bumin in the aorta at 30 seconds were due to intravascular albumin on intimal endothelium or in vasa vasorum were given in that study. The similarity of the concentrations for labeled albumin to the concentrations for labeled lipoprotein suggests that the latter concentrations also are due to intravascular protein.

The concentrations in the aortic wall of labeled lipoprotein that had penetrated beyond the endothelium into the tissue (Fig. 1) were computed from the concentrations at 30 seconds after injection and the data at subsequent intervals. The concentration of labeled lipoprotein increased more rapidly in the inner layer than in the outer layer ($p < .05$ by a two-tailed t-test). Since the inner layer is almost free of vasa vasorum (*6*), this suggests that lipoprotein enters the inner layer directly across the intimal endothelium rather than from the vasa vasorum of the outer layer.

In the inner aortic layer the concentration of labeled lipoprotein increased faster in the ascending thoracic aorta than in the descending thoracic aorta, and faster in the descending thoracic aorta than in the abdominal aorta (by a two-tailed t-test $p < .02$ for the difference between the ascending aorta and the abdominal aorta). Thus, the rates of entry of lipoprotein into the inner layer of the aorta formed a gradient. Similar gradients of the rates of entry into the inner layer have been observed both for labeled cholesterol fed to normal dogs (*7*) and for labeled albumin injected intravenously into such dogs (*5*). All these gradients are similar to the gradient formed by the accumulation of cholesterol in the inner layer of the aorta during the first month of the development of atherosclerosis in dogs fed thiouracil and cholesterol (*8*).

No evidence of trapping of lipoprotein in arterial tissue was found in the present study. It seems unlikely, of course, that there is much trapping of lipoprotein in the aorta of the normal dog. If there were, the concentration of some constituent of lipoprotein should increase in the aorta with age. The only evidence for this is a very slow increase in the concentration of cholesterol with age (*9*).

The similarity of the gradient formed by the accumulation of cholesterol early in experimental atherosclerosis to the gradient formed by the rates of entry of lipoprotein is compatible with the filtration theory of atherosclerosis, but does not prove that the theory is correct. The present data offer no explanation of the fact that later in the course of experimental atherosclerosis, the concentration of cholesterol in the abdominal aorta exceeds that in the thoracic aorta. Some possible explanations of that fact were suggested earlier (*8*).

Many factors may have contributed to the failure of a small fraction of the labeled protein in the plasma to float at a density of 1.063. The possibility that the fraction not floating was thyroid hormone labeled in the thyroids of the injected dogs was excluded by the results in the dogs fed potassium iodide. The amount fed should have almost completely blocked the utilization of radioiodine by their thyroids. The failure of a small fraction to float at 1.063 does not affect any of the conclusions drawn from the data. A contaminating labeled protein would have affected the validity of the conclusions most of all if it had been a rapidly diffusing protein like albumin. The concentrations of labeled albumin in the aorta following its intravenous injection are known (*5*). Even if all the labeled protein not floating at 1.063 had been albumin, that albumin would have made an insignificant contribution to the concentrations found in aortic tissue.

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11 September 1963

Convulsant Drug Action on Neuronally Isolated Cerebral Cortex

Abstract. *After topical application of thiosemicarbazide, semicarbazide, or isoniazid to the surface of an isolated region of cortex, convulsive responses to electrical stimulation appeared only after a finite number of normal responses had been elicited, and disappeared again if stimulation was interrupted for about 10 minutes. With any of the other convulsant drugs tested, development of the convulsive pattern was independent of whether or not the cortex was stimulated. The two groups of drugs evidently have different modes of action.*

Many different substances are known to produce convulsive neuronal activity when topically applied to the cerebral cortex. In the intact spontaneously active cortex they all produce much the same kind of regional alterations in electrical activity, but it is unlikely that they all act by the same pharmacological mechanism. This has been inferred from the fact that some of the drugs, when systemically administered, produce different topographic distributions of convulsive activity within the central nervous system (*1*). This study affords a demonstration of differences in their action on the cortex itself. These differences became apparent when a number of these drugs were applied to the unanesthetized neuronally isolated cortex, in which neuronal activity does not usually occur spontaneously but can be elicited by electrical stimulation. Under these conditions it could be shown that the drugs could be divided into two groups according to whether or not the frequency with which neuronal activity was elicited influenced the development of convulsive effects.

