However, we have not been able to trace that any research of this kind has been carried out on insects, whereas mammals have been the subject of many important works [20, 21].

The ultrastructural alterations observed in PARSONS' study on mice subjected to various doses of whole-body irradiation (7-200 r) of course varied in degree, according to the stage of development of the egg. These alterations mostly concern the nucleus, in which phenomena of kariolysis and pyknosis and nuclear alterations occur; they also concern the mitochondria, which decrease in number and size, and the RNA granules, which are reduced in numbers.

The present paper concerns the study of post-irradiation alterations in the ovary of *Dacus oleae* Gmel.; this insect has already been the subject of insect control tests using the sterile-male method [21], and of research on the effects of radiation on mesenteron. The object of this study is the evaluation of the lesions that various doses of certainly sterilizing radiation induce in the female germinal cells. We have employed the most modern methods of cytological investigation, such as the electron microscope and some histochemical reactions. Since research of this kind is completely absent in the literature, not only on irradiated insects but even on normal insects, we have had to begin our work by a study of the ultrastructure of the ovary in normal *Dacus oleae* females.

MATERIALS AND METHODS

The insects were irradiated in the pupal stage by the technique we have already described in earlier papers [22, 23]. We used a cobalt-60 teletherapy unit with F. D. 80 cm and r/m 30. For irradiation the pupae were placed side-by-side in a depression cut into the top of a block of paraffin-wax to a depth equal to the width of the pupae. The depression was covered with a layer of Plexiglass 5 mm thick. In this way we obtained a homogeneous irradiation of the insects and were able to keep the quantity of diffused radiation constant in the various tests.

We irradiated 8 lots of pupae from the Palermo area and administered in a single run, by separate groups, doses of 2, 4, 5, 8, 10, 12, 20 and 30 kr for times varying from 1 h 7 min. to 16 h 40 min. The irradiated pupae were then left to develop along with appropriate controls. When the adults

had emerged from the puparium, we selected, as in earlier studies, those that had been irradiated in the middle period of the pupal stage.

Some samples were fixed alive in Duboscq Brazil, embedded in paraffin, sliced in series and stained with Feulgen and Brachet reagents. Others were studied with the electron microscope. The fixation was carried out at +4°C, with 1% osmium tetroxide buffered at pH 7.2 injected *in vivo*. The ovaries were removed by dissection, embedded in methacrylate and sliced with the Porter-Blum ultra-microtome.

The sections were then stained with 1.5% uranylacetate, covered with a thin protective coating of carbon and examined under a Hitachi HS-6 electron microscope.

RESULTS

(A) Normal structure of the ovary

The female of *Dacus oleae*, immediately after the emergence, usually shows well-developed ovaries containing mature eggs ready to be fertilized.

Each ovary is more or less conical in appearance, consists of 15 to 20 ovarioles converging into the calyx of the lateral oviducts, and generally speaking measures about $700 \times 500 \mu\text{m}$. Each ovariole is composed of a string (vitellarium) of 4-7 ovarian chambers that are quite easily identifiable and of a distal germarium containing the oogonia, the segmentation of which is less clear. The wall of each ovariole is formed by a single-layer follicular epithelium (Figs. 1 and 2), composed of small cuboidal cells. These cells are easily differentiated only in the vitellarium, where they have octoploid nuclei [24] that are rather small (about $4.5 \mu\text{m}$ in diameter), spheroid, full of positive Feulgen substance arranged in thin granular filaments, and contain only one nucleolus. The cytoplasm is rather sparse but rich in RNA, as is demonstrated by the pyroninophilia which is easily identifiable by Brachet's method and is homogeneously distributed around the nucleus.

Under the electron microscope (Fig. 9), these cells are seen to be extraordinarily rich in ergastoplasm (the cause of the pyroninophilia), in the shape of more or less twisted reservoirs and of free ribosomes.

