terioration. After 24 hours in this mixture, the activity had already dropped too low for accurate quantitative assay, and in a few days the clotting time in the Ac-G assay system was approximately the same as the saline control. Rapid deterioration of Ac-G also takes place when versene is used as the anticoagulant. This finding is in agreement with the report of Olwin(2).

Discussion. The substrate provides an excess of both prothrombin and fibrinogen so that the test is not influenced by reasonable fluctuations in the level of these substances in the material to be tested. Dependable results are obtained on plasma from which the prothrombin has been removed by adsorption, as well as with prothrombin concentrates containing many times the prothrombin content of normal plasma.

In the actual performance of the test, failure to allow a reasonably constant interval of incubation in the constant temperature bath or block before picking up the tube to observe the end point may give rise to erratic results.

When beef plasma is used as the standard, the clotting time of the 20% standard should be determined within a few minutes of making the dilution, since an appreciable drop in activity may occur. This is particularly true of the more dilute standard, although an occasional lot of beef plasma has been encountered which seems to be labile even at a dilution employed for the 100% standard. The cause for this occasional instability has not been determined.

Summary. A simple one-stage procedure has been devised for the determination of accelerator globulin activity in human plasma. The components of the assay system are of human origin. The test is specific for accelerator globulin activity, and is unaffected by large fluctuations in prothrombin or fibrinogen content of the material to be tested.

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Action of Shellfish Poison on Peripheral Nerve and Skeletal Muscle. (20739)

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(Introduced by J. B. Bateman.)
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Shellfish poisoning, the paralytic type of poisoning which follows the ingestion of shellfish which have ingested poisonous plankton, has been adequately described by Sommer and Meyer(1). The causative substance of the paralysis has been characterized as a neurotoxin by Kellaway(2), who briefly described the effects of this toxic principle on peripheral nerve and muscle. He noted that this poison would render a skeletal muscle unresponsive to stimulation via its nerve just as does curare and that motor nerve endings were very susceptible to and readily blocked by this material.

The present investigation was initiated in an effort to characterize further the effects of shellfish poison on peripheral nerve and skeletal muscle.

Materials and methods. The preparations used were the sartorious muscle with its nerve and the gastrocnemius muscle-sciatic nerve of the frog, *Rana pipiens.* The apparatus and experimental protocol for recording action potentials are the same as those described previously in detail by Stover, Fingerman, and Forester(3) with the sole exception that in the present experiments the potentials were

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timed by signals obtained from a Tektronix time mark generator. These preparations, maintained at 25°C, were stimulated indirectly via the nerve with short (0.1-0.3 msec.) pulses of 1.5 volt amplitude. The electrical responses of both the nerve and the muscle could be recorded after suitable amplification from the screen of a triple beam cathode ray oscilloscope. When the muscles exhibited no response to indirect stimulation, they were stimulated directly with a 1.5 volt dry cell, and the presence or absence of even the slightest contraction was ascertained.

Experiments. The nerve-muscle preparation was removed from the frog, allowed to equilibrate for 30 minutes, and then placed in a lucite chamber in such a manner that the nerve could be stimulated and the action potentials of the nerve and muscle or muscle only could be recorded. The preparation was bathed in a frog Ringer solution of one to 5 intraperitoneal mouse units/ml of clam poison. The first noticeable effect is a gradual decrease in the height of the nerve and muscle action potentials when the nerve is stimulated. The electromyogram disappears before the nerve action potential does and there is no noticeable twitch of the muscle in response to a single indirect stimulus (Fig. 1). The first deflection in these records is the artifact resulting from the application of the stimulus. The interval of time between the short pips on the time base, bottom trace, represents one msec.

It has been possible in 7 experiments, however, to record end plate potentials from a muscle which has just ceased to exhibit a visible contraction in response to a single stimulus (Fig. 2). If 2 stimuli are given in rapid succession, *e.g.*, at an interval of 7 msec, then the end plate potentials produced in response to each stimulus will summate to produce a muscle action potential and an associated twitch (Fig. 3). Soon these end plate potentials disappear and only the nerve action potential can be recorded. At this stage one cannot elicit a contraction in response to 2 stimuli given in rapid succession (Fig. 4). There is no evidence that shellfish poison alters the latent period of the end plate potential. The muscle was then stimulated directly with 1.5 volts and there was no response in 5 of the 7 experiments. Occasionally, however, a slight twitch was observed. Finally, the nerve also ceased to conduct an impulse.

