THE MODIFICATION OF REPRODUCTION IN INSECTS TREATED WITH ALKYLATING AGENTS. II. DIFFERENTIAL SENSITIVITY OF OOCYTE MEIOTIC STAGES TO THE INDUCTION OF DOMINANT LETHALS

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Differential sensitivity among cell stages is commonly encountered in radiobiological studies. In investigations of chemical mutagens, similar differences in sensitivity are to be expected if these agents are truly radiomimetic. In the experiments reported herein, a study of the induction of dominant lethal mutations in the female germ cells of the screw-worm fly (*Cochliomyia hominivorax* (Coquerel); Diptera, Calliphoridae) was conducted to determine whether the sensitivity pattern was the same or similar for alkylating agents and gamma radiation. Comparisons of mutagenic efficiency were also made between a bifunctional and two trifunctional alkylating agents and between the two trifunctional agents having the same number and kind of functional groups but differing in the nature of the prosthetic group. Monofunctional alkylating agents are weak mutagens (Fahmy and Fahmy, 1956, 1958), and are relatively inefficient in the sterilization of insects (Crystal, 1963) and the induction of chromosome breaks (Alexander, 1960).

The present studies were also intended to supplement the rapidly accumulating amount of information on the production of sterility in insects by chemical means (LaBrecque, 1961; Knipling, 1962; Crystal, 1963). It is of interest to study the effects of chemosterilants on formed oocytes, since sterility need not be the result of reduced egg production but may be attained by dominant lethality in the zygotes. It has been shown that a chemical agent that produces infecundity in flies treated at a stage in which only immature germ cells are present may not be able to inhibit fecundity at a later stage; in such a situation sterility must then be the result of inviability of the eggs (see Crystal and LaChance, 1963). Finally, it is not enough to determine whether a chemical is mutagenic or not; quantitative data are needed to provide a basis for comparison of effectiveness between different sterilizing agents.

Many different types of compounds have been studied for their effects on the hereditary material of male *Drosophila* (Fahmy and Fahmy, 1956; Purdom, 1960) and of mice (Bateman, 1960; Moutschen, 1961). To date, the evidence is overwhelming that many of these agents are capable of inducing changes in the hereditary material very similar to radiation-induced changes (*i.e.*, point mutations, deficiencies, recessive lethals, dominant lethals, and chromosome breaks). Nevertheless, there is also evidence that the position, proportion, and time of induction of these primary genetic changes are different for radiation and chemical mutagens (Fahmy and Fahmy, 1956, 1960). Chemical mutagens themselves differ greatly with re-

spect to their mutagenicity (Fahmy and Fahmy, 1956). With regard to varying sensitivity of female germ cells, Löbbecke and von Borstel (1962) have shown that when *Habrobracon* females are treated with nitrogen mustard or ethyl methanesulfonate, the oocytes in first metaphase are much more sensitive than oocytes in prophase I. To our knowledge, the three alkylating agents discussed in this report have not been studied for their effects on the induction of dominant lethals in meiotic oocytes of insects.

Materials and Methods

Adult females of the screw-worm fly are ideal for testing the effects of mutagens on oocytes in different stages of maturation. The female reproductive system of this insect contains two ovaries, each of which consists of 100–150 ovarioles, and development of the egg in each of these ovarioles occurs synchronously. By treatment of a single female, it is thus possible to treat hundreds of oocytes all in the same stage of development. Gravid females deposit as many as 200–250 eggs in a single egg mass. Oogenesis in this species has been described (LaChance and Bruns, 1963), and the different stages of meiosis, correlated with the age of the female, have been fairly well defined (LaChance and Leverich, 1962). From these previous studies it is known that 3-day-old females reared at 80° F. contain oocytes each with the nucleus in early prophase of the first meiotic division, that 4-day-old females contain almost fully mature eggs each with the oocyte nucleus in metaphase I, and that 5-day-old females contain a large number of fully mature oocytes in which meiosis has progressed up to anaphase I. Further growth and changes in the egg and its nucleus are arrested in this stage until the egg is laid.

