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).

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 (%)	
Experiment 1				
Parent	5	96		2
1	5	89		20
2	5	75		48
3	5	59		3
4	5	46		6
5	15	72		2
Experiment 2				
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

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_9 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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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. Polydactyly in offspring of mice injected during pregnancy with 5-bromodeoxyuridine.

Daily dose (mg/kg)	Treatment (days of gestation)		No. of mothers	Total progeny	Average litter size	No. with polydactyly*
	Start	End				
124	9	17	2	10	5.0	2 (20)
124	9	16	2	12	6.0	4 (33)
124	9	14	4	22	5.5	12 (55)
124	9	13	2	10	5.0	0
124	6	14	2	9†	4.5	1 (11)
90	6	15	15	80‡	5.3	5 (6.3)
90	3	13	3	16	5.3	0
60	6	14	8	48	6.0	1 (2.1)
20	6	13	14	102	7.3	0
0			29	225	7.8	0

*Numbers in parentheses indicate percentages. †Two (22 percent) of these animals exhibited closed eye. ‡One (1.3 percent) animal exhibited Danforth's duplication; and one exhibited luxoid.

variety of dosages was injected over several days, commencing on the 3rd, 6th, or 9th day of pregnancy. Animals injected with saline were used as controls for each group. Food and water were available all the time. Mice were examined macroscopically at time of birth for malformations and at the time of weaning for other somatic effects. Eight mice showing abnormalities were interbred for three generations. These mice were also bred to normal mice of strain A for one generation and then backcrossed to the abnormal parent for two more generations.

In the study for possible carcinogenicity, 59 strain-A mice of both sexes were injected with BUDR. When under 24 hours of age, the mice received an injection of 1 mg; at 1 week, 4 mg; and at 2, 3, 4, 5, and 6 weeks, 5 mg of BUDR. The approximate weight of mice receiving 1, 4, and 5 mg was 1, 5, and 12 grams, respectively. All treated animals were killed at 8 months of age and examined for malignancies.

Information on the number of treated females that had offspring, the mean litter size, and the abnormal progeny is presented in Table 1. Of the 225 progeny from 29 mothers injected with saline (average litter size, 7.8) none had any visible abnormalities. Among the animals from treated mothers, the majority of the abnormal progeny had uncomplicated polydactylism with the exception of one case of luxoid and of posterior reduplication (Danforth's reduplication) (*3*), which resemble certain cases of duplicitas posterior in man. In two mice, only the left foot was affected; in two other mice both feet were affected; and in the rest of the mice just the right foot was affected. The abnormality was limited to the big toe of the hind foot, with most animals showing the anomaly on only the right side. The number of mice with closed eye (failure of lids to open) was too small for consideration. Although this anomaly was not seen in the controls, it has been observed frequently in experiments with the mice of strain A.

When four different pairs of abnormal mice were bred and the resulting progeny backcrossed to the abnormal parents for two consecutive generations, no polydactylous mice were found among the 234 young examined. Experiments in which other abnormal mice were outcrossed to normal strain A mice failed to show any cases of polydactylism even when the subsequent progeny were bred for two additional generations to the abnormal parent.

The anomalies produced in these experiments must be considered due to a biochemical lesion, in contrast to the polydactylisms described by Chase (*4*) and Holt (*5*) which were attributed to a recessive gene and multiple factorial inheritance, respectively. In all the experiments described here, the 5-bromodeoxyuridine was injected during the period in which the anterior and posterior limb bud swellings occur. Abnormal progeny were noted when the drug was used at all concentrations except 20 mg per kilogram per day; however, most of the polydactyl animals occurred when the highest dosage was used. As might be expected, litter size was most influenced when the treatment was begun at day 3, near the time of implantation, or at day 6 of gestation, during early development. Comparison of the average litter size from treated mothers with that from untreated mothers indicates that treatment does reduce the litter size.