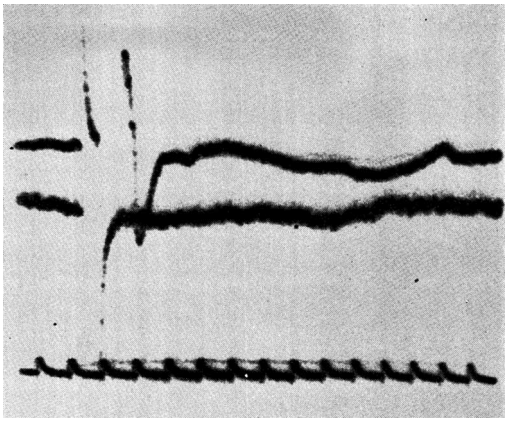

FIG. 1. A nerve action potential (upper trace) which has been recorded after the muscle has ceased to produce an action potential (middle trace). Time base (bottom trace): 1 msec. from short pip to short pip.

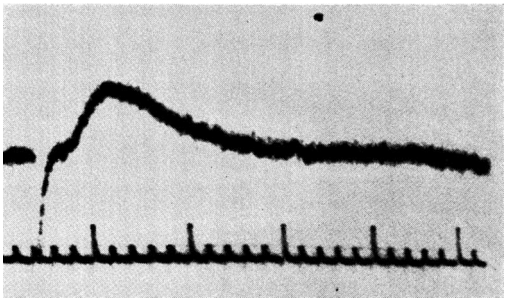

FIG. 2. An end plate potential recorded from a poisoned muscle which no longer contracts in response to a single stimulus. The electrode used to record the end plate potential is the same one used to record the middle trace of Fig. 1. Time base (bottom trace): 1 msec. from short pip to short pip.

The higher the dosage of shellfish poison, the more rapid is the sequence of events. Also the larger the muscle, the longer the time required for total paralysis. This is true because the time required to build up a lethal concentration within a large muscle is obviously greater than in a small one. For example, end plate potentials were recorded from the sartorius muscle in as short an interval of time as 2 minutes following the application of the poison. However, the gastrocnemius muscle always required a longer

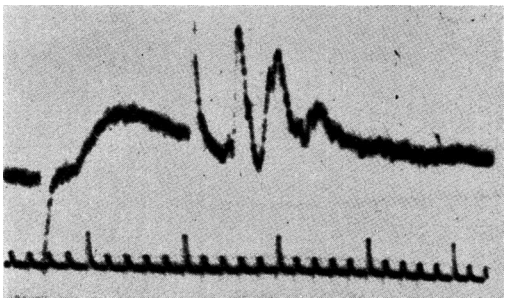

FIG. 3. The summation of end plate potentials to produce a muscle action potential. Time base (bottom trace): 1 msec. from short pip to short pip.

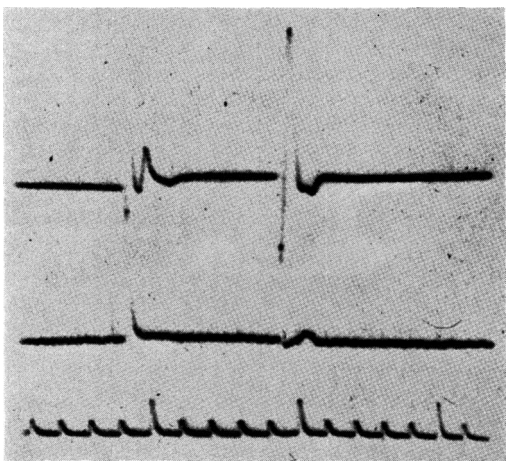

FIG. 4. After end plate potentials can no longer be recorded, the nerve action potential can still be recorded (upper trace) but no muscle action potential (middle trace) is observed even if two stimuli are applied in rapid succession. Time base (bottom trace): 1 msec. from short pip to short pip.

period. In one experiment, for example, 1.5 hours were required to block the sciatic nerve and eliminate the irritability of the gastrocnemius muscle to a stimulus of 1.5 volts.

