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.
3. P. O. Chatfield and C. P. Lyman, *Am. J. Physiol.* 177, 183 (1954).
4. M. A. Rothenberg, *Biochim. et Biophys. Acta* 4, 96 (1950).

15 March 1957

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.

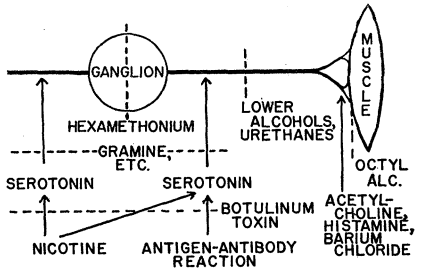

Fig. 1. Hypothetical sites of action of stimulants and inhibitors upon smooth muscle and the associated nerve structures.

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References and Notes

1. W. H. Schultz, *J. Pharmacol. Exptl. Therap.* 2, 221 (1910); H. H. Dale, *ibid.* 4, 167 (1913).
2. B. C. Seegal, *Ann. N.Y. Acad. Sci.* 50, 681 (1949).
3. W. B. Geiger, E. M. Hill, M. Thompson, *Proc. Soc. Exptl. Biol. Med.* 92, 793 (1956).
4. This work was supported by grants from the National Science Foundation (G-2500), and from the Helen Hay Whitney Foundation.
5. N. Ambache, *Arch. intern. pharmacodynamie* 97, 427 (1954).
6. W. Blume, *Naunyn-Schmiedeberg's Arch. exptl. Pathol. u. Pharmakol.* 110, 46 (1925); M. G. Larrabee and J. M. Posternak, *J. Neurophysiol.* 15, 91 (1952); F. Crescitelli, *Am. J. Physiol.* 155, 82 (1948).
7. M. A. Fink, *Proc. Soc. Exptl. Biol. Med.* 92, 673 (1956).
8. W. Feldberg, *J. Physiol. (London)* 113, 483 (1951).
9. N. Ambache and A. W. Lessin, *ibid.* 127, 449 (1955).
10. H. H. Dale and J. H. Gaddum, *ibid.* 70, 109 (1930).
11. N. Ambache, *ibid.* 110, 164 (1949); H. Necheles *et al.*, *J. Pharmacol. Exptl. Therap.* 108, 61 (1953).

28 February 1957

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

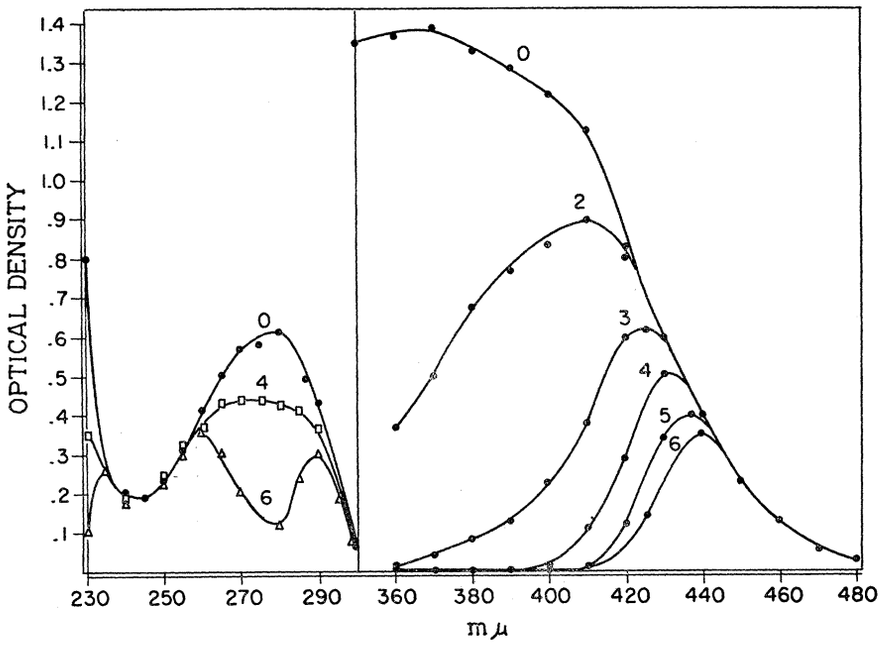

Fig. 1. Difference absorption spectra of tryptophan and 2,4-dinitrophenol, obtained with Beckman DU spectrophotometer and photomultiplier. (Left) Tryptophan. Each curve represents the difference spectrum due to $1 \times 10^{-4}M$ tryptophan in $0.05M$ phosphate, *p*H 7.3, and 0.005 percent Versene obtained as the difference between 0 and $1 \times 10^{-4}M$ (●), 4 and $5 \times 10^{-4}M$ (□), and 5 and $6 \times 10^{-4}M$ (△) respectively. (Right) 2,4-Dinitrophenol. Each curve represents the difference spectrum due to $1 \times 10^{-4}M$ dinitrophenol in $0.05M$ phosphate, *p*H 7.8, obtained as the difference between 0 and $1 \times 10^{-4}M$, 1 and $2 \times 10^{-4}M$, 2 and $3 \times 10^{-4}M$, and so forth. The indicated figure represents the lower concentration.

observed that, with solutions of only moderately high absorbance, such difference spectra may be seriously misleading.