Since the results of the preliminary experiments in this study were somewhat variable (compare experiments 3 and 4, Table I), it was decided to further standardize the treatment procedure to eliminate as many variables as possible. Although the synchronous development of many oocytes within a single female makes the screw-worm fly highly suitable for this kind of investigation, any factor that retards or accelerates the physiological aging of the female also affects the state of maturation of her oocytes. Thus, although within a given female all oocytes in the first egg chamber are in the same stage of development, variation among females of the same age will sometimes be encountered, due to many unknown factors that influence physiological age. Therefore, for experiments 5–14 reported in Table I, the testing procedure was slightly modified and standardized to minimize the introduction of extrinsic variables due to handling, crowding, anesthesia, and preparation of the chemical solutions. In these experiments the general procedure adopted was: (1) Flies were allowed to emerge from the puparia for a period of four hours, after which they were sexed. Emergence of the adults from the puparia was not retarded by storing the pupae at low temperatures (a common practice), since this might have affected the physiological age of the females. Thus, all of the females in a given group were within ± two hours in age. (2) The insects were maintained in an 80° F. colony room throughout the experiments. On the morning of the third day after emergence a solution of the chemical was prepared in an organic solvent and a given quantity was applied topically with a microapplicator to the dorsal thorax of one group of anesthetized females. This procedure was repeated on the fourth and fifth day after emergence with

the same amount of freshly dissolved chemical applied topically to two other groups of females. Approximately 50 females were treated in each group. (3) All batches of the chemical to be used on the three successive days were weighed out by the same person on the same day. The solvent was added just before the chemical was applied, and, although a very small amount of solution was used in each test, a final volume of 10 ml. was made up to reduce errors in weighing and dilution. The chemicals were stored at freezing temperatures, yet at times they polymerized. When there was any evidence of polymerization, the chemical

TABLE I

The induction of dominant lethal mutations in meiotic oocytes of Cochliomyia hominivorax by three alkylating agents

Females treated topically with the chemicals at 3, 4, or 5 days containing oocytes in early prophase, metaphase, or anaphase, respectively, subsequently mated with untreated males, and egged at 6 days. Assume dominant lethals equal 100 minus % corrected egg hatch.

Experiment number	Chemical	Amount applied (μl.)	Concentration		Total number of eggs scored	Hatchability of eggs (as percentage of controls) from females treated at		
			Per cent	$M \times 10^{-3}$		3 days	4 days	5 days
1	Tretamine	2.4	0.5	24.48	8,858	0.66	1.13	0.27
2		2.4	0.1	4.89	7,456	0.41	0.21	0.16
3*		2.4	0.01	0.489	9,585	31.39	26.0	3.50
4		2.4	0.01	0.489	9,917	90.77	37.64	15.24
5		2.4	0.01	0.489	9,145	96.39	50.08	1.99
6**		2.4	0.01	0.489	8,782	76.57	75.59	1.41
7		2.4	0.01	0.489	14,073	100.7	19.69	12.04***
8		2.0	0.01	0.489	10,738	100.4	33.69	3.44
9	Benzoquinone derivative	2.0	0.01	0.295	6,444	91.0	87.5	92.5
10		2.0	0.1	2.95	6,298	45.6	8.2	0.0
11		2.0	0.1	2.95	6,306	49.0	8.0	0.0
12	Thiotepa	2.0	0.01	0.529	6,137	93.5	78.6	67.7
13		2.0	0.01	0.529	6,281	117.1	79.2	60.8
14		2.0	0.05	2.65	6,098	100.5	27.6	1.2

* Pupae kept at 60° F. to retard emergence.

** High mortality, even among controls, attributed to overcrowding; control hatchability was low, and suggested that females were younger physiologically than chronologically at four days.

*** Females treated at 5 days egged at 8 days.

was recrystallized from carbon tetrachloride just before use. (4) Anesthesia prior to treatment of the females was accomplished by chilling for a very brief period rather than by knock-down with CO_2. (5) Untreated males were added to the cages of treated females approximately 16 hours before the females were egged instead of immediately after treatment. This procedure greatly reduced the chances of males becoming contaminated from chemical still on the thorax of the females or of sperm being exposed to the chemical within the spermathecae of the females. Studies on the metabolism of a related alkylating agent (metepa) indicated that

house flies, mosquitoes, stable flies, and screw-worm flies absorbed and metabolized the topically applied chemical very quickly (Plapp *et al.*, 1962; Chamberlain, unpublished). (6) When the females were 6 days old, oviposition was induced by presenting each female with a small piece of lean meat and keeping her at a temperature of 90–96° F. for a few hours. At the low concentrations of chemicals used in these experiments, toxicity was not encountered, and the females deposited the normal numbers of eggs.