The loss of the end plate potentials may be due to either or both of the following: (a) the terminal fibrils have been blocked or (b) the "transmitter" mechanism of the end plate has been interfered with. The former hypothesis seems more likely for Kellaway has demonstrated that the unmyelinated motor nerve endings are very susceptible to shellfish poison and are readily blocked. The relative resistance of a nerve to blocking may well be due to insulation by the myelin sheath for several investigators including Crescitelli(4) have shown that the nerve sheath is a barrier to locally applied substances. It is also obvious from these results that shellfish poison has a direct effect on muscle as well as nerve. This direct effect on nerve may well be due to diffusion in from the cut end as well as through the nerve sheath. Kellaway also observed this direct effect on muscle, but he states that the muscles did not completely lose their irritability to direct stimulation during the time they were observed.

In a second series of 3 experiments, frogs paralyzed by shellfish poison and double-pithed non-poisoned frogs were treated with 0.01 to 10 mg of acetyl choline in .1 ml frog Ringer solution. This acetyl choline solution was applied directly to the muscle or indirectly via the circulation. Their leg muscles were then observed for signs of contraction. In both instances the non-poisoned animals exhibited twitching of the leg muscles, but the poisoned animals showed no such response to the acetyl choline.

Discussion. Shellfish poison has a strong effect on both peripheral nerve and skeletal muscle. The action of this agent on muscle is similar to that of curare in 2 respects. Firstly, the poisoned muscle does not respond to stimulation via its still unpoisoned nerve and is unresponsive to directly applied acetyl choline. Secondly, end plate potentials can be recorded and summated to produce a twitch for a period of time following the application of this material. However, the effects of these dosages of shellfish poison on the isolated preparations are not reversible during an hour of observation following paralysis and the removal of the poison as are the effects of curare.

Shellfish poison has an even more general action than curare for it not only decreases the excitability of muscle to direct stimulation, but also blocks nerve conduction. In view of these actions, this agent should be referred to as a neuromuscular toxin rather than merely as a neurotoxin.

The action of shellfish poison is decidedly different from that of botulinum toxin. The latter neither blocks nerve conduction nor de-

creases the irritability of muscle(5) but probably inhibits the release of acetyl choline(3). Furthermore, muscle which has been paralyzed by botulinum toxin will contract in response to a dose of acetyl choline(6). The paralytic action of shellfish poison seems to be due primarily to a prevention of the muscle from responding to acetyl choline (curare-like effect), whereas the primary action of botulinum toxin is probably to prevent the release of acetyl choline.

Summary. Shellfish poison has a curare-like action, preventing the skeletal muscles from responding to liberated acetyl choline. Secondarily, it blocks peripheral nerve conduction and decreases the excitability of these muscles to direct stimulation. This material should henceforth be termed a neuromuscular toxin.

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Parallel Inhibition of Granulation Tissue by Cortisone, Hydrocortisone, Dibenamine and Banthine (R).* (20740)

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Hormonal influences upon granulation tissue formation have been well established. Stimulating and inhibiting effects can be observed, depending upon the hormone administered(1). Cortisone and hydrocortisone are preeminent as inhibiting factors and have aroused a great interest because of their clinical utility. In this laboratory it has been demonstrated that the responsiveness of small blood vessels to autonomic nervous stimuli is dependent upon the simultaneous presence of cortisone(2,3). The synergistic and potentiating effects of cortisone and certain neurohumors have been also shown to apply to a number of other phenomena, apparently not related to primary vascular mechanism. These seem to operate in the eosinophile fall(4), in certain aspects of fat transport(5) and other processes under investigation at present. Autonomic blocking agents on the other hand protect experimental animals deprived of their adrenals and subjected to stress from the ensuing shock(6). We have, therefore, investigated the effects of the adrenalins on the one hand, and of dibenamine and banthine on the other, upon granulation tissue formation in order to explore any possible relationship to the action of cortisone. To obtain comparable specimens untreated as well as cortisone and hydrocortisone treated animals were examined in the same manner, and new observations recorded.

Methods. Male, white rats of the Sprague-Dawley strain, weighing about 150 g were used. As in our previous experiments(7), one-half cc of turpentine USP was injected subcutaneously into the region of the right scapula and several animals of each series were killed daily beginning on the first to the 6th day after induction of the abscess. The dry freezing technic of tissue fixation was utilized, hoping that more definite information could be gained in regard to the structure and staining

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