immediately to the brain-stimulation lever at the termination of this period. The figure in parenthesis gives the number of tone presentations before the animal switched to the food lever; this figure is followed by the time (in minutes) which elapsed between the first tone presentation and the response on the food lever.

Figure 2 summarizes the data for all animals during their last four tests. Bar graphs were plotted for the mean elapsed time and for the mean tone presentations before the switch to the food lever. The animals required an average of 2.1 tone A presentations, with an average elapsed time of 0.07 minutes, before switching to the food lever, while they rarely switched to the food lever during the tone B period. For purposes of calculation, 1.5 minutes was scored when the animals did not respond on the food lever during a tone period.

The results demonstrate not only that the animals were capable of distinguishing between the two tones during intracranial stimulation, but that, at least under certain conditions, they were capable of terminating self-stimulation to respond to other reinforcements. In subsequent experiments animals were found to differentiate between stimuli appearing simultaneously with brain stimulation, but whether or not they responded depended upon the reinforcing effect of the brain stimulation and the consequence of responding to the second lever.

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Allelic Genes in the Housefly Producing Modified Enzymes That Cause Organophosphate Resistance

Abstract. In all of six phosphate-resistant strains of *Musca domestica* L. a mutant gene is present which produces an altered ali-esterase. The modified enzymes are no longer irreversibly inhibited by the oxygen analogs of the insecticides to which the strains are resistant but can slowly convert them. In five of the strains the resistance is caused by this gene only.

It is generally conceded that the fundamental effect of gene mutation is the production of altered proteins. However, in only a few cases has this actually been demonstrated (*1*). From the study presented here, it appears that organophosphate resistance in the housefly, *Musca domestica* L., is mainly due to the production of such an altered protein. The adaptive value of this change is of great importance to the species under the altered conditions of life.

In the housefly the use of the organophosphate insecticides Parathion, Diazinon, and Malathion (*2*) has led to the development of resistant strains (*3*). Since these phosphorothionates, after conversion to the corresponding oxygen analogs paraoxon, diazoxon, and malaoxon, act as strong inhibitors of different esterases in the insect, a comparison was made between the esterase activities shown by homogenates of resistant and susceptible strains. A curious difference was found: homogenates of all the phosphate-resistant strains studied showed a very low ali-esterase activity toward substrates such as methyl- and phenylbutyrate, whereas high activity was found in all the susceptible strains investigated (*4*). Low activity has now been observed in five strains resistant to Parathion and Diazinon from Europe and North America and in two strains from North America resistant to Malathion. High activity is present in six organophosphate-susceptible strains, some of which are resistant to the chlorinated hydrocarbons DDT and γ-BHC (benzene hexachloride).

The low esterase activity was found to be caused by an autosomal gene (*5*) (indicated as *a*; the corresponding gene for high esterase activity is indicated as a^+). The mean esterase activities toward methylbutyrate of a homogenate of single male flies of the genetic constitutions *aa*, aa^+, and a^+a^+, respectively, are about 50, 175, and 300 (expressed as microliters of CO_2 produced in the Warburg manometer in 30 minutes under certain conditions). Furthermore, it was found that in four out of five strains studied the phosphate resistance depends mainly on one gene (*5*, *6*), which is identical with the *a* gene. In the fifth strain the *a* factor is responsible for only a part of the resistance; in addition, one or more other resistance genes are present which do not affect esterase activity.

Although the *a* genes seem to be equal in their effect on the esterase activity, they differ widely in the specificity and degree of resistance which they confer on the strains. At least three different *a* genes have been discerned so far (Table 1, strains D, C, and G). The fact that they have the same influence on the esterase activity and all cause resistance suggests that they may be alleles. Nguy and Busvine (*6*) studied this possibility for the Parathion-Diazinon resistant strain C and the Malathion resistant strain H. Hybrids of these strains were backcrossed with the susceptible strain. Since the offspring consisted of about 50 percent Malathion resistant and 50 percent Parathion-Diazinon resistant flies (resistance is semidominant), these workers concluded that the two genes are allelic or otherwise closely linked.

We studied the possible allelism in the Parathion-Diazinon resistant strains F and C and the Malathion resistant strain G, estimating the esterase activity instead of resistance. The crosses F × G and C × G were made, and f_1 and f_2 generations were obtained. The f_1 flies had low esterase activity. Low

Table 1. Susceptibility and in vitro breakdown capacity in some strains of houseflies. The in vitro breakdown was measured in the following way. To a series of samples of homogenates, increasing amounts of inhibitor were added. After 2 hours of incubation the presence or absence of inhibitor was tested by adding fresh cholinesterase. The breakdown capacity was calculated by taking the mean of the amount of the inhibitor that had been converted completely and the next higher amount which had not yet been completely degraded. In strains without a breakdown enzyme a certain amount of inhibitor was bound by the homogenate. However, this amount (indicated by an asterisk) does not increase with time.

LD_{50} Diazinon (μg/jar)	Breakdown diazoxon (mμg/2 hr)	LD_{50} Malathion (μg/fly)	Breakdown malaoxon (mμg/2 hr)
	Strain S		
2	5*	0.6	4*
	Strain D		
12	15		
	Strain C		
62	72	2.5	13
	Strain G		
4	7*	25	130
	Strain F		
100	24		
	Strain F_a		
11	17		
	Strain F_b		
11	5*		

activity was also found in all of the 98 individual flies tested of the f_2 of the cross F × G and in all of the 52 flies tested of the f_2 of C × G. If two nonallelic genes for low esterase activity had been present, ¼ of the f_2 flies would have had only one *a* gene (intermediate esterase activity) and 1/16 would have had no *a* genes (high esterase activity). Since low activity was found, the above data indicate allelism or close linkage.

