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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References and Notes

1. G. Pontecorvo, *Trends in Genetic Analysis* (Columbia Univ. Press, New York, 1958), p. 70.
2. Parathion, $(C_2H_5O)_2P(S)OC_6H_5pNO_2$; Diazinon $(C_2H_5O)_2$ P(S)O-2-isopropyl-6-methyl-4 pyrimidyl; Malathion, $(CH_3O)_2$ P(S)SCH $(COOC_2H_5)CH_2(COOC_2H_5)$.
3. J. Keiding, *Science* **123**, 1173 (1956); G. Sacca, *Riv. parassitol.* **18**, 289 (1957); R. W. Fay, J. W. Kilpatrick, G. C. Morris, *J. Econ. Entomol.* **51**, 452 (1958).
4. K. van Asperen and F. J. Oppenoorth, *Entomologia exptl. applicata* **2**, 48 (1959).
5. F. J. Oppenoorth, *ibid.* **2**, 303 (1959).
6. V. D. Nguy and J. R. Busvine, *Bull. World Health Organization*, in press.
7. We are greatly indebted to Dr. R. D. O'Brien, Pesticides Research Institute, London, Ontario, for providing a sample of malaoxon.
8. K. van Asperen and F. J. Oppenoorth, *Entomologia exptl. applicata* **3**, 68 (1960).

11 February 1960

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

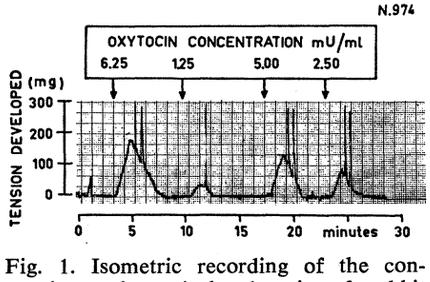

Fig. 1. Isometric recording of the contractions of an isolated strip of rabbit mammary gland. There are no spontaneous contractions. Concentrations of oxytocin from 1.25 to 6.25 milliunits (mU) per milliliter evoke responses which are seen as slow increases in tension, recovery from which takes several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

system (Sanborn Poly Viso). If kept at 3°C in Tyrode's solution, such strips retain for several days their ability to react to oxytocin.

The strips of mammary gland show no spontaneous contractions. They respond consistently to oxytocin, developing tensions which may reach values of the order of 500 mg (Fig. 1). Contraction develops slowly, taking about 1 minute from the onset to the peak. Relaxation occurs even if oxytocin is not washed out from the bath. The lowest concentration of oxytocin detected by the mammary gland strip has been 0.1 milliunit/ml. Within a range from 0.5 milliunit/ml to 10 milliunit/ml the tension developed by the contraction of the strip is in direct linear relationship with the concentration of oxytocin (Fig. 2). The dose-response curve remains remarkably constant for several hours if the resting tension is readjusted to a constant value before each observation. The most commonly used values of resting tension are between 50 and 100 mg. Furthermore, the mammary gland strip does not contract when heparinized blood or plasma is added to the bath, thus making possible the direct determination of oxytocin in these fluids.

The linearity and the stability of the dose-response curve of the mammary strip offer considerable advantages over other tests currently employed for the assay of oxytocin. Moreover, with strips of mammary tissue, as with the mammary gland studied in vivo, specificity is high and spontaneous activity absent. Because of these properties the mammary strip compares favorably with the isolated rat uterus which frequently exhibits spontaneous activity and also responds to a great variety of substances occurring naturally in normal blood.

