Reports

Enhancement of Activity of Nerves by X-rays

No report of the response of excised mammalian nerves during x-irradiation has heretofore been published (*1*). Recordings made at the end of irradiation or at considerable periods after irradiation fail to show the dynamic changes that take place during irradiation which are reported in this paper (*2*). Nerves transferred from irradiation chambers to recording chambers have failed to give the highly reproducible values that are necessary in quantitative work of this type.

The paired ventral caudal nerves of the rat were chosen because they possess a uniform fiber spectrum (*3*) and therefore exhibit sharp, compact spike potentials. They are long nerves with a minimum of branching, well adapted to studies on conduction velocity. Their activity is not highly dependent on temperature.

Each nerve was irradiated on platinum electrodes in a specially constructed, sealed chamber, which was affixed to the x-ray tube. A fine spray of oxygenated Ringer-Locke's solution was capable of keeping the nerve in excellent condition for periods well over 24 hours, with adequate provision to keep the electrodes from shorting out. The stock of solution which served as the source of the fine spray was itself not irradiated.

X-irradiation was carried out at 280 kv (peak) and 20 ma at a dose rate of 6 kr/min. Electrophysiological apparatus was employed to procure records of the response of the nerve to square-wave stimulation during irradiation. Single supramaximal stimuli were applied only at the time a recording was made. Simultaneous recordings from two pairs of electrodes, one near the proximal end of the nerve, the other near the distal end of the nerve, separated by 50 mm, were displayed on the face of a cathode-ray tube. Photographic records of the responses of the nerves were made from these tracings; they were later projected on a screen for exact determination of values. The amplitude of spike potential and the conduction velocity were established for control nerves and for nerves under irradiation.

Irradiation was begun only after a period of stabilization during which the output of the nerve was shown to be constant for a period of time exceeding that of the period of irradiation. Figure 1 shows the result of x-irradiation on the spike amplitude and conduction velocity of a typical nerve. An almost immediate response to x-irradiation is obvious in Fig. 1, but it was not anticipated that the effect would be to enhance the activity of the nerve. There was an initial small increase in conduction velocity followed by a steady decrease which was quite independent of the increase in spike amplitude. The increase in spike amplitude, which has now been recorded in more than 50 cases, has been as great as 60 percent, with a mean of 25.6 percent; in only 6 percent of the cases did the nerve fail to show an increase in spike amplitude as a result of irradiation. Conduction velocity, on the other hand, has shown an average increase of only 5.8 percent, with 20 percent of the nerves failing to show an increase. In one sense, the conduction velocity was more sensitive to x-rays than the spike amplitude, for it began to decrease long before the spike amplitude began to decrease; in another sense, it was less sensitive, for the response was less and, at expiration of the nerve, when spike amplitude was essentially zero, conduction velocity was still approximately 50 percent of the original velocity.

Factors that affect the ability of the nerve to respond as a result of x-irradiation are under extensive investigation. It may be noted that the increase in spike amplitude is greatest for nerves that are kept in the solution the shortest time prior to irradiation. After 24 hours, the increase in spike amplitude, in response to x-irradiation, is less than 5 percent. A second factor of importance in the magnitude of the response of the nerve to x-irradiation is the dose rate. At higher dose rates, the increase in spike amplitude is considerably less than it is at lower dose rates. This suggests that there may be two competing processes proceeding at different rates. One tends to increase the output of the nerve, and the other tends to destroy the activity of the nerve. It is the latter that is observed in most biological responses to x-irradiation.

The increased output of the nerve is not a reversible phenomenon, but is the result of an altered condition of the nerve, possibly an alteration in the permeability of the membrane, and is not dependent on concomitant bombardment by x-rays. This was proved by the fact that nerves continued to respond at high levels when, during the course of irradiation, the x-ray beam was turned off.

An explanation of the increase in output of the nerve under irradiation may be sought in the fact that x-irradiation may increase the ionic permeability of the membrane, which, in turn, may increase the magnitude of the action potential. After the increase in permeability which is manifested by an increase in the action potential, further increase in permeability, to the point of impairment of the activity of the nerve, would then lead to a decline in activity and ultimate

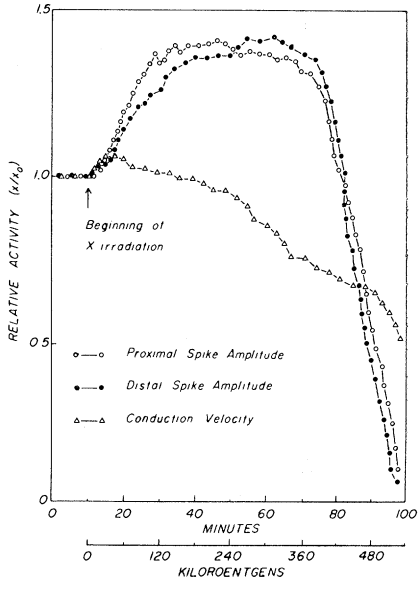

Fig. 1. Relative activity of caudal nerve of a rat as a function of time and dose of x-rays. Two pairs of electrodes were utilized to give the values. One pair was located proximally and the other distally, separated by 50 mm. The value of proximal spike amplitude, distal spike amplitude, or conduction velocity at time t is represented by x, while the corresponding value at the beginning of irradiation is represented by x_0.

