doses of 200 mg/kg/24 hr produced a fall in tissue CO_2 and O_2 tensions, effects due to the increased alveolar ventilation and relatively greater increase of tissue oxygen consumption than tissue perfusion.

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A Study of the Mechanism of the Schultz-Dale Reaction.* (22617)

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(Introduced by Ann G. Kuttner.)

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The Schultz-Dale reaction(1,2), which consists of the contraction of smooth muscle from an antigenically sensitized animal on re-exposure *in vitro* to the antigen, has a manipulative simplicity that makes studies of mechanism particularly inviting. The mechanisms that have been proposed fall into 2 overlapping groups: (1) liberation of a stimulant such as histamine or acetylcholine as a result of the antigen-antibody(3,4,5) and (2) involvement of nerve, a possibility that has been considered both favorably(6) and unfavorably(7,8,9), without very final evidence either way. The literature concerning the effects of pharmacodynamic agents and enzyme inhibitors on this reaction gives useful preliminary information bearing upon questions of mechanism, but the data are scattered and were obtained under a wide variety of experimental conditions. Therefore a preliminary study was made of the effects of a selected group of drugs and enzyme inhibitors upon the reaction, since it seemed likely that indications might be obtained as to which physiological or enzymological systems were involved in the reaction.

Gray, Pedrick and Winne(10), using guinea-pig ileum, found that 0.8% ethyl alcohol blocked the Schultz-Dale reaction, which suggested to us that nerve might be involved, and further experiments to test this possibility were performed. Results that support the hypothesis that nerve is involved are presented below.

Materials and methods. Guinea pigs weighing about 500 g received intraperitoneally 2.0 mg of crystalline egg albumin on 2 successive days. After 21 to 24 days, the animals were killed, and the ileum was removed and washed with Tyrode's solution. Portions of the ileum about 15 mm long were set up in a muscle bath stirred by aeration and arranged for washing by overflow. It was found important to keep the lumen open at both ends. The bath and all solutions were kept at 37.5°C. Contractions were recorded with an ink-writing lever that exerted a tension of about 0.5 g, and gave an amplification of 1:4.5. The usual method of testing the effect of a substance of interest upon the action of antigen and other stimulants was to add the substance to the bath containing the ileum, and after 15 minutes, to add the stim-

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TABLE I. Effect of Various Substances upon the Schultz-Dale Reaction.

Inhibitor	Concentration	Reactivity, in presence of inhibitor, to: Antigen (see text for conc.)	Acetylcholine bromide, $1:2.5 \times 10^7$	Histamine dihydrochloride, $1:2.5 \times 10^6$
A. Metabolic poisons:				
Potassium cyanide	.0004 M	+	+	+
Sodium azide	.0014 M	+	+	+
" iodoacetate	.00004 M	—	+	+
" fluoride	.002 M	+	+	+
" fluoroacetate	.001 M	+	+	+
Dinitrophenol	.0001 M	+	+	+
B. Autonomic drugs:				
Atropine sulfate	1: 7,000	+	—	+
Trasentine	1: 11,000	+	—	+
Hexamethonium bromide	1: 7,000	+	+	+
Mytolon chloride	1: 50,000	+	+	+
Ephedrine	1: 10,000	+	+	+
Epinephrine	1:100,000	+	+	±
Tripelennamine hydrochloride	1:100,000	—	+	—
C. Other substances:				
Ethyl alcohol	1.0%	—	+	+
n-Butyl alcohol	.2%	—	+	+
Urethane	1.0%	—*	+	+
Nupercaine	1:200,000	—*	+	+
Botulinum toxin	(See text)	—	+	±

* Muscle sometimes contracted during subsequent washing with Tyrode's solution.

ulant. Ordinarily each stimulant was applied only once to a particular portion of ileum. All experimental results reported were confirmed with several animals. Additional experiments in which the order of addition of the stimulants was changed showed that the results were independent of this factor. It was found important to remove a stimulating contaminant, apparently ammonium sulfate, from the egg albumin before use. This was done by dialyzing the egg albumin solutions against Tyrode's solution. As the amount of egg albumin necessary for a satisfactory response varied from one animal to another, the level to be used was chosen by first determining the minimum concentration of egg albumin needed for a just perceptible response, and then using 10 times this concentration in the tests with this particular animal.

The experiments with botulinum toxin required a slight modification of the usual technic. The procedure adopted consisted in injecting about 2×10^7 units of toxin diluted with Tyrode's solution into the lumen of a short length of ileum tied shut at both ends, and after 30 minutes at room temperature, washing out the toxin, arranging the ileum in the muscle bath, and then performing the tests with antigen or other stimulants. The toxin used was a type D preparation kindly furnished by Dr. George G. Wright, of Camp Detrick, Frederick, Md., and was stated to contain 10^8 mouse units per ml. A recheck of this figure after our experiments had been completed indicated little or no deterioration.

Results. Our first approach to the mechanisms by which antigen stimulates smooth muscle from a sensitized animal was to study the effect of a number of enzyme inhibitors upon the process. A similar approach to related problems has been used by others(11,12,13). Our experiments (Table I) showed that inhibitors that block respiration (cyanide, azide), other oxidative processes (fluoroacetate, dinitrophenol) and glycolysis (fluoride) did not, on short exposure, have an important effect upon the ability of antigen to cause contraction of smooth muscle from sensitized animals. Neither did these substances, under similar conditions, alter contraction due to acetylcholine or histamine. These results led us to a tentative working hypothesis that res-

piratory and glycolytic processes do not form part of the main chain of events in the Schultz-Dale reaction, although they are undoubtedly essential to the integrity of the tissue and to the recovery process following contraction.

