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.
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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.

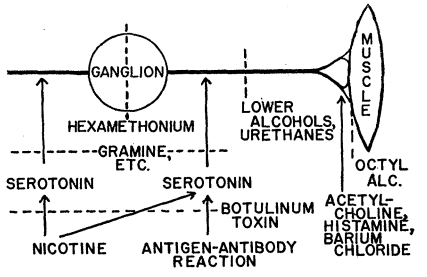

Fig. 1. Hypothetical sites of action of stimulants and inhibitors upon smooth muscle and the associated nerve structures.

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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