All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

extinction. A sound foundation for this hypothesis is given by the work of Rothenberg (*4*), who showed that x-irradiation increased the permeability of the squid axon to sodium-24; the importance of Na^+ ions in the propagation of the nerve impulse is well known. The problem still to be answered, however, is why the conduction velocity falls during irradiation while the spike amplitude is rising.

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References and Notes

1. This report is based on a paper read at the first National Biophysics Conference at Columbus, Ohio, 4 Mar. 1957.
2. This research was performed under contract No. AT(11-1)-205 between the U.S. Atomic Energy Commission and the University of Notre Dame.
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Mode of Action of Antigen and Other Smooth-Muscle Stimulants

Smooth muscle from an antigenically sensitized animal contracts upon reexposure *in vitro* to the antigen (*1*). This phenomenon, the Schultz-Dale reaction, may form a basis for several hypersensitive conditions (reviewed by Seegal, *2*). Because the Schultz-Dale reaction is prevented by botulinum toxin, and by certain substances that are capable of interfering with conduction in nerve, involvement of nerve in the process seems likely (*3*). Ganglionic blocking agents do not prevent the reaction. This report (*4*) offers information concerning the relationship of the Schultz-Dale reaction to the action of other smooth-muscle stimulants.

Ileum from guinea pigs sensitized to egg albumin was set up in a muscle bath containing Tyrode's solution and arranged for kymographic recording of the contractions of the longitudinal muscle as previously described (*3*). Supposed inhibitors and stimulants were added to the bath. The concentration of antigen chosen was 10 times that which produces a just-perceptible contraction of the ileum. Concentrations of the other stimulants were as follows: serotonin, 2.0 μg/ml; nicotine, 2.0 μg/ml; acetylcholine, 0.02 μg/ml; barium chloride, 0.2 mg/ml; and histamine, 2.0 μg/ml. Several concentrations of each inhibitor were used; the concentrations given in subsequent paragraphs are those that illustrate most clearly the difference between the actions of the various stimulants.

Our present interpretation of the results is given in terms of a diagram (Fig. 1), patterned after one of Ambache (*5*), which attempts to indicate mechanisms consistent both with our data and with much of the enormous pertinent literature. Solid arrows indicate hypothetical pathways of stimulation, and dashed lines indicate points at which inhibition is believed to take place. The principal steps in the development of the relationships thus expressed follow:

Contraction of muscle owing to antigen, to serotonin, or to nicotine is prevented by low concentrations of alcohols and urethanes. This suggests a common step in the mechanisms of the actions of these three stimulants, probably conduction in nerve, since alcohols and urethanes block conduction in the concentrations that were used (*6*).

Stimulation by antigen and by serotonin (but not by acetylcholine, histamine, or barium chloride) is prevented by structural analogs of serotonin, such as gramine, yohimbine, and bufotenine (all 0.02 mg/ml). This suggests that antigen may act by liberating serotonin, as Fink (*7*) has concluded from studies with mouse uterus.

Stimulation by antigen is blocked by botulinum toxin, but stimulation by serotonin is not so blocked (*3*). This suggests that liberation of serotonin by antigen is the process blocked by botulinum toxin.

The mechanism of stimulation by nicotine seems to be more complex, since ganglionic blocking agents are capable of inhibiting, often without completely abolishing, the response to this substance (*5, 8*). Moreover, nicotine stimulation is abolished by butolinum toxin (*9*). Nicotine stimulation was also found to be prevented by the structural analogs of serotonin, so nicotine may also act by liberating serotonin.

Lower alcohols (ethyl, 1.0 percent; propyl, 1.0 percent; butyl, 0.4 percent; and amyl, 0.2 percent) do not prevent the contraction of muscle owing to acetylcholine or histamine, whereas higher alcohols (hexyl, 0.04 percent; heptyl, 0.02 percent; and octyl, 0.02 percent) prevent stimulation by acetylcholine but not by histamine. Thus, histamine seems to act at a site closer to the contractile mechanism than does externally applied acetylcholine. This observation recalls the demonstration by Dale and Gaddum (*10*) that the site of action of externally applied acetylcholine is probably not identical with that of the acetylcholine liberated by cholinergic nerve endings. The data also confirm results of others (*9, 11*) that suggest that the site of action of barium chloride, often supposed to be a direct muscle stimulant, may be close to that of acetylcholine.

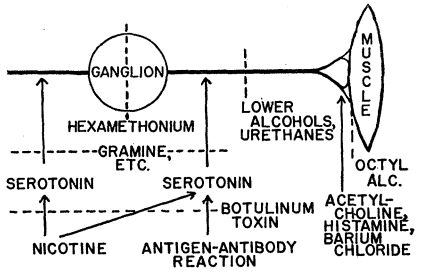

Fig. 1. Hypothetical sites of action of stimulants and inhibitors upon smooth muscle and the associated nerve structures.

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Instrumental Artifacts in the Determination of Difference Spectra

A. H. Mehler (*1*) has warned of the serious errors that may arise because of the unavoidable stray light within the monochromator of single-beam spectrophotometers when a photomultiplier detector is employed to measure the difference in optical density of two solutions of relatively high absorbance. This was illustrated by the apparent deviation from Beer's law when the absorbance of a constant amount of each of various materials was determined as the difference between two solutions of increasing absolute concentration.

This report seeks to extend this warning to the practice of determining the absorption characteristics of a given compound in the presence of other absorbing species by using an appropriate blank to "zero out" the absorption owing to the extraneous compounds and thus to obtain a "difference spectrum." We have