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Chemosterilization - A New Field of Research in Tsetse Fly Control

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The success achieved in eradicating the screw-worm (Cochliomyia hominivorax (Coquerel)), first on the island of Curacao (Baumhover et al., 1955) and then throughout its range in the southeastern United States (Knipling, 1960a) established the practicability of Knipling's concept of the sterile-male technique (Knipling, 1960b). This concept involves the use of insects for the destruction of their own species through the induction of sterility in a large proportion of the males, and by utilization of their mating behavior to reach female insects that would not be affected by the usual insecticide techniques.

The success of the screw-worm program also intensified interest in the possible use of chemicals in adaptations or extensions of the sterility approach to insect control. Knipling (1959) discussed the theoretical advantages of the use of chemicals to induce sterility in a large segment of the natural population. Shortly thereafter, the demonstration by LaBrecque (1961) that both male and female house flies (Musca domestica L.) could be sterilized by radiomimetic chemicals directed attention to the possible advantages of chemosterilants over radiation in applying the sterile-male technique.

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At this meeting our interest in chemosterilants lies primarily in their potential usefulness for the control of tsetse flies, and in the research needed to explore the various ways in which they might be used against these pests. Before we begin a discussion of what we need to learn about the effects of chemosterilants on Glossina, however, it would probabl be well to review what we have already learned about their effects on other species of Diptera.

Perhaps it would be well, at this point, to define what we mean by the term chemosterilant, since this is a relatively new word. A chemosterilant is a chemical capable of causing sexual sterility, that is, failure to reproduce, in insects or other organisms.

Insect chemosterilants may act in several ways. They may cause the insects to fail to produce ova or sperm; antimetabolites, when they are also chemosterilants, act in this way. Compounds that cause the death of sperm and ova after they have already been produced would also be considered chemosterilants. A third type of action, and the one in which we are most interested at the present time, is that shown by the radiomimetic compounds. These compounds apparently injure the genetic material in the sperm and ova so severely that, although they remain alive and the sperm retain full motility, the zygotes, if formed, do not complete development into mature progeny. This type of action is desired because the males sterilized in this manner compete readily with normal males for the available females and transfer motile sperm to the spermathecae of the females, with the result that the mating requirements of the females are satisfied to the same extent as in a mating with a normal male.

The three radiomimetic compounds which have been the most widely tested as chemosterilants are apholate, tepa (aphoxide; APO) and metepa (methaphoxide; MAPO).

In practical control operations chemosterilants might be used in two basic ways -- as a substitute for radiation to sterilize insects that had been reared for release in large numbers or as a means of inducing sterility in a large proportion of the natural population, thus avoiding the necessity for rearing and releasing large numbers of insects. For the former purpose chemicals may prove to be more economical than radiation or to cause less general injury in treated males, and they almost certainly will permit greater mobility and dispersion of the rearing and sterilization facilities. However, there are many species of insects which could not readily be reared and released in overwhelming numbers, either because the species is not adaptable to laboratory rearing, because the numbers required to overwhelm the normal population would be too enormous, or because the released insects would themselves be dangerous, destructive, or annoying. To control or eradicate such species, it would be highly advantageous to be able to induce sterility chemically in a large proportion of the natural population in such a way that the sterile males would mate with, and thus render infertile, the females that escaped the sterilizing chemical. In tsetse fly control this might be accomplished if a suitable attractant could be developed for use with the chemosterilants.

Chemosterilants may be effective when given in the food of insects, when applied topically, when used as residual treatments on surfaces on which the insects walk, or when applied to the immature stages or in the larval medium. Not every compound is effective by all the various methods of administration, and species vary in the optimum method of treatment. In house flies apholate, tepa, and metepa all caused sterility in females and males at concentrations of 0.5 to 1.0 per cent when the sexes were separated at emergence, given treated food, and then crossed with untreated flies of the opposite sex, but much lower concentrations induced sterility when both sexes were given treated food from the time of emergence (LaBrecque, 1961; LaBrecque et al., 1963a). Tepa and metepa also sterilized both males and females of the screw-worm fly when fed to either sex separately or to both sexes simultaneously (Crystal, in press). Apholate in the adult diet has also been found to sterilize the screw-worm fly at 0.5 per cent (Chamberlain, 1962), *Drosophila melanogaster* Meigen at 1 per cent (Cantwell and Hennebery, in press), *Anopheles quadrimaculatus* Say at 0.5 per cent, and *Aedes aegypti* (Linnaeus) at 0.1 per cent (Weidhaas et al, 1961).