Careful examination of coat color of all treated progeny, normal and abnormal, revealed no mosaic patterns which would be expected if treatment had been successful in converting the heterozygous condition for agouti, black, or dilute coat color to the homozygous condition.

Autopsy of strain A mice treated postnatally with BUDR revealed six lung adenomas and one breast adenocarcinoma in 59 mice. This can be compared with six lung adenomas in 70 control mice. Consequently, under the conditions of the experiments, BUDR cannot be considered carcinogenic.

Since organization of the embryo occurs early in gestation, interference with essential metabolic processes would lead to a variety of monstrosities. Most of these would be incompatible with life, so only the less severe ones would be expected to survive. Consequently, direct comparison cannot be made of results obtained in these experiments with those obtained with nitrogen mustard (*6*), nicotine (*7*), or thalidomide (*8*) in which the effects were usually determined by examination of the fetuses prior to expected delivery. Despite the different examination procedure, it is interesting to point out the results obtained by Dagg (see *9*) with 5-fluorouracil. Deformities of the tail were most prevalent followed by limb defects which included both hyperphalangism and reduction in size of the hind feet. Other deformities were also found, dependent upon time of treatment and dosage.

The metabolic pathways of 5-bromouracil, 5-bromodeoxyuridine, and 5-fluorouracil have been studied extensively (*1*). 5-Bromouracil is a mutagen which can compete with adenine as well as thymine. When thymine is lacking, addition of 5-bromouracil or BUDR to the growth medium of microbial systems leads to the quantitative replacement of thymine by 5-bromouracil in DNA. 5-Bromodeoxyuridine is considered a more effective mutagen than 5-bromouracil because it may be more readily converted into the deoxynucleotide triphosphate and interferes less with the formation of uracil and cytosine. 5-Fluorouracil is rapidly metabolized and excreted (*10*). It has a number of biochemical effects; the most important may be the inhibition of the methylation reaction leading to the formation of thymidine or thymidylic acid (*11*).

These results show that 5-bromodeoxyuridine acts as a highly specific teratogen in mice. The absence of any other somatic effects or inheritance of the induced polydactylism indicates,

with a high degree of certainty, that the mutagen interferes with developmental process. The difference in effect obtained with BUDR and 5-fluorouracil may be due to the different strains of mice used as well as to the nature of the compounds and methods of observation.

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3 June 1964

Histochemical Demonstration of Uptake of Exogenous Norepinephrine by Adrenergic Fibers in vitro

Abstract. *Adrenergic fibers in isolated iris preparations were demonstrated histochemically by means of the formaldehyde condensation technique. In the presence of adenosine triphosphate (ATP) there was a net uptake of norepinephrine added in vitro by the adrenergic fibers producing a striking increase in fluorescence. In the absence of ATP only a slight increase in fluorescence was observed when the tissue was exposed to high concentrations of norepinephrine.*

Several reports indicate that exogenous norepinephrine can be taken up by tissues containing adrenergic fibers (*1*); however, the site of norepinephrine concentration within the tissue is not established. Studies with tritiated norepinephrine indicate that at least part of the exogenous norepinephrine is found in the norepinephrine-containing particles within adrenergic nerve fibers (*2*). These findings suggest that exogenous norepinephrine can enter the adrenergic storage sites but do not show conclusively whether the presence of tritiated norepinephrine in the adrenergic "granules" reflects true net uptake or simple exchange diffusion. Other studies indicate a net uptake of norepinephrine by certain tissues (*3*). A decrease in the spontaneous release of norepinephrine contained within isolated adrenergic nerve "granules" in vitro has been shown by von Euler and Lishajko (*4*). This was found to be potentiated by the presence of adenosine triphosphate (ATP) in the medium.