It has been found that homogenates of flies resistant to Parathion and Diazinon contain an enzyme which can degrade the oxygen analogs of these insecticides (*7*). From the fact that the activity of this enzyme parallels the degree of resistance in two of the strains in which resistance is caused by one gene only, we concluded that the enzyme is made under the influence of the *a* gene (Table 1, strains D and C). The breakdown enzymes in the resistant strains thus would be modifications of the ali-esterase normally made under the influence of the a^+ gene. Two sets of data provide evidence to support this conclusion.

First, a substrain F_a was obtained in which the *a* factor of the resistant strain F was incorporated into the genome of the susceptible strain S. This was done by crossing strain F with strain S and by subsequent repeated backcrossing to strain S. Concurrently, selection for low ali-esterase activity was made. Homogenates of the F_a flies showed a breakdown capacity which was only a little lower than that of the F flies. This shows that breakdown capacity and low ali-esterase activity are dependent on one and the same gene. This was further borne out by the absence of breakdown in substrain F_b (Table 1). This strain showed about the same resistance as strain F_a but contains the a^+ allele, and its resistance must be brought about by one or more other factors (strain F also has more than one resistance factor). In a similar experiment attempts were made to transfer the *a* gene from the highly resistant strain G into strain S. This strain should have shown high resistance and breakdown capacity. However, it proved impossible to obtain such a strain in this case, probably as a result of the presence of a lethal factor (*5*).

Second, since the *a* genes are alleles and in strains resistant to Parathion and Diazinon a breakdown enzyme is produced for the corresponding phosphates, Malathion resistant strains should produce an enzyme capable of degrading malaoxon. This was found to be the case (*7*).

The mutant enzymes present in the strains resistant to Parathion and Diazinon have been shown to possess a very high affinity for the oxygen analogs of these organophosphates. Nearly complete saturation of the enzyme is obtained with substrate concentrations of the order of $10^{-8}M$. Since it has been found that the ali-esterase is readily phosphorylated by these organophosphates (*8*), the main difference is in the rate of dephosphorylation. This is practically zero for the ali-esterase, and thus there is an irreversible inhibition, while a slow but definite turnover is present in the breakdown enzymes. If it is assumed that the concentration of the "mutant" enzymes is equal to that of the ali-esterase, the turnover number must be of the order of 0.1 per minute.

Although most of the activity of "resistant homogenates" toward substrates such as methylbutyrate is brought about by other enzymes, there is evidence that a small part of the activity is due to the breakdown enzyme. This evidence was obtained by comparing the hydrolytic activities of homogenates in the presence of the organophosphates with the activities of other homogenates in which the added phosphate had been completely degraded. Though the difference was small, the activities of the latter homogenates were significantly higher. This may well be explained by the assumption that the breakdown enzyme, which will be saturated by the organophosphates as long as these are present, regains its reactivity to methylbutyrate after the degradation of the organophosphates has been completed. This activity is only 1 to 3 percent of the activity of the ali-esterase of the susceptible flies, but, still, this finding supports the view that ali-esterase and breakdown enzymes are related.

It can be calculated that the turnover number of the breakdown enzyme for methylbutyrate is at least 10^4 times that for the organophosphates. Yet there seems little doubt that the degradation of the organophosphates constitutes a physiologically very important feature of the breakdown enzyme.

The natural function, if any, of the ali-esterase in the flies is unknown. It is possible that the breakdown enzymes are as active as the ali-esterase in converting some unknown natural substrate. Whatever the function may be, evidently it can be dispensed with for at least some hours, since the enzyme is blocked as long as it is protecting the insect from intoxication by degrading the organophosphate.

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New Test for the Biological Assay of Oxytocin

Abstract. A strip of mammary gland is removed from a lactating rabbit and suspended in a bath. The contractions of the strip are recorded isometrically. The strip shows no spontaneous activity and responds with reasonable linearity and stability to oxytocin at concentrations ranging from 0.5 to 10 milliunits per milliliter.

Lactating rabbits from the 15th to the 30th day postpartum are anesthetized with a short-acting barbiturate. A mammary gland is separated from skin and abdominal fascia and a radial strip of gland tissue removed, two parallel cuts being made from the periphery to the teat. Strips so obtained are usually 3 to 4 cm long, 0.5 cm wide, and 2 to 4 mm thick. The strip is suspended in a bath of small volume (1.5 ml) containing Tyrode's solution at 38°C. Isometric contractions of the strip are recorded with a strain gauge of high sensitivity (Statham Model, G7A ± 0.15 oz) and a recording galvanometer

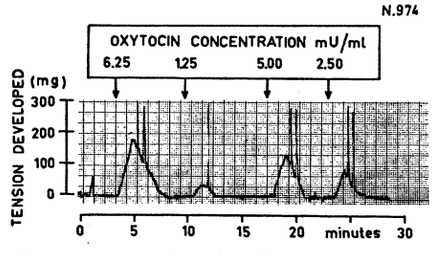

Fig. 1. Isometric recording of the contractions of an isolated strip of rabbit mammary gland. There are no spontaneous contractions. Concentrations of oxytocin from 1.25 to 6.25 milliunits (mU) per milliliter evoke responses which are seen as slow increases in tension, recovery from which takes several minutes. The brief spikelike effects are due to changing the bath fluid.