Irradiation in the pupal stage was the method employed to induce sterility in the screw-worm flies released in the eradication programs. Chemosterilants have also shown some promise for application in the pupal stage. Chamberlain (1962) produced sterility of 40% or higher in adults of the screw-worm fly by dipping pupae of various ages in solutions of apholate. Shaw and Sanchez Riviello (in press) found that the Mexican fruit fly could be sterilized by dipping the puparia for 60 seconds in a 5% solution of tepa in methanol, provided the adults were allowed to emerge on the treated puparia, but not when the puparia were washed and drained. Presumably the fruit flies are actually sterilized in the adult stage as the result of contact with the treated pupae cases but this does not detract from the practical usefulness of the puparial application for this species. Stoch (in press) found that house flies can be sterilized by dipping the pupae in 2.5 to 5 percent ethanol solutions of tepa, metepa, or apholate, but the age of the pupae was very critical, the results were not entirely consistent, and there was sometimes more mortality than desirable. Penetration of the puparium and pupal integument presents a problem that will no doubt be the subject of considerable study in connection with the development of practical chemosterilization procedures.

Some chemosterilants have been highly effective as residual applications for treatment of the adult insects. Such contact chemosterilants would be especially useful for the treatment of insects with piercing-sucking mouthparts or insects that do not feed in the adult stage. Weidhaas (1962) found that males and females of A. quadrimaculatus were sterilized by exposure for 4 hours on deposits of tepa at 10 mg./sq. ft. on glass surfaces; the treatment was equally effective against virgin females and males less than 24 hours old, males that were 4 days old, and females collected in nature that were already inseminated, some of which had already laid one to four batches of eggs when they were collected. Tepa and metepa at 250 mg./sq. ft. on glass surfaces sterilized male and female house flies in exposures of 2 to 4 hours, even after the residues had been aged for 30 days, but apholate was ineffective (Meifert et al., 1963). Apholate deposits on glass were effective against the stable fly, Stomoxys calcitrans (Linnaeus) -- adults exposed for 48 hours on deposits of 10 mg. in a half-pint jar, or for 1 hour on deposits of 100 mg., were completely sterilized, even when the deposit was 24 weeks old (Harris, 1962).

Topical applications, though perhaps not adaptable to large scale sterilization programs, may prove useful in obtaining more precise comparisons of compounds on the basis of the dosage required to produce sterility. However, Couck et al. (in press) in their experiments with topical applications found that when 10 per cent solutions of the chemosterilants were applied to house flies and screw-worm flies at 1 and 2.2 - 2.4 microliters, respectively, per fly, more compounds caused mortality and fewer caused sterility than when the same compounds were given in the adult food

The sexual competitiveness of chemosterilized males is, of course, of the utmost importance. In several series of experiments male house flies sterilized by feeding on 1.0 per cent apholate have been confined in 4-foot cages with untreated males and virgin untreated females at ratios ranging from 1 to 10 treated males per normal male. At all ratios the treated males appeared to be more highly competitive sexually than the untreated males, as the ratio of sterile to fertile eggs was always higher than the ratio of sterile to normal males, even after the data were adjusted to allow for the normal proportion of sterile eggs (LaBrecque et al, 1962a). Males of A. aegypti sterilized by feeding on 1 per cent of apholate in honey solution successfully inseminated normal females, and when normal males and females were caged at various ratios with sterile males, the chemosterilized males mated as readily as the normal males and were equally competitive (Weidhaas and Schmidt, 1963). Males of the Mexican fruit fly that emerged from tepa-treated puparia were sterilized permanently with no deleterious effect on their sexual aggressiveness (Shaw and Sanchez Riviello in press).

The longevity of chemosterilized insects, particularly the males, is also of importance. Murvosh et al. (in press) found that male and female house flies maintained continuously on diets containing 0.5 and 1 per cent of apholate and metepa showed increased mortality after the tenth day of adult life, but by this time the greater part of the sexual activity of the males would already have been accomplished.