The mitochondria, on the other hand, are small, sparse and poor in cristae; the multivesicular bodies and other organelles are few. The Golgi fields are constituted by swarms of tiny vesicles. The cell membranes often appear interrupted and show signs of frequent exchanges. In the nucleus, the chromatin is in the form of granules, and is homogeneously distributed; the karyotheca is provided with many pores with annuli and continually produces ergastoplasmic cysts by means of vesiculations of its lamina externa.

In the germarium (Fig. 3) the follicular cells do not seem separable from the oogonia, which will be described further on.

The follicular epithelium lies on a thin basal membrane (Figs. 9 and 13), which has been found under the electron microscope to be $2000-3000 \text{ \AA}$ thick and made up of very thin filaments (about 200 \AA thick) of indefinite length. These filaments show along their length an alternation of dark and light stripes, every 600 \AA or so, and are quite similar to those that

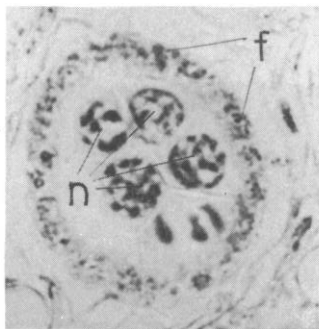


Fig. 1

Cross-section of ovariole of a normal *Dacus oleae* Gmel. female
(n), nuclei of the nurse-cells; (f) nuclei of the follicular cells. Feulgen's reagent, 560 diam.

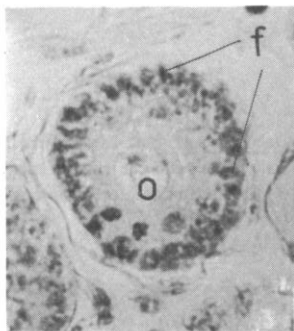


Fig. 2

Cross-section of the same ovariole, on another level
(f), follicular cells; (o), nucleus of oocyte. Feulgen's reagent, 560 diam.

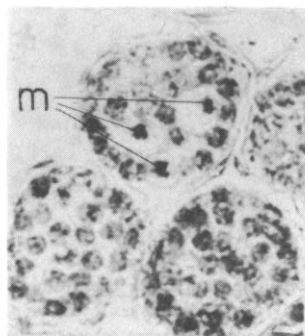


Fig. 3

Cross-sections of three ovarioles in a normal *Dacus oleae* Gmel. female, sectioned at the level of the germarium
Many of the oogonial nuclei are in mitosis (m). Feulgen's reagent, 560 diam.

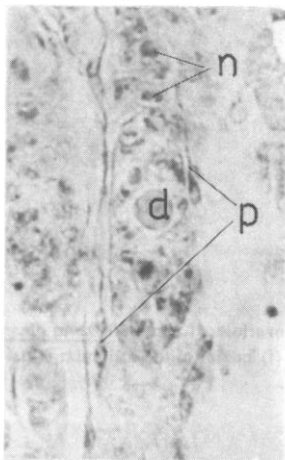


Fig. 4

Longitudinal section of ovariole of a Dacus oleae Gmel. female irradiated at the pupal stage with 2 kr. In the inner part of the peritoneal membrane (p) one can observe many undifferentiated oogonia (n) and a few cells in degeneration (d). Pyknotoses are completely absent. Feulgen's reagent, 560 diam.

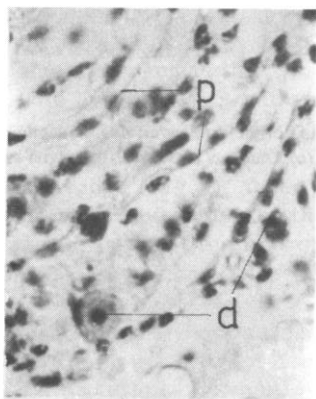


Fig. 5

Oblique section of a clump of ovarioles in an adult Dacus oleae Gmel. female irradiated at the pupal stage with 12 kr

Abundant nuclei can be seen in the peritoneal membrane (p) and, in the inner part, a paucity of cells, of which a few are degenerating (d), can be observed. Feulgen's reagent, 560 diam.