To insure that eggs deposited by unfertilized females were not included in any egg sample used for hatchability studies, each female was allowed to oviposit individually. After producing an egg mass the female was sacrificed and the spermathecae examined for the presence of sperm. Only egg masses from inseminated females were used. Egg masses were separated and plated for hatchability studies according to the method described by LaChance and Leverich (1962). All hatchability figures were based on counts of approximately 2000 eggs for each dose and age group. Hatchability of the eggs was scored after incubation for 28–30 hours at 80° F. After correction for the number of natural deaths in the controls, it was assumed that all unhatched eggs were the result of a dominant lethal change induced in the oocyte of the treated female. Sex-linked recessive lethal changes would also have contributed to death in the F_1 male embryos; but insemination by normal, untreated males prevented detection of autosomal recessive lethal changes, and hatchability tests cannot detect dominant lethal changes expressed beyond the egg stage.

The experiments reported in this paper were performed over a period of 7 months, during which 16 different sets of controls were examined. Control hatchability ranged from 66% to 95%. The data in Table I have been corrected for the respective controls. A total of 126,886 eggs from treated and control females was examined for embryonic dominant lethals.

The three chemicals used in these tests were: (a) 2,4,6-tris(1-aziridinyl)-*s*-triazine(= tretamine = TEM = triethylenemelamine); (b) 2,5-bis(1-aziridinyl)-3,6-bis(2-methoxyethoxy)-*p*-benzoquinone; and (c) tris(1-aziridinyl)phosphine sulfide (= thiotepa = thio-TEPA = triethylenethiophosphoramide). These chemicals will hereafter be referred to as tretamine, benzoquinone, and thiotepa. All three are alkylating agents. Both tretamine and thiotepa have three aziridinyl alkylating groups but differ in that in tretamine these three groups are carried on a triazine ring, whereas in thiotepa the three groups are attached to phosphine sulfide. The benzoquinone compound has only two aziridinyl alkylating groups attached to the quinone ring in the 2,5 positions, but also carries two methoxyethoxy chains in the 3,6 positions.

Results

The first chemical to be studied in this series was tretamine. Results of the first two experiments performed (experiments 1 and 2 in Table I) indicated that concentrations of 0.5% and 0.1% were highly mutagenic to all oocyte stages, inducing nearly 100% dominant lethals. At 0.01% (Table I) some viable eggs were laid. This finding indicated that a level had been reached at which differential sensitivity of the meiotic stages could be investigated.

The experiment was repeated five times with 2.4 μl. of tretamine applied at a concentration of 0.01% and the results are summarized in Table I (experiments

3–7). At this concentration, great differences among the meiotic stages occurred in their sensitivity to the chemosterilant. In all experiments, the oocytes in prophase I were very resistant to tretamine, whereas the oocytes in anaphase I (5-day-old females) had large numbers of dominant lethals. In general, high rates of dominant lethals were also induced in oocytes treated in metaphase I (4-day-old females), but the sensitivity of this stage seemed to be more variable. The reasons for the variability of the metaphase I stage will be discussed below.

The data in Table I establish a clear trend in oocyte sensitivity to tretamine, from resistance in early prophase I to high sensitivity in anaphase I. Only in experiment 3 were a moderate number of dominant lethals induced in early prophase I oocytes. In this series, retardation of emergence of the adults by chilling (not as carefully avoided as in later trials) could have altered the physiological age of the females and consequently the speed of maturation of the oocytes; thus, although 3-day-old females were treated, oocyte maturation had perhaps progressed further than in other groups of comparable chronological age. The equal numbers of dominant lethals induced in 3- and 4-day-old females in experiment 6 can, to some extent, be attributed to the very crowded cages used in this experiment; these conditions perhaps delayed aging and maturation in the females so that the sensitive period was not reached until the females were more than four days old.

In order to further test the hypothesis that the stages in oocyte maturation differ in sensitivity to the induction of dominant lethals by chemical mutagens, similar experiments were conducted with thiotepa and benzoquinone. The results of these experiments are presented in Table I. In general, they agree remarkably well with the results obtained with tretamine. At the higher concentrations, early prophase I oocytes were extremely resistant to both of these chemicals, and metaphase and anaphase I were very sensitive. Neither of these chemicals was very effective in inducing dominant lethals at a concentration of 0.01%, but thiotepa was definitely more damaging than benzoquinone. Thiotepa was very mutagenic to anaphase I oocytes at 0.05%. This intermediate concentration was not tested with benzoquinone because the earlier test at 0.01% indicated low mutagenic efficiency of the compound. However, at a concentration of 0.1% dominant lethals were induced in half of the prophase I oocytes, whereas 92% and 100% dominant lethals were induced in the other two stages. Thus, these three chemicals produced different amounts of genetic damage when equal concentrations were tested. In descending order of mutagenic efficiency, tretamine was first, thiotepa second, and benzoquinone third.

