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## Resistance to the Chemical Sterilant, Apholate, in *Aedes aegypti*

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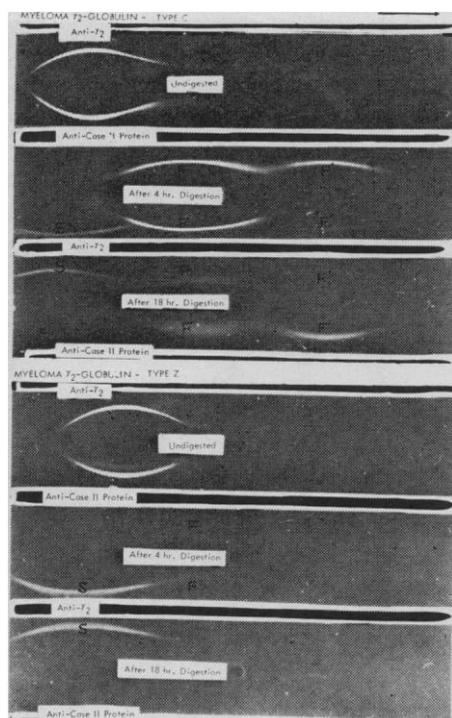


Fig. 3. Immunoelectrophoretic analyses of type C and type Z myeloma  $\gamma_2$ -globulins before and after digestion with papain for 4 and 18 hours.

as the  $F'$  arc is unchanged in intensity as compared with the pattern of the 4-hour digest. In contrast to this behavior, the case V (Z) protein, after 4 hours of papain digestion, shows a marked diminution in intensity and a cathodal displacement of the major precipitin arc ( $F^*$ ) without the development of an  $F'$  arc. Treatment of the case V (Z) protein for 18 hours destroyed all material precipitable by the antiserum to the case II protein.

We then studied the comparative effects of papain treatment on six type C and the two type Z myeloma  $\gamma_2$ -globulins. Representative results of these studies are shown in Fig. 3. These patterns were made with the antiserum to the case II protein, which develops only the F and  $F'$  arcs, and an antiserum to normal 7S  $\gamma_2$ -globulin (anti- $\gamma_2$ ) which also demonstrates the S (slow) fragment. The type C myeloma  $\gamma_2$ -globulin, after 4 hours of papain digestion, shows strong F and  $F'$  arcs in addition to an S arc. After 18 hours of digestion, these three arcs are still visible, although attenuated, and it is evident that the F arc has been diminished to a greater degree than the  $F'$  and S arcs. In contrast to this behavior, the type Z myeloma  $\gamma_2$ -globulin, after 4 hours of papain treatment, shows a prominent S arc, only a very faint trace of an F arc, and no  $F'$  arc. After di-

gestion for 18 hours, only the S arc remains. The faint inner arc visible in the pattern of the undigested type Z myeloma  $\gamma_2$ -globulin developed with the antiserum to the case II protein probably represents a small amount of normal 7S  $\gamma_2$ -globulin, and the very faint F arc seen after 4 hours of digestion may have been derived from that source.

These observations establish the existence of major structural differences between type C and type Z myeloma  $\gamma_2$ -globulins which are comparable to the demonstrated differences in the type C and Z proteins in "Heavy ( $H_{\gamma_2}$ ) chain" disease. These structural differences are reflected in greater susceptibility to papain digestion of type Z than of type C proteins, and the development of an  $F'$  component after papain treatment of type C but not of type Z proteins. Because of the greater susceptibility of type Z molecules to papain degradation, it would be anticipated that the isolated F fragment of papain-treated pooled normal 7S  $\gamma_2$ -globulin would contain exclusively type C molecules, and this has been demonstrated.

Within the obvious and generally recognized limitations of the nomenclature systems now used, the structural differences demonstrated in these studies are tentatively assigned to the H-chains, and more specifically to the portion of the H-chain contained in the F fragment of papain-treated 7S  $\gamma_2$ -globulin.