The experiments were performed on a total of 18 cats. In each case, a high midbrain section was carried out under ether anesthesia, and then the anesthetic was discontinued (cerveau isolé preparation). A region of cortex about 5 by 15 mm in area was isolated in the suprasylvian gyrus, with the subpial incision technique described by Burns (*2*). Agar-saline wick electrodes were used for monopolar surface re-

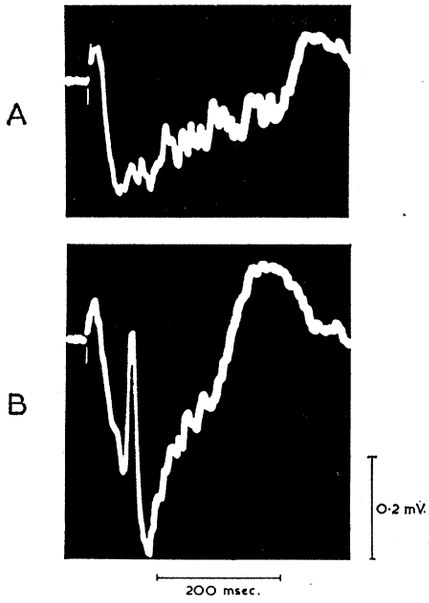

Fig. 1. Responses recorded from surface of isolated region of cortex, elicited by a single stimulus. *A*, Normal response; *B*, response elicited 20 minutes after application of 1.5-percent solution of thiosemicarbazide to cortical surface near focal recording electrode. Monopolar recording negativity of focal electrode produces an upward deflection. Amplifier time constant is 100 msec. Distance between stimulating electrodes and focal recording lead is 4 mm. (Stimulus artifacts retouched.)

cording, with the reference electrode on an area of dead cortex outside the isolated region. For application of the drugs, small squares of filter paper soaked in the appropriate solution (usually 1- to 2-percent concentration, buffered with phosphate to *p*H 7.4 when necessary) were placed on the surface of the cortex close to the focal recording electrode. Activity was produced by applying a 1-msec square wave through a pair of platinum ball-tip electrodes placed 3 to 5 mm from the focal recording lead.

A single stimulus applied to the surface of the isolated cortex is known to elicit a burst of activity which spreads throughout the isolated region (*2*). During the burst, the surface of the active portion of the cortex becomes positive with respect to an indifferent point, while displaying an irregular oscillation with a frequency of about 60 cy/sec (Fig. 1*A*). The burst response may last from 0.3 to 2 seconds, the surface-positivity usually being maximal near the beginning of the response, decreasing somewhat as the activity continues, then terminating abruptly, sometimes with a surface-negative overshoot (irrespective of the amplifier time constant). Local application of a convulsant agent produced a modification of the response in the treated region, the first manifestation of which was the appearance of a negative "spike," lasting about 25 msec and usually occurring at the time corresponding to the peak of surface-positivity in the normal response (Fig. 1*B*). If the drug action continued, the number of such spikes per response increased, but spikes were never seen during the silent intervals between bursts, except in a few cases in which continuous epileptiform activity developed when too strong a concentration of drug was used, or when the drug was left too long in contact with the cortex. These conditions leading to self-maintained activity were carefully avoided in all of the experiments described below.

With one group of drugs, of which strychnine is an example, the spikes appeared in each burst response soon after application of the drug-soaked filter paper, gradually increased in amplitude, then declined after the filter paper had been removed. The time course of growth of the spikes was the same regardless of whether responses were elicited once a minute or once every 3 seconds, and recovery after removal of the drug was slightly more rapid when the faster rate of stimulation was used (Fig. 2). If the strychnine was allowed to remain in contact with the cortex in the absence of stimulation for 30 minutes, the spikes were still found to be present in responses elicited at the end of this period. These features showing the action of strychnine to be independent of the rate of elicited cortical activity were also displayed by picrotoxin, metrazol, penicillin, *d*-tubocurarine, and thiamine.