In order to assess the role of molecular structure in mutagenic activity, it was thought desirable to compare the activity of different compounds on the basis of molar concentration. The data on chemical concentrations in Table I have been expressed in both per cent concentration and molarity. A comparison of the compounds indicates that tretamine was the most effective mutagen of the three. However, on the basis of molarity, benzoquinone appears to be at least as potent a mutagen as thiotepa, but further tests are needed to clarify this point.

Before a meiotic stage can be assumed to represent a stage of high sensitivity, one must rule out the possibility that one stage has had more time to recover from the treatment than another before having to cope with the crises of the meiotic divisions, syngamy and embryogenesis, which follow fertilization of the egg. Such

a possibility must be ruled out especially with regard to results obtained with 3-day-old females, which were allowed three days for recovery between treatment and egging in contrast to 5-day-old females, which had only one day to recover. In order to study this possibility, experiment 7 was performed in a manner slightly different from the others. In experiment 7 the females treated at three or four days of age were egged at 6 days, and those treated at 5 days were egged at 8 days. The results showed a high incidence of dominant lethals induced in 5-day-old females, but a virtual absence of lethals in females treated at three days of age. Thus, the time elapsing between treatment and oviposition did not alter the results. Also, in *Habrobracon* oocytes there is no evidence for recovery from nuclear damage induced by nitrogen mustard (von Borstel, 1955). It can therefore be concluded that the meiotic stage of the oocyte nucleus at the time of treatment does condition the amount of dominant lethality induced in the oocytes. Similar results have been obtained with gamma radiation (LaChance and Leverich, 1962).

Discussion

Treatment of various meiotic stages in the oocytes of screw-worm flies has demonstrated consistent trends in sensitivity to three chemical mutagens. Although some variation existed in the different replicates, especially with treatment of 4-day-old females (Table I), these variations did not obscure the major trend demonstrated, the progression from resistance to susceptibility to chemical mutagens during maturation of the oocytes. It must be emphasized that varying sensitivity of the metaphase I stage was encountered only with treatments at the borderline of effectiveness. At more damaging concentrations (those that resulted in 100% dominant lethals in anaphase I oocytes), the metaphase I oocytes also exhibited high sensitivity (Table I, experiments 7, 8, 10, 11, 14).

The present data, as well as those obtained in tests with gamma radiation (LaChance and Leverich, 1962), show that during the period between three and four days in the adult life of screw-worm females, the oocytes are changing from resistance to sensitivity to both types of mutagens. However, the energy from gamma radiation can be delivered and effect damaging results in a matter of minutes, but chemical mutagens probably induce genetic damage over a longer period, during which the oocytes could be continuing their transition from resistant to sensitive stages. This extended period of activity would, of course, result in some variability, which would be more evident at the lower concentrations.

The chronological age of the females were controlled to $\pm$ two hours, but it is the physiological age of the female that determines the progress of development in the oocytes. Even within closely timed groups, some females may be more advanced in development than others. Because of the transitional nature of the oocytes between three and four days, perhaps occasionally some 4-day-old females were treated before the oocytes entered the period of sensitivity. The data obtained by sampling approximately 2000 eggs from many females were representative of an average of the stages in oocyte development present at the time of treatment. Thus, data taken when the females were four days old should not be interpreted as representing a period of intermediate sensitivity but, rather, one of a transitional nature, in which most but not all of the females had reached the sensitive stage

in oocyte development. This conclusion is supported by unpublished cytological observations on ovarioles dissected from females of this age group.

Although the cytological difference between metaphase and anaphase I is very slight, there seemed to be a constant difference between them in response to chemical mutagens. It was surprising to find that 4-day-old oocytes were somewhat less sensitive than 5-day-old oocytes; in radiation experiments the reverse has been consistently true (LaChance and Leverich, 1962). Perhaps this response is indicative of some basic difference in the mode of action between the two types of mutagens.