Falck's method (*5*) for the histochemical demonstration of norepinephrine in isolated tissues of rat iris was used. This method depends on the production of fluorescent condensation products of norepinephrine with formaldehyde (*6*). The freshly removed rat iris was washed in Tyrode's solution, mounted on a slide, and allowed to dry in room air for 10 minutes. It was subsequently exposed to paraformaldehyde fumes in a closed vessel at 80°C for 35 minutes and examined under the fluorescence microscope (*7*). A mercury vapor lamp was used as the light source with a Schott BG 12 or a Corning 5-58 filter. A Kodak No. 15 filter was used at the ocular. This arrangement permits the observation of the green fluorescence of the condensation product after excitation with a light spectrum which includes mainly the 405 mμ and 436 mμ mercury lines.

Figure 1 shows an iris preparation photographed at optimum exposure for the demonstration of the fluorescent fibers. It shows the characteristic network of the terminal adrenergic plexus. When similar preparations were first exposed to norepinephrine in the presence of ATP and subsequently treated with formaldehyde, a striking increase in the intensity of the fluorescence of the fibers was observed. This increase was so marked that it was impossible to obtain clear photographs of these tissues if the same exposure and development times were used as in the controls. The photomicrographs in Figs. 2 and 3 were therefore taken at a reduced photographic exposure. Figure 2*a* shows a typical area of an iris treated with paraformaldehyde immediately after removal. The characteristic network of fluorescent fibers can be seen in this preparation. No change was observed when similar preparations were exposed to a buffered glucose-free Tyrode's solution at *p*H 7.4. Figure 2*c* shows another iris exposed to a similar solution at the same *p*H but containing 50 μg/ml of norepinephrine and subsequently

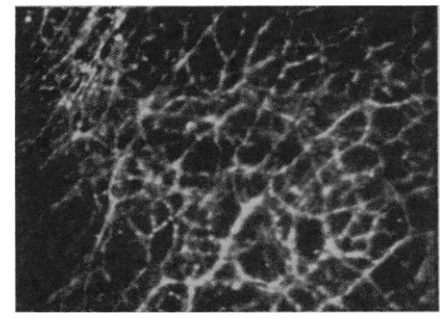

Fig. 1. Adrenergic plexus in rat iris (× 200). Polaroid No. 10,000 with 7-second exposure. Control.

washed in four changes of Tyrode's solution for 15 minutes each. For these records, identical exposure and development times were used. Only a slight increase in the fluorescence intensity of the fibers was seen in this last preparation (Fig. 2*b*) or in several other similar preparations exposed to different concentrations of norepinephrine. However, Fig. 2*b* shows that

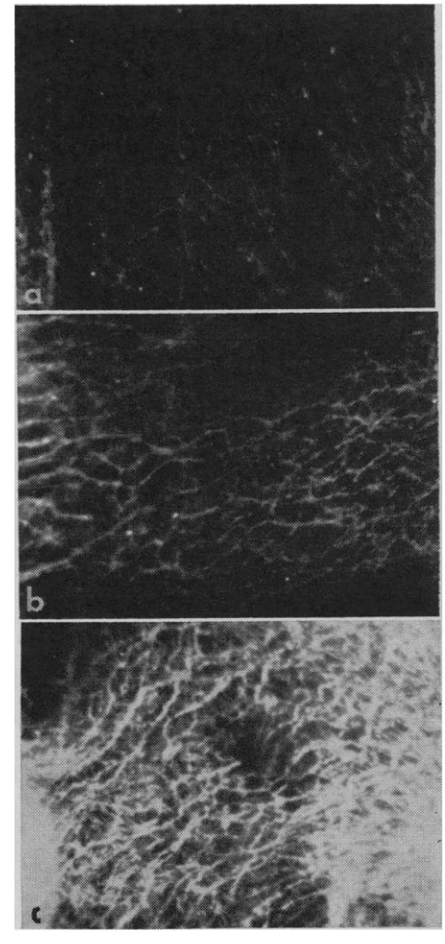

Fig. 2. Rat iris preparations at 4-second exposure. *a*, Control; *b*, in Tyrode's solution with 50 μg/ml of norepinephrine; *c*, 50 μg/ml of norepinephrine plus 1 μg/ml of ATP.