The action of the convulsant hydrazides, that is, thiosemicarbazide, semicarbazide, and isoniazid, differed from that of strychnine in the following respects. (i) After the initial application of the drug to a preparation which was stimulated once every few seconds, there was invariably a latent period of 10 to 30 minutes during which the burst responses were normal in configuration, regardless of the frequency of stimulation (*3*). In the complete absence of stimulation, convulsive activity did not develop at all even after a period of 50 minutes had elapsed. (ii) After the latent period, the negative spikes usually appeared in the burst responses quite abruptly, being immediately of considerable amplitude, although occasionally the preparation went through a period of a few minutes in which normal and convulsive responses alternated. (iii) After the spikes were constant in appearance, cessation of stimulation for about 10 minutes caused the responses to return to their normal form. (iv) Resumption of stimulation caused the spikes to reappear.

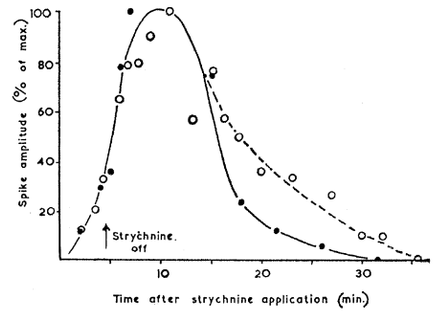

Fig. 2. Amplitude of convulsive spikes in burst responses after application of 0.05-percent strychnine to surface of cortex, for responses elicited once every 3 seconds (●——●) and responses elicited approximately once a minute (O— —O).

The sequence of alternating periods with and without stimulation could be repeated a number of times in a single preparation. With different rates of stimulation, it was found that the faster the rate, the more rapidly did the convulsive spikes reappear. With stimulus

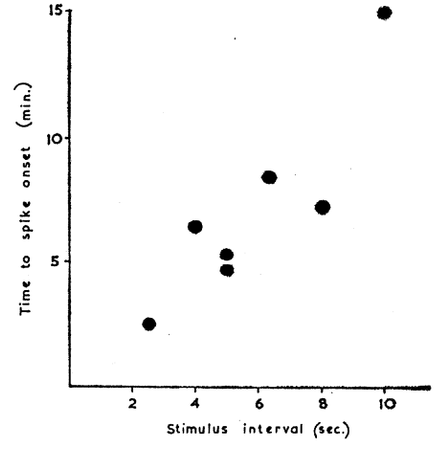

Fig. 3. Relationship between rate of stimulation and time of onset of convulsive spikes following application of 1.5-percent thiosemicarbazide to surface of cortex. The drug was in contact with the cortex for about 1 hour before stimulation trials were begun. After each trial, an interval of approximately 20 minutes without stimulation was permitted to elapse, during which time the convulsive spikes disappeared and the responses resumed their normal configuration.

intervals between 2.5 and 10 seconds the relationship appeared to be approximately linear (Fig. 3), that is, the spikes reappeared after a constant number of stimuli regardless of the frequency of stimulation. With longer intervals, however, for example 15 or 20 seconds, the spikes did not reappear even after 30 to 40 minutes of stimulation. These results suggest that during each of the series of normal responses to stimulation some change occurred which outlasted the stimulus interval (provided this interval was 10 seconds or less), and which built up during successive responses until a critical level was reached, triggering the convulsive activity pattern. This change presumably dissipated itself during periods without stimulation.