The effectiveness of the chemosterilized males in neutralizing the reproductive potential of the normal females in a population will probably depend on their ability to transmit motile sperm, although possibly female of some species will not mate again after a copulation in which they receive seminal fluid without sperm or with lifeless sperm. It is therefo important to know whether chemosterilized males retain the capability of transmitting motile sperm thoughout their lives. We have unpublished data showing that male house flies sterilized by feeding for 5 days on a diet containing 1 per cent of apholate mated repeatedly with virgin females over a period of 28 days and still carried motile sperm in the testes; females inseminated throughout the period laid sterile eggs. On the other hand Cantwell and Henneberry (in press) report the cessation of sperm development in males of D. melanogaster fed 0.25 and 1 per cent apholate, although no observable differences were found in fecundity of inseminated males for one week after they were sterilized.

The pick-up, translocation and fate of chemosterilants in insects is, of course, of the greatest importance. Plapp et al. (1962) found that in larvae and adults of the mosquito, Culex tarsalis Coquillet, the degradation of P^{32}-labeled metepa was complete within 48 hours of administration. Adult house flies degraded 50 per cent of large dosages within 2 hours, and metepa and one major breakdown product were found in the excretory products. When the metepa was injected thoracically at the rate of 2 µg./fly there was no selective accumulation in ovarian tissue.

Dame and Schmidt (in press) found that house flies and two species of mosquitoes, A. quadrimaculatus and A. aegypti, absorbed approximately 7, 7, and 2.5 µg., respectively, during a 4-hour exposure on glass surfaces treated at 10 mg./sq. ft., whereas A. quadrimaculatus absorbed only 27 mµg. from masonite treated at 100 mg./sq. ft. After 3 days of feeding on treated food, the amounts of the chemosterilant absorbed were 3.0, 3.7, and 1.7 µg. in A. quadrimaculatus, house flies, and A. aegypti, respectively. Exposure of mosquito larvae from the third instar through pupation in water treated at 10 p.p.m. resulted in low metepa uptake. The distribution of metepa in the insects was rapid and apparently non-selective, and excretion was rapid in insects exposed to residual deposits. Insects exposed to treated larval medium and food retained a high percentage of their original radioactivity over prolonged periods, but this activity represented detoxified metepa.

Field experiments with chemosterilants have been restricted to a few small tests to explore possible methods of application and evaluation. LaBrecque et al. (1962b, 1963b) and Gouck et al. (in press) obtained encouraging results with corn meal baits containing fly food and 0.5 per cent of tepa or metepa or 0.75 per cent of apholate on garbage dumps and in a poultry house for the control of house flies. Female flies collected in the treated areas showed more than 90 per cent sterility, male flies showed 10 to 100 per cent sterility, and good to excellent reductions in house fly abundance were obtained.

In a male-release experiment Shaw and Sanchez Riviello (in press) released 2-1/4 million tepa-treated puparia of the Mexican fruit fly between February and July in a 10-acre mango grove and obtained substantial protection of the major part of the crop.

Chemosterilants may also prevent the reproduction of disease organisms in vector species. Altman (1963) found that holding mosquitoes (Aedes aegypti) on a tepa residue of 10 mg./sq. ft. either immediately before or after they fed on chicks infected with malaria (Plasmodium gallinaceum) caused a reduction in the percentage of mosquitoes that became infected, a reduction in the mean oöcyst count, and reductions in malaria transmission rates. Tepa also reduced the malaria transmission rates when mosquitoes infected for more than 14 days were held on a tepa residue of 10 mg./sq. ft.

Aziridines are highly reactive compounds, and this characteristic presents some difficulties in their practical application. Their reactivity increases sharply with rising temperature and acidity of the medium. Since this reaction is usually connected with a disappearance of the physiological activity, any attempt to formulate and apply a chemosterilant preparation must be guided by this important characteristic.

The high reactivity of aziridine chemosterilants can, on the other hand, be of great advantage in problems connected with removal of unwanted residues from plants, animals, or any objects contaminated during the treatment.