Buffered solutions of 2,4-dinitrophenol and of tryptophan were prepared whose concentrations were $1 \times 10^{-4}M$, $2 \times 10^{-4}M$, $3 \times 10^{-4}M$, and so forth. Difference spectra were determined between succeeding pairs in the series so that in each case the absorption spectrum due to a $1 \times 10^{-4}M$ solution was obtained. The results are shown in Fig. 1.

It is clear that the nature of the *apparent* difference absorption spectrum changes as the absolute concentration of the absorbing material increases. The deviations from the true absorption spectra of these compounds are due entirely to the stray polychromatic light within the monochromator, which causes the apparent difference in optical density due to a given level of absorbing compound to decrease progressively as the absolute concentration increases. The use of a photomultiplier detector that permits determination of difference spectra between solutions of high absolute absorbance increases the likelihood of this instrumental artifact. Since this effect is most pronounced at the absorption maxima and is of least significance at the absorption minima, the entirely misleading data of Fig. 1 result. If these were difference spectra that had been obtained with a view toward identifying an unknown compound, they would be unrecognizable. In consequence, only in double-beam optical systems where the stray light is negligible may difference spectra obtained at high absolute absorbances be determined with confidence (*2*).

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References and Notes

1. A. H. Mehler, *Science* 120, 1043 (1954).
2. These observations were made in the course of studies variously supported by the National Institutes of Health, the U.S. Atomic Energy Commission, and the National Science Foundation.

12 March 1957

Photosensitive Pigments from Retinas of Deep-Sea Fishes

In November 1956, a new group of visual pigments was described briefly by Denton and Warren (*1*) from several species of deep-sea fishes. The new pigments were named "chrysopsins" or visual golds and characterized as having maximum sensitivity to light at a wavelength about 20 mμ less than the $\lambda_{max.}$ of rhodopsin. The chrysopsins were not extracted from the retina, but were demonstrated by determining the density changes when fresh retinas were bleached with white light.

Through the kindness of Carl L. Hubbs of the Scripps Institution of Oceanography, bathypelagic deep-sea fishes were collected on an expedition made by the Scripps vessel *Paolina-T* in February 1957. The fishes were caught at night with a midwater trawl at depths of 280 to 380 fathoms near Guadalupe Island, Baja California, Mexico. Hubbs generously sorted the trawl collections and made field identifications by dim red light (*2*). The retinas were removed, and digitonin extracts were prepared by standard methods (*3*). Spectrophotometric examination and bleaching with narrow-band colored light allowed analysis of the retinal pigments present in these extracts (*4*).

In general, the results of Denton and Warren were confirmed, $\lambda_{max.}$ of the photosensitive pigment of *Argyropelecus affinis* Garman being 478 mμ (Fig. 1). The pigment was demonstrated to be homogeneous by partial bleaching experiments (*3*). The absorption spectrum and the hydroxylamine difference spectrum of this retinal extract were in good agreement with the theoretical curve constructed from Dartnall's nomogram (*5*) for a visual pigment with $\lambda_{max.}$ of 478 mμ. The greater absorption of the extract (curve 1) in the short-wavelength portion was caused by the presence of light-absorbing impurities. The difference spectrum (curve 3) falls below the theoretical curve in this region because of the appearance of the violet-absorbing product of bleaching. The absorption of the product of bleaching was maximal at 375 to 380 mμ (*p*H 8.3) and at 365 to 370 mμ when hydroxylamine was added, indicating that retinene$_1$ was the chromophore of the light-sensitive pigment (*6*). This pigment is therefore provisionally called pigment 478_1 (the subscript means that retinene$_1$ is the chromophore).

A retinal extract of *Sternoptyx obscura* Garman of the same family, Sternoptychidae, had a different photosensitive pigment with $\lambda_{max.}$ at 485 mμ, but with the same product of bleaching. In the unrelated *Bathylagus wesethi* Bolin (family Argentinidae), however, a 478_1 pigment like that of *Argyropelecus* was mixed in the extract with a rhodopsin ($\lambda_{max.} = 500$ mμ) in the ratio of about 4/1. This was determined by selectively bleaching the red-sensitive rhodopsin with red light and then bleaching the 478_1 pigment with orange and yellow light. The products of bleaching of the two pigments were spectrally identical, suggesting that retinene$_1$ was the chromophore of each.

Further indications of diversity were found in extracts of other deep-sea fishes, each of which had only a single