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#### References and Notes

1. Since the precise structure and polypeptide composition of the  $\gamma$ -globulins are unknown, the designations of the enzymatically and chemically produced subunits as well as the subunits which are found in the various plasma cell dyscrasias, including this disease, must be recognized as tentative.
2. E. C. Franklin, M. Meltzer, F. Guggenheim, J. Lowenstein, *Federation Proc.* **22**, 264 (1963).
3. E. F. Osserman and K. Takatsuki, *Medicine* **42**, 357 (1963).
4. E. C. Franklin, J. Lowenstein, B. Bigelow, M. Meltzer, *Am. J. Med.*, in press.
5. E. F. Osserman and K. Takatsuki, *ibid.*, in press.
6. It is a useful coincidence that the initial, C, of Franklin's first case corresponds to the "common" antigenic type, and that the initial of our case which is representative of the rare antigenic type is Z.
7. R. E. Ballieux, G. Bernier, K. Tominaga, F. W. Putnam, unpublished observations.
8. The group of 18 myeloma  $\gamma_2$ -globulins which were demonstrated to be of type C included proteins of both antigenic group I and group II with respect to their constit-

uent L-chains. The two type Z myeloma  $\gamma_2$ -globulins were of antigenic group I. At present, therefore, there appears to be no correlation between the antigenic types of the constituent H-chains (that is, C or Z) and the constituent L-chains (I or II) of these proteins.

9. Papain digestion was carried out according to the procedure of R. R. Porter, *Biochem. J.* **73**, 119 (1956). We used twice-crystallized papain (Worthington Biochemical Corp.) (1 mg papain per 100 mg protein) in the presence of 0.01M cysteine and 0.002M ethylenediamine-tetraacetic acid (EDTA) at pH 5.6 and at 37°C.
10. These studies were supported by NIH grant CA-02332.

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### Resistance to the Chemical Sterilant, Apholate, in *Aedes aegypti*

Abstract. Increased resistance to the sterilizing effects of apholate was observed in two colonies of *Aedes aegypti* (L.) exposed in the larval stage of each generation to concentrations of apholate that induced about 90 to 40 percent sterility in the eggs laid by the ensuing adults.

Since the inception of research on the potential of chemosterilants as insect control agents, many persons have asked whether insects could develop resistance to the sterilizing effects of these compounds. On the other hand, because the action of certain chemosterilants influences the induction of dominant lethal mutations, it has been theorized that continued exposure to substerilizing dosages or to dosages causing only partial sterility might result in accumulation of genetic defects and ultimate death of a colony.

In 1962 efforts were initiated to determine whether the yellow fever mosquito, *Aedes aegypti* (L.), could develop resistance to apholate (1). Weidhaas *et al.* (2) had reported that sterility could be induced in *A. aegypti* by feeding adult mosquitoes on honey solution containing 0.1 percent of apholate. Weidhaas (3) had also demonstrated that exposure of larvae of *A. aegypti*, from the third instar to pupation, in water containing 10 parts of apholate per million produced about 90 percent sterility in the ensuing males and about 50 percent sterility in females. When both sexes were treated, sterility was about 98 percent. In later experiments at this laboratory sterility induced by this larval treatment sometimes reached 100 percent. For our experiments in development of resist-

ance, selection pressure was exerted by treatment in the larval stage.

Two different colonies of *A. aegypti* were subjected to selection. Treatment of the first colony began in December 1962, and a second colony was subjected to selection pressure beginning in July 1963.

The strain of *A. aegypti* used in both experiments was from our regular colony, maintained at this laboratory for more than 20 years without deliberate exposure to insecticides. In each generation several hundred third-instar larvae were placed in shallow exposure pans that contained 1 liter of a solution of apholate (5 ppm) in tapwater. In the first experiment the number of larvae in each pan varied, depending on the quantity available; in the second experiment 300 larvae were always used in each pan. After 24 hours, food consisting of powdered dog biscuit was added, and larvae were allowed to continue development in the treated water until they reached the pupal stage. Pupae were removed to untreated water, and emerging adults were allowed to remain together in a screen cage for about 5 days to permit mating. Females were then given a blood meal on a guinea pig. A day later a container which had been lined with filter paper, and containing water to keep the filter paper damp, was placed in the cage for oviposition. Eggs deposited by all the laying females

were used to produce the next generation of the colony. In addition, the percentage hatch of eggs laid in each generation was determined separately. In the first experiment females were individually confined for oviposition, and the percentage hatch was calculated for all eggs laid. In each generation 1000 to 5000 eggs were checked for sterility. In the second experiment a sample of about 200 eggs, taken at random from eggs laid by all females about 1 week after deposition, was used to determine percentage hatch. A complete cycle from third instar to third instar required about 3 weeks.