The activity of chemosterilants is not restricted to insects and the effects on other organisms intentionally or potentially exposed to such materials must be carefully evaluated. Toxicological data are available on a few compounds used in cancer therapy but more must be learned about their chronic toxicity and about possible delayed effects of small dosages. Toxicological studies are now in progress and it would be premature to forecast the conclusions they will produce. The most that can be said at present is that no chemosterilants have yet been recommended for the control of any insect, that they must be considered as hazardous, and that experimental use should avoid the possibility of contact with man or beneficial animals.

Chemosterilants can almost certainly be used in the control of some species to sterilize reared insects for release among natural populations. No doubt they will also prove acceptable for use with various baits and attractants, which would result in the exposure of the pest species and no others. The demonstration of a somewhat greater degree of safety would permit the use of chemosterilants in more general, but still highly selective, methods of application. The question of paramount interest to us today is, what prospect do chemosterilants hold for use in tsetse fly control? We can make some preliminary estimates now, but we need the findings of a thoroughgoing research program to provide a definite answer.

A detailed appraisal of the potential role of the sterility principle for tsetse fly eradication in the light of our present knowledge has been given by Knipling (1963). He assumes fly densities might range from more than 1,000 to less than 200 per square mile, and presents a computation on the effects of releasing sterile males in a low-level area. Beginning with releases at a ratio of 3 sterile males to 1 normal male, and decreasing the release rate by half in each successive 3-month period, the natural population would be reduced to less than 1 per square mile at the end of 12 months, assuming that the normal rate of increase in the absence of control measures would have been 50 per cent in each generation.

The difficulties and assumed high cost of rearing tsetse flies are regarded as a major obstacle to the use of the method, and costs are considered in the appraisal cited above. Through a concerted research effort it might well be possible to rear, or perhaps collect, the insects at a cost that would fall well within the range of practicability. In this connection, it might be pointed out that the difficulties in the mass rearing of screw-worms were considered insurmountable by many when Knipling first proposed the method in 1937.

A third element considered in Knipling's appraisal is the use of sterile males as an adjunct to other tsetse fly control methods. Spraying, for example, might be used to reduce populations to levels at which sterile male releases would become the most economical, and perhaps the ~~only~~ most effective means for the final steps in eradication.

One can hardly fail to agree with the conclusion reached in Knipling's study that the sterile-male principle does hold sufficient promise for practical usefulness in tsetse fly control to merit major attention and research support.

I need not point out to this group that a vast amount of information on the behavior and bionomics of the tsetse fly has been accumulated over the years, much of which has a direct bearing on the practicability of the sterile-male technique, or that research is now under way in various agencies with this possible approach more specifically in mind.

Relatively little has been published on the actual sterilization of tsetse flies. Potts (1958) has shown that gamma radiation can be used to produce sterility in tsetse flies, and that males so sterilized are reasonably competitive in mating. Further radiation studies are needed however, and it will be particularly interesting to compare the cost and efficiency of radiation and chemicals to induce sterility in the male flies.

A research program on the effect of chemosterilants on tsetse flies is now being organized by the Agency for International Development and the Agricultural Research Service of the United States Department of Agriculture in cooperation with the Department of Veterinary Services, Agricultural Research Council of Rhodesia and Nyasaland. Laboratory studies will be headquartered at Salisbury and field studies will be conducted in favorable areas, still to be selected. The work will be supported by necessary resources and services supplied by the Gainesville, Florida, and Beltsville, Maryland, laboratories of the Entomology Research Division, ARS.

Promising chemosterilants developed by the Entomology Research Division in its screening program will be tested for their effectiveness in sterilizing both sexes of at least one of the important tsetse fly species. Glossina morsitans West. has been chosen as the primary species for such evaluation but the investigations will not necessarily be limited to this species.

The first studies will include experiments to determine the relative effectiveness of different methods of application, e.g., administration in the food, exposure of adult flies on treated surfaces, ~~and~~ treatment of puparia, or treatment of the adults. The results will serve as a basis for subsequent investigations to determine how to utilize chemosterilants in practical field trials, that may be undertaken in cooperation with appropriate research agencies in Africa.

The effect of various chemosterilants on the behavior, vigor, and longevity of treated flies in comparison to that of non-treated flies will be determined. Male behavior, particularly sexual competitiveness, will be studied. Since another research group at the same laboratory will be investigating radiation effects on this species it is hoped that comparisons of the two methods of sterilization can be made.