It was of interest to note that concentrations of tretamine as low as 0.5% completely inhibited ovarian growth when administered to newly emerged females, but when administered when the flies were 24 hours old, had little effect on ovarian growth (Crystal and LaChance, 1963). However, although a normal number of eggs was produced after treatment of 3–5-day-old females, more than 99% contained dominant lethals. These findings illustrate the varied and powerful effects of this mutagen on insect reproduction.

The data indicate that tretamine is a more potent mutagen than thiotepa. Since both of these compounds have three aziridinyl groups that react with nucleoproteins by alkylation, the difference in activity between them serves to reemphasize the role of the prosthetic group in mutagenicity (Fahmy and Fahmy, 1956, 1960) and in chemosterilization (Crystal, 1963). The benzoquinone compound is bifunctional and, although it was less effective than thiotepa at a concentration of 0.01%, the higher concentration (0.1%) gave definite evidence of being mutagenic, even to resistant prophase I oocytes. The evidence is sufficient to classify these three chemicals as fairly powerful alkylating agents that are radiomimetic, at least to the extent of showing the same pattern of effect on developing oocytes. The basic cause for the greater sensitivity of some meiotic stages to mutagens cannot yet be ascertained, no more than it can definitely be stated why these same stages are more sensitive to radiation (see Whiting, 1945; Bozeman and Metz, 1949; Sparrow, 1951; LaChance and Leverich, 1962).

It is fairly certain that dominant lethals are a manifestation of chromosome breakage in the meiotic oocytes (von Borstel, 1955). Fahmy and Fahmy (1954) have shown that dominant lethals induced in *Drosophila* sperm by tretamine are due to primary breaks that undergo noneucentric rearrangements. The end result of such chromosome aberrations in oocytes would be lethality in the zygotes due to failure of completion of the meiotic division, or chromosome loss or imbalance in the embryo. Whether the meiotic stages differ in the amount of genetic damage initially induced or in the degree of recovery or repair of the genetic damage is still unknown. Possibly permeability to chemicals differs in the various stages due to changes involving the nuclear membrane. Also the varying ability of broken chromosomes to rejoin after treatment of different meiotic stages has been offered as a plausible explanation for differences in radiosensitivity (Whiting, 1945). To date no better hypothesis has been put forth, and this explanation may therefore be equally tenable for radiomimetic substances, providing that the chromosome breaks are induced in fully formed chromosomes shortly after treatment and not at some later stage, such as during chromosome replication for cleavage divisions in the zygote. Clearly the basis for the differential sensitivity of meiotic stages

to alkylating agents depends on whether these chromosome breaks become actual immediately or at some later stage of development in the egg.

The results are likely to be misleading if dominant lethals are assessed on the basis of inviability alone without subsequent cytological analysis (Fahmy and Fahmy, 1958; Purdom, 1960). This is an important point when adult males are treated and subsequently mated to untreated females, since inviability in such a situation could be due to aspermia. However, inviability in our experiments was certainly due to dominant lethality, since females were treated at stages in which there is no germinal selection against damaged oocytes and mated to untreated males. All eggs studied were deposited by females that had been examined for the presence of motile sperm.

A comparison of mutagenic efficiency between chemical mutagens and ionizing radiation is highly speculative. The 50% dominant lethal dose for *Cochliomyia* oocytes irradiated in early prophase I is 7,939 r (LaChance and Leverich, 1962); about 60% dominant lethals were induced by 10,000 r. Thus, treatment with 2.0 μl. of 0.1% benzoquinone appears to be about as mutagenic as 7,939 r, and 2.4 μl. of 0.1% tretamine (4.89×10^{-3} *M*) is vastly more damaging to resistant oocytes.

The present data are consonant with those of Löbbecke and von Borstel (1962) in which the metaphase I oocytes of *Habrobracon* were found vastly more sensitive than prophase I oocytes to treatment with nitrogen mustard and ethyl methanesulfonate when both dominant and recessive lethals were studied.

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Summary

When adult females of the screw-worm fly, *Cochliomyia hominivorax,* are topically treated with alkylating agents, dominant lethal mutations are induced in the oocytes. Since the meiotic stage of the oocytes is correlated with the age of the female, it is possible to treat oocytes in either early prophase, metaphase, or anaphase of the first meiotic division. When such treatment is made, fewer dominant lethals are induced in prophase oocytes than in oocytes in the other two stages. This trend was consistently found in studies with three chemical mutagens: (a) tretamine (2,4,6,-tris(1-aziridinyl)-*s*-triazine), (b) a benzoquinone derivative (2,5,-bis(1-aziridinyl)-3,6,-bis(2-methoxyethoxy)-*p*-benzoquinone), and (c) thiotepa(tris(1-aziridinyl)phosphine sulfide). In addition to demonstrating the differential sensitivity of various oocyte meiotic stages to these agents, this report compares the mutagenic efficiency of the agents and discusses the possible basis for differential sensitivity of cell stages to mutagens.