In the second experiment, beginning with the  $F_7$  generation of the apholate-treated colony, larvae from the regular colony were treated with the same dosage of apholate as those in the experimental colony. This procedure provided a direct comparison between the sterility induced in the selected and in the unselected colonies. The percentage sterility in eggs from untreated mosquitoes from the regular colony was also determined in each generation (Table 1).

In the first experiment sterility of eggs from females exposed to 5 ppm of apholate gradually declined from 96 percent in the parent generation to 46 percent in the  $F_4$  generation. Larvae from the  $F_5$  generation were exposed to 15 ppm, a dosage which usually causes complete or almost complete sterility. Resulting sterility of eggs from the females was 72 percent or 24 percent less than that obtained with the initial selection dosage of 5 ppm. The percentage sterility in the control colony ranged from 2 percent to 48 percent (average 13.5 percent); however, in 4 out of the 6 hatchings sterility was 6 percent or less.

In the second experiment, sterility ranged from 77 percent to 91 percent for the parent and first five generations, but decreased in the next three generations (38 percent to 52 percent). In the  $F_6$  generation the selection concentration was increased to 10 ppm. This concentration caused 81 percent and 88 percent sterility in the next two generations, but in the  $F_{11}$  generation sterility dropped to 59 percent. At this selection concentration, complete sterility was obtained with treated mosquitoes from the regular colony. In addition, these females produced far fewer eggs than females from the apholate colony. Sterility among untreated control females ranged from 0 percent to 19 percent.

These data indicate that *A. aegypti* can develop resistance to apholate. The degree of resistance encountered to date is not great, probably between four and five times that of the normal strain.

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#### References and Notes

1. The chemical name of apholate is 2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis-(1-aziridinyl)-1,3,5,2,4,6-triazatriphosphorine.
  2. D. E. Weidhaas, H. R. Ford, J. B. Gahan, C. N. Smith, *New Jersey Mosquito Extermination Assoc. Proc.* 48, 106 (1961).
  3. D. E. Weidhaas, *Nature* 195, 786 (1962).
  4. Supported in part by funds transferred from the U.S. Army Medical Research and Development Command, Office of the Surgeon General.
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### Polydactylism in the Offspring of Mice Injected with 5-Bromodeoxyuridine

Abstract. *The intraperitoneal injection of multiple doses of 5-bromodeoxyuridine (BUDR) into pregnant mice produced abnormalities limited to the hind limbs of the offspring. This effect is probably a result of a metabolic lesion. When injected into newborn mice, BUDR did not increase the incidence of tumors.*

A mutagen, 5-bromodeoxyuridine (BUDR), was investigated for possible somatic effects on hybrid mice. The potential carcinogenicity of this mutagen was also studied by direct treatment of newborn inbred mice. The BUDR was selected because its mutagenic action is well understood (1), and because it enters the DNA of mammalian cells while 5-bromouracil does not (2).

Mice of strains A and C3H-MF were used. The breeding mice were 3 to 5 months old and all the females were virgin. The somatic effect of BUDR was studied in hybrid embryos of C3H-MF males and strain A females. At the time of discovery of vaginal plug, considered as zero time in gestation period, the pregnant females were isolated in individual cages. The drug was dissolved in isotonic saline, and a

Table 1. Sterility of eggs from a colony of *Aedes aegypti* exposed in the larval stage of each generation to selection pressure with apholate and from treated and untreated mosquitoes from the regular colony.

Generation of apholate colony	Concentration apholate (ppm)	Sterility of mosquitoes exposed to apholate		Sterility of eggs from untreated regular colony (%)
		Apholate colony (%)	Regular colony (%)	
<i>Experiment 1</i>				
Parent	5	96		2
1	5	89		20
2	5	75		48
3	5	59		3
4	5	46		6
5	15	72		2
<i>Experiment 2</i>				
Parent	5	91		6
1	5	85		1
2	5	90		0
3	5	82		1
4	5	85		2
5	5	77		19
6	5	38		15
7	5	52	84	8
8	5	50	93	17
9	10	81	100	4
10	10	88	100	18
11	10	59	100	4