Such investigations on techniques of rearing and handling tsetse flies under laboratory conditions as are necessary to carry out the investigations will be conducted.

The age at which mating occurs in males and females, the number of times each sex will mate, and, if the females mate more than once, the effect of multiple matings with normal and sterile males on the fertility of the females, will be determined.

Studies will be made to determine whether females booby-trapped with chemosterilant-treated pads on the abdomen will induce sterility in males that attempt to mate with them, and whether males will attempt to mate with females that have previously been inseminated, as has been observed with house flies.

Preliminary studies of attractants, particularly sex attractants, will be initiated if sufficient tsetse flies and time are available. Such attractants might be used in connection with chemosterilants to sterilize a large proportion of the natural population without the necessity of mass rearing or collecting.

The ability to establish artificial environmental conditions for normal behavior, reproduction, and survival of tsetse flies will require a detailed knowledge of the ecology of the fly in its natural environment. Intensive ecological investigations will be undertaken with special attention to ways of duplicating favorable natural environmental conditions for rearing tsetse flies. Special attention will be given to developing proper environments for fly production during different times of the year, taking into account the relationship of rainfall, temperature, vegetative cover and other factors affecting reproduction and survival. The habits of the insect with respect to rate and extent of migration during different times of the year will be investigated. The preferred sites for pupation and

resting habits of adults during different times of the year will also be studied. All such information will be utilized in efforts to simulate conditions for rearing the insect in the laboratory or in the field. Primary attention will be given to Glossina morsitans.

Research will be undertaken in large outdoor cages up to 1 acre in size to develop ways of rearing tsetse flies in large numbers. Conditions in the screened cages will simulate the most suitable natural environments. Vegetative cover, suitable resting sites for adults, suitable sites for larviposition and pupation will be provided. Optimum moisture conditions will be maintained by appropriate water management procedures, including stream diversions and impoundments. Different host animals will be placed in cages so that the flies can feed naturally. Pupae or adult flies will be collected from natural populations and placed in such cages. The rate of reproduction will be closely investigated. The objective will be to determine if G. morsitans and possibly other important species can be reared in simulated natural environments with a minimum of care by operators. The ultimate objective will be to rear the insects in large numbers with a minimum of labor and harvest up to 90% of the males for sterilization and release. All females will be used for reproduction purposes.

A 2-year study is planned for the aforementioned phases of the investigations. If the results of the study suggest that the sterile-male technique does indeed hold promise as a practical method of tsetse fly control additional field experiments will be planned.

Literature Cited

Altman, R. M. 1963. The effects of tepa on Plasmodium gallinaceum in Aedes aegypti. Am. J. Hygiene 77: 221-7.

Baumhover, A. H., A. J. Graham, B. A. Bitter, D. E. Hopkins, W. D. New, F. H. Dudley, and R. C. Bushland. 1955. Screw-worm control through the release of sterilized flies. Jour. Econ. Ent. 48: 462-6.

Cantwell, G. E., and T. J. Henneberry. (In press) The effects of gamma radiation and apholate on the reproductive tissues of Drosophila melanogaster. Jour. Insect. Path.

Chamberlain, W. F. 1962. Chemical sterilization of the screw-worm. Jour. Econ. Ent. 55: 240-3.

Crystal, M. M. (In press) The induction of sexual sterility in the screw-worm fly by antimetabolites and alkylating agents. Jour. Econ. Ent.

Dame, David A., and C. H. Schmidt. (In press) Uptake of metepa and its effect on two species of mosquitoes (Anopheles quadrimaculatus, Aedes aegypti) and house flies (Musca domestica) Jour. Econ. Ent.

Gouck, H. K., M. M. Crystal, A. B. Borkovec, and D. W. Meifert.(In press) A comparison of techniques for screening chemosterilants of house flies and screw-worm flies. Jour. Econ. Ent.

Gouck, H. K., D. W. Meifert, and J. B. Gahan. (In press) A field experiment with apholate as a chemosterilant for the control of house flies. Jour. Econ. Ent.

Gouck, H. K. (In press) Chemosterilization of house flies by treatment in the pupal stage. Jour. Econ. Ent.

Harris, R. L. 1962. Chemical induction of sterility in the stable fly. Jour. Econ. Ent. 55: 882-5.