The manner in which the manifestation of the pharmacological action of the convulsant hydrazides depends on on-going neuronal activity is reminiscent of the action of low doses of the hemicholinium HC-3 in blocking neuromuscular transmission (*4*). In this latter case, block of the neuromuscular junction results from the arrest of acetylcholine formation (*5*), and becomes apparent only after a finite number of impulses have traversed the junction and depleted the pre-existing store of the transmitter. This may be contrasted with the block produced by substances like curare and decamethonium, whose mechanism of action does not depend on whether or not the junction is active (*6*). The difference between the modes of action of the convulsant hydrazides and strychnine may be analagous, although there is no evidence that both these types of drugs necessarily act on the same neuronal system. Strychnine may act in the cortex, as it does in the spinal cord (*7*), by suppressing the action of inhibitory neurones, and it is possible that the convulsant hydrazides also act on an inhibitory system, blocking the synthesis of an inhibitory substance which is normally released during neuronal activity. Their convulsant action would not be evident until the pre-existing store of this material had been depleted by normal activity, and if the block of synthesis was only a partial one, the material would re-accumulate during a period in which no activity occurred. On the other hand, it would also be possible to explain the action of the hydrazides in terms of the accumulation of some substance that had an excitatory effect on the cortical neurones. The hypothesis that they act by blocking an inhibitory system recalls the proposal by Killam and Bain (*8*) that the action of the hydrazides is to block activity of enzymes catalyzed by vitamin B_6, and thus interfere with the production of gamma-aminobutyric acid, which has an inhibitory action on cortical neurones. This study suggests that if this were the case, it should be possible to demonstrate a net destruction or release of gamma-aminobutyric acid during normal neuronal activity in the cortex (*9*).

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Sharks: Attraction by Low-Frequency Sounds

Abstract. *Large sharks (Carcharhinidae, Sphyrnidae), in their natural environment, were attracted to low-frequency (predominantly 20 to 60 cy/sec) pulsed sounds, but apparently not to higher frequency (400 to 600 cy/sec) pulsed sounds, or to low-frequency continuous sounds. The sharks apparently detected and oriented to the sounds in the acoustic far field.*

In a recent study conducted on the reefs off Miami, Florida, sharks were attracted to low-frequency pulsed sounds resembling those of struggling fish. The appearance of sharks in the vicinity of wounded or struggling fish is a phenomenon that has long been noted by fishermen and skin divers. Hobson (*1*) and Tester (*2*) have shown that olfaction plays a major role in the attraction of sharks. In some instances, however, the rapid appearance of sharks precludes the possibility that olfactory substances, which are carried at a relatively slow rate by currents, formed the initial attractive stimulus. Because vision is limited by poor visibility underwater, and because the struggling fish is sometimes hidden from view, it appears reasonable that some form of mechanoreception is involved.

The existence of the sense of hearing in sharks has been well established since the days of Parker (*3*), who obtained responses from the smooth dogfish, *Mustelus canis*, by striking the side of the tank with a hammer. More recently, Vilstrup (*4*) obtained from the spiny dogfish, *Acanthias vulgaris* (= *Squalus acanthias*), conditioned responses to sound; and Moulton (*5*) conditioned *Mustelus canis* to an oscillator tone. Clark (*6*) succeeded in establishing instrumental conditioning in large lemon sharks, *Negaprion brevirostris*, and observed that they responded to a submerged bell. Dijkgraaf (*7*) trained dogfish, *Scyliorhinus canicula*, with sound and electric shock. His preliminary results indicate that perception of a 180 cy/sec tone occurs mainly in the labyrinth. Olla (*8*) obtained responses from trained small hammerhead sharks at frequencies between 100 and 600 cy/sec. Kritzler and Wood (*9*) obtained an audiogram for a captive bull shark, *Carcharhinus leucas*. The shark responded to frequencies between 100 and 1500 cy/sec and was most sensitive to the band between 400 and 600 cy/sec. With identical sound sources at three positions, they observed that the shark was able to localize the source from a distance of at least 6.5 m (*10*).

Hobson (*1*) attempted to evoke responses from sharks to sounds in the field at Eniwetok Atoll. He played back recordings of various sounds through an underwater speaker when sharks were in the area and visible to observers. There was no indication that the sharks detected or responded to the sounds.

The initial phase of our study consisted of making recordings in the field to determine the frequency composition and pulse characteristics of the sounds of struggling fish. We used a Sony SRA-2, 262 D tape recorder and