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 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*).

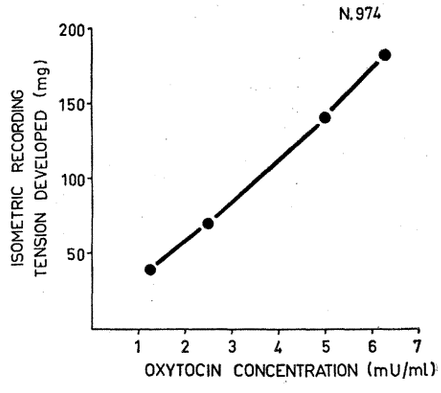

Fig. 2. The responses illustrated in Fig. 1 are plotted to show the linear relationship between concentration of oxytocin and tension recorded from the isolated strip of mammary tissue.

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8 February 1960

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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9 May 1960

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

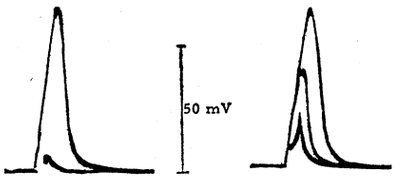

Fig. 1. Effect of clam poison on the mononodal action potential of a single nerve fiber preparation of the frog sciatic nerve (*Rana pipiens*). At $3 \times 10^{-10}M$ concentration (10^{-4} μg toxin per milliliter of solution), this toxin blocks conduction in 30 seconds. After return to Ringer's solution, conduction was restored within 1 minute (*p*H, 7.7; temperature, 23°C).

known but not their structure. Knowledge of the mode of action of these toxins is rather limited, but it is known that some of them block conduction and neuromuscular transmission reversibly (*1*). On the suggestion of B. Jandorf we have tested, on the electrical activity of conducting membranes, the effects of two of the toxins (*2*): the clam poison, prepared according to the method of E. J. Schantz *et al.* (*3*), and the puffer-fish poison.

The action of both toxins was tested on Ranvier nodes of a single frog sciatic nerve fiber prepared according to the method of Staempfli (*4*), and on the single isolated electroplax of *Electrophorus electricus*, preparaed with the method developed by Schoffeniels (*5*). In the innervated membrane of the latter cell there are many synaptic junctions, although most of the surface area is conducting membrane; the two types of membranes can be readily distinguished by electrical characteristics.

On exposure of Ranvier nodes to either of the toxins in concentrations of about $3 \times 10^{-10}M$ (10^{-4} μg/ml), electrical activity was rapidly and reversibly blocked within 30 seconds (See Fig. 1). With higher or lower concentrations the period of time required changed correspondingly. When the electroplax was exposed to either of the two toxins, a marked difference in the effect was observed. Clam poison, in a concentration of 5×10^{-4} μg/ml, blocked the response to neural stimulation in 10 to 20 minutes. With higher toxin concentration (0.1 μg/ml) the inhibition occurred in seconds. A concentration of 0.2 μg/ml was required to block the response to direct stimulation. Puffer-fish poison, in a concentration of 0.025 μg/ml, blocked the response to both direct and indirect stimulation simultaneously. The presence of curare (50 μg/ml) did not change the concentrations required for blocking the response to direct stimulation. Inhibition by puffer-fish poison at concentrations of 0.1 to 0.25 μg/ml, recorded with intracellular electrodes, occurred without depolarization.

The extraordinary toxicity of the compounds, which is several orders of magnitude higher than that of the most toxic nerve gases, raises the interesting and challenging problem as to the underlying mechanism. The primary role of the acetylcholine system in the generation of bioelectric potentials prompts the question of whether that system may be affected by these toxins. A reaction of these toxins with any one of the members of the system is thus conceivable. However, the affinity of the toxins to acetylcholinesterase is so low that a reaction with this enzyme may be excluded as a casual factor (*6*). A few tentative tests by S. Ehrenpreis with the receptor were inconclusive; considerable modifications of techniques are required for analysis. Action on the storage protein leading to a release of the ester appears unlikely, since there seems to be no depolarization, and since the blocking effect persists as long as the toxins are present. Obviously, the toxins may react with entirely different constituents of the membrane. At present, no satisfactory explanation can be given of the underlying chemical reaction. But the data reported promise to provide biology with a new potent tool for the analysis of events associated with nerve activity (*7*).

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Eating or Drinking Elicited by Direct Adrenergic or Cholinergic Stimulation of Hypothalamus

Abstract. A double cannula system, allowing repeated stimulation of central structures with crystalline chemicals, was developed. This technique was employed to study the effects of adrenergic and cholinergic stimulation of the lateral hypothalamus of rats. Drug-specific effects on the feeding and drinking mechanisms, respectively, were observed.

The exploration of the central nervous system by means of electrical stimulation has provided a wealth of information of great interest to physiologists and psychologists alike. The usefulness of this technique is limited, however, because the effects of stimulation are not restricted to synaptic junctions but affect fibers of passage, causing conduction in both normal and antidromic directions.

It has long been recognized that chemical stimulation avoids these problems, but the technique has in the past been plagued by the problem of uncontrolled spread, which raises a serious objection to the injection of chemicals in solution. Attempts to control for this factor by minimizing the injected quantities have apparently not been completely successful in preventing the escape of the fluid along the shank of the needle, following the path of least resistance.

Depositing chemicals in solid form has been shown to reduce this problem greatly (*1*), but this method has not allowed repeated stimulation of a selected locus. In the present study, a technique was developed which avoids this objection.

A double cannula system, consisting of two modified syringe needles, was permanently implanted unilaterally, by means of a stereotaxic instrument, into the lateral hypothalamus of each of 12 albino rats. Histological verification of the intended placements showed the tip of the cannula to be located in a circumscribed perifornical region at the same rostrocaudal coordinate as the ventromedial nucleus (see Fig. 1), an area corresponding to the ventral portion of Anand and Brobeck's "feeding area" of the lateral hypothalamus (*2*).

After 5 days of postoperative recuperation, the inner cannula was removed and minute amounts (1 to 5 μg) of crystalline chemicals were tapped into its tip before it was returned to its usual position. Successive treatments were administered to all animals in a counterbalanced order, with a minimum of 3 days between injections. Both food and water were freely available throughout the experiment. The food and water consumption of satiated rats was re-