Knipling, E. F. 1959. Sterile-male method of population control. Science 130: 902-4.

Knipling, E. F. 1960a. The eradication of the screw-worm fly. Scientific American 203 (Oct.): 54-61.

Knipling, E. F. 1960b. Use of insects for their own destruction. Jour. Econ. Ent. 53: 415-20.

Knipling, E. F. 1963. Potential role of the sterility principle for tsetse fly eradication. WHO/Vector Control/27, 17 mimeo pp.

LaBrecque, G. C. 1961. Studies with three alkylating agents as house fly sterilants. Jour. Econ. Ent. 54: 684-9.

LaBrecque, G. C., D. W. Meifert, and Carroll N. Smith. 1962a. Mating competitiveness of chemosterilized and normal male house flies. Science 136: 388-9.

LaBrecque, G. C., C. N. Smith, and D. W. Meifert. 1962b. A field experiment in the control of house flies with chemosterilant baits. Jour. Econ. Ent. 55: 449-51.

LaBrecque, G. C., D. W. Meifert, and H. K. Gouck. 1963a. Effectiveness of three 2-methyl-aziridine derivatives as house fly chemosterilants Fla. Ent. 46: 7-10.

LaBrecque, G. C., D. W. Meifert, and R. L. Fye. 1963b. A field study on the control of house flies with chemosterilant techniques. Jour. Econ. Ent. 56: 150-2.

Meifert, D. W., R. L. Fye, and G. C. LaBrecque. 1963. Effect on house flies of exposure to residual applications of chemosterilants. Fla. Ent. 46: 161-8.

Murvosh, C. M., G. C. LaBrecque, and C. N. Smith. (In press). The effect of three chemosterilants on longevity and sterility in the house fly, Musca domestica L.,Jour. Econ. Ent.

Plapp, F. W.,Jr., W. S. Bigley, G. A. Chapman, and G. W. Eddy. 1962. Metabolism of methaphoxide in mosquitoes, house flies, and mice. Jour. Econ. Ent. 55: 607-13.

Potts, W. H. 1958. Sterilization of tsetse flies (Glossina) by gamma irradiation. Ann. Trop. Med. Parasit. 52: 484.

Shaw, J. G., and Sanchez Riviello, M. (In press) Tepa-treated puparia of the Mexican fruit fly for field-release tests. Jour. Econ. Ent.

Weidhaas, D. E. 1962. Chemical sterilization of mosquitoes. Nature 195: 786-7.

Weidhaas, D. E., H. R. Ford, J. B. Gahan, and C. N. Smith. 1961. Preliminary observations on chemosterilization of mosquitoes. N. J. Mosq. Extermin. Assoc. Proc. 48: 106-9.

Weidhaas, D. E., and C. H. Schmidt. 1963. Mating ability of male mosquitoes, Aedes aegypti (L.), sterilized chemically or by gamma radiation. Mosq. News 23: 32-4.

List of Questions for Discussion

1. Are adequate methods available for determining the number of tsetse flies per square mile with sufficient accuracy to compute the number of sterile males that would have to be released to obtain a given ratio of sterile to normal males?
2. Is the available information on the flight range of the various species adequate to predict the width of a fly-free zone, or a zone saturated with sterile males, that would be required to isolate an experimental or control area?
3. Is the information on bionomics adequate to predict whether a decrease in the reproduction rate of, for example, 75% would result in an increased rate of survival for the remaining 25% of the flies?
4. What is the minimum number of flies per square mile required to maintain the species?
5. What is the normal rate of increase from generation to generation? From year to year?
6. Which line of research holds the greatest promise for producing tsetse flies at low cost -- research on indoor colonization, research on outdoor colonization in cages of an acre or more in size, or research on methods of field collection of pupae, including methods to concentrate larviposition by wild flies in artificially improved sites?
7. Laboratory experiments indicate that females may mate more than once. Is there evidence that this does, in fact, occur regularly in nature, where the flies are not in enforced proximity?

8. What known attractive elements in the environment of tsetse flies, either chemical or otherwise, living or lifeless, might be treated with chemicals to sterilize large numbers of flies congregating on them without hazard to humans or beneficial animals? Chemicals that sterilize either by tarsal contact or by ingestion could be used, if appropriate.