LITERATURE CITED

Alexander, Peter, 1960. Radiation imitating chemicals. *Scientific American,* **202**: 99–108.

Bateman, A. J., 1960. The induction of dominant lethal mutations in rats and mice with triethylenemelamine (TEM). *Genetical Research,* **1**: 381–392.

Bozeman, M. L., and C. W. Metz, 1949. Further studies on sensitivity of chromosomes to irradiation at different meiotic stages in oocytes of *Sciara*. *Genetics,* **34**: 285–314.

CRYSTAL, M. M., 1963. The induction of sexual sterility in the screw-worm fly by antimetabolites and alkylating agents. *J. Econ. Ent.*, **56**: 468–473.

CRYSTAL, M. M., AND L. E. LaCHANCE, 1963. The modification of reproduction in insects treated with alkylating agents. I. Inhibition of ovarian growth and egg production and hatchability. *Biol. Bull.*, **125**: 270–279.

FAHMY, O. G., AND M. J. FAHMY, 1954. Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. II. The mechanism of induction of dominant lethals by 2:4:6-Tri(Ethyleneimino)-1:3:5-Triazine. *J. Genetics*, **52**: 603–619.

FAHMY, O. G., AND M. J. FAHMY, 1956. Cytogenetic analysis of the action of carcinogens and tumour inhibitors in *Drosophila melanogaster*. V. Differential genetic response to the alkylating mutagens and x-radiation. *J. Genetics*, **54**: 146–164.

FAHMY, O. G., AND M. J. FAHMY, 1958. Discussion: Mutagenic effects of alkylating agents. *Ann. N. Y. Acad. Sci.*, **68**: 736–748.

FAHMY, O. G., AND M. J. FAHMY, 1960. Mutagenicity in the sperm of *Drosophila* and the structure of the "nitrogen-mustard" molecule. *Heredity*, **15**: 115–128.

KNIPLING, E. F., 1962. Potentialities and progress in the development of chemosterilants for insect control. *J. Econ. Ent.*, **55**: 782–786.

LaBRECQUE, G. C., 1961. Studies with three alkylating agents as house fly sterilants. *J. Econ. Ent.*, **54**: 684–689.

LaCHANCE, L. E., AND A. P. LEVERICH, 1962. Radiosensitivity of developing reproductive cells in female *Cochliomyia hominivorax*. *Genetics*, **47**: 721–735.

LaCHANCE, L. E., AND S. B. BRUNS, 1963. Oogenesis and radiosensitivity in *Cochliomyia hominivorax* (Diptera: Calliphoridae). *Biol. Bull.*, **124**: 65–83.

LÖBBECKE, E. A., AND R. C. VON BORSTEL, 1962. Mutational response of *Habrobracon* oocytes in metaphase and prophase to ethyl methanesulfonate and nitrogen mustard. *Genetics*, **47**: 853–864.

MOUTSCHEN, JEAN, 1961. Differential sensitivity of mouse spermatogenesis to alkylating agents. *Genetics*, **46**: 291–299.

PLAPP, F. W., JR., W. S. BIGLEY, G. A. CHAPMAN AND G. W. EDDY, 1962. Metabolism of methaphoxide in mosquitoes, house flies and mice. *J. Econ. Ent.*, **55**: 607–613.

PURDOM, C. E., 1960. Mutagenic effects of nitrogen mustard derivatives of azobenzene compounds in *Drosophila melanogaster*. *Biochem. Pharmacol.*, **5**: 206–218.

SPARROW, A. H., 1951. Radiation sensitivity of cells during mitotic and meiotic cycles with emphasis on possible cytochemical changes. *Ann. N. Y. Acad. Sci.*, **51**: 1508–1540.

VON BORSTEL, R. C., 1955. Differential response of meiotic stages in *Habrobracon* eggs to nitrogen mustard. *Genetics*, **40**: 107–116.

WHITING, A. R., 1945. Effects of X-rays on hatchability and on chromosomes of *Habrobracon* eggs treated in first meiotic prophase and metaphase. *Amer. Naturalist*, **79**: 193–227.