The sensitivity of the mammary strip

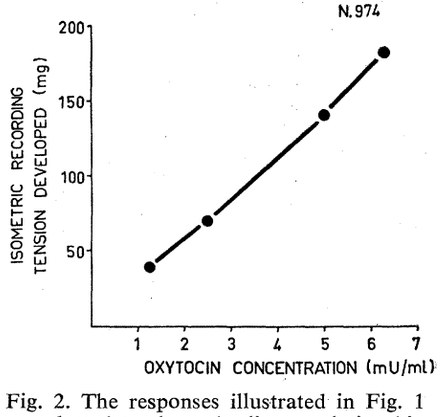

Fig. 2. The responses illustrated in Fig. 1 are plotted to show the linear relationship between concentration of oxytocin and tension recorded from the isolated strip of mammary tissue.

test is 5 to 10 times greater than that of the response of the intact mammary gland to oxytocin given intravenously. Sensitivity is, however, less than that obtained in vivo when the oxytocin is injected into the arteries supplying the mammary gland (*1, 2*). It is also less than the sensitivity of the superfused rat uterus (*1, 3, 4*).

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References and Notes

1. R. J. Fitzpatrick in *Oxytocin* (Pergamon, London, in press).
2. V. González-Panizza, Y. Sica-Blanco, C. Méndez-Bauer in *ibid.*
3. R. J. Fitzpatrick, *The Neurohypophysis* (Butterworths, London, 1957), p. 203.
4. This work was aided by a grant from the Rockefeller Foundation and from the Josiah Macy, Jr. Foundation, New York.

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Feather Mites and Ornithosis

Abstract. Ornithosis virus has been isolated from several species of poultry ectoparasites, suggesting for the first time that this too may be a vector-borne infection.

A virus of the ornithosis group, for which we prefer the designation *Bedsonia* rather than *Miyagawanella*, has been isolated by mouse passage from ectoparasites collected under two epizootiologically different circumstances in two widely separated geographical areas.

In the first case there had been some serologic, but no clinical, evidence of this infection in a chicken flock in the preceding 3 years. When an observer reported an extensive infestation with mites, the Hooper Foundation requested that some of these be collected so that they could be examined for the ornithosis virus. The ectoparasites were collected from a rooster with an indirect complement fixation titer of 1:16, and in the first intraperitoneal mouse passage and in the subsequent ones there was gross and microscopic evidence of the virus. These insects were not identified, but in the further pursuit of this very interesting observation, lice, some identified by an entomologist to be *Menopon gallinae (M. pallidum)* were collected from ten hens and again the virus was isolated.

Although it has been known since early in the 1930's that activation of latent infection accounts for the sporadicity of this infection, in some cases this explanation has not sufficed, in incubator-hatched poultry for example. This isolation suggested a hitherto unrecognized virus-perpetuating system. But the misleading interference of masked infections in laboratory mice, although there was no special reason to suspect them in this observation, and the fact that the isolation was made in a laboratory where other work on this virus is being carried on, made it necessary to make some further studies.

Since ornithosis had been occurring annually and inexplicably in a turkey flock, the next step was to see whether there might be infected ectoparasites on the premises of that flock. A public health official who had been participating in investigations of the flock collected miscellaneous material, including insects, from nests in which there had been no turkeys for about 2½ months. Most of the insects were still alive when they were identified and separated for the isolation tests. Mouse passage of 117 pools has again revealed the virus, despite the fact that the mites could not have fed on infected turkeys for at least 3½ months before the test. Two of the infected pools consisted of Glycyphagidae, Berlese, probably *Glycyphagus domesticus*. The other was a mixture of *Haemogamasus, Haemolaelaps, Ornithonyssus*, and *Cheyletus*. Isolations have been made also from *Cheyletus* and a *Mesostigmata*, possibly Arctacaridae.

This information is given to stimulate others to investigate this possibility further and to consider such ectoparasites as potential reservoirs or vectors of other infections.

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Rapid and Reversible Block of Electrical Activity by Powerful Marine Biotoxins

Abstract. Puffer-fish poison and clam poison reversibly inhibit conduction in single nerve fiber preparation of frog in a concentration of $3 \times 10^{-10} M$. In the isolated electroplax of *Electrophorus electricus* higher concentrations block both transmission and conduction. Neither toxin is a potent acetylcholinesterase inhibitor. The mechanism of action of these toxins in blocking transmission and conduction has not yet been established.

Extremely toxic compounds have been isolated from certain marine animals, fish and invertebrates. Some of these toxins have been isolated and purified. Their molecular weights are