The effect upon the contraction that results from antigen, acetylcholine, and histamine of a number of pharmacodynamic agents known to alter the activity of smooth muscle was also investigated. These results (Table I) generally confirm the numerous reports in the literature, and were useful in interpretation of data described in a later paragraph.

During this work certain observations led us to suspect the existence of a step involving nerve as a part of the reaction:

(1) During storage of ileum that had been removed from animals, ability to be stimulated by antigen was lost sooner than ability to be stimulated by acetylcholine or histamine. This behavior seemed to indicate a longer chain of events in the antigen stimulation. Because of the well-known lability of nerve, this hinted that some nervous element might be involved in the process as a whole.

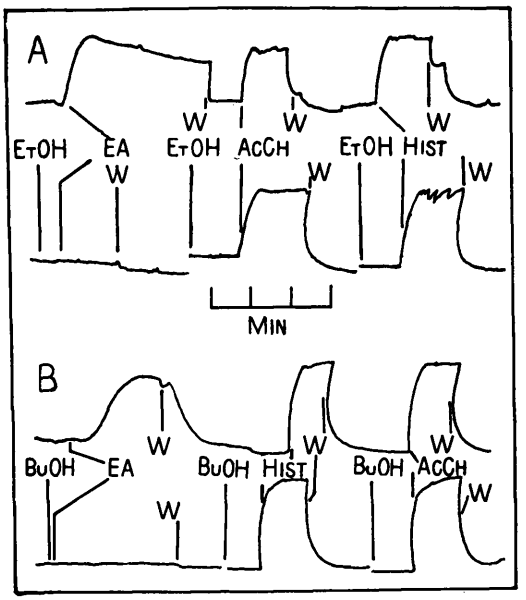

FIG. 1.* A. Effect of ethyl alcohol upon Schultz-Dale reaction. B. Effect of n-butyl alcohol upon Schultz-Dale reaction.

* Abbreviations used in figures are: EtOH, ethyl alcohol, 1.0%; EA, egg albumin, 1:2.5 × 10^7; Hist, histamine dihydrochloride, 1:2.5 × 10^6; BuOH, n-butyl alcohol, 0.4%; Ur, urethane, 1.0%; Min, time in min. The recording drum was stopped for 15 min. immediately following each addition of an alcohol or of urethane.

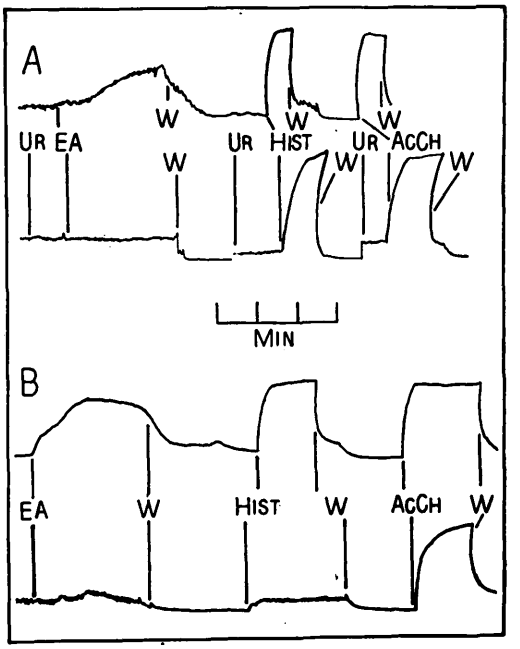

FIG. 2.* A. Effect of urethane upon Schultz-Dale reaction. B. Effect of type D botulinum toxin upon Schultz-Dale reaction. Lower tracing was obtained with ileum treated with botulinum toxin as described in text.

* Abbreviations: See footnote Fig. 1.

(2) Iodoacetate (0.0004 *M*) had much the same effect as aging. This also seemed to point to a chain of events involving nerve.

(3) The results obtained with the various pharmacodynamic agents listed in the table were at least consistent with a mechanism involving nerve.

An attempt was therefore made to determine whether substances known to block transmission in nerve were capable of blocking the Schultz-Dale reaction. It was found that ethyl alcohol (1.0%) and *n*-butyl alcohol (0.2%), blocked the Schultz-Dale reaction, but did not affect contraction due to acetylcholine and histamine (Fig. 1, A and B). Nupercaine and urethane (Fig. 2, A) also blocked the contraction resulting from antigen, but when the bath fluid was being replaced with fresh Tyrode's solution, con-

traction sometimes took place. Nupercaine and urethane did not alter the effects of acetylcholine and histamine.

These narcotics and anaesthetics are probably not very specific in their action, but seem to be able to block transmission in different kinds of nerves rather unspecifically(14,15), and it seemed desirable to locate the site of action more exactly if possible. The nervous structure involved in stimulation of contraction of the ileum includes preganglionic fibers that enter ganglia of Auerbach's plexus, whence postganglionic fibers lead to nerve endings associated with the smooth muscle. Preliminary evidence that the site of stimulation by antigen may be the postganglionic fibers was the observation (Table I) that the Schultz-Dale reaction remains unchanged in the presence of agents that block at the ganglia, hexamethonium bromide and benzoquinonium (Mytolon).

As Ambache(16,17) and others(18,19,20) have demonstrated, there exists a substance with a very specific paralyzing action upon postganglionic cholinergic fibers, namely, botulinum toxin. Studies with the type D toxin showed clearly that the antigen acts by stimulating the postganglionic fibers, since the toxin blocked the Schultz-Dale reaction, but not stimulation by acetylcholine (Fig. 2, B).

The ability of the toxin to suppress stimulation by histamine was highly variable. Sometimes the toxin suppressed stimulation by histamine rather markedly, (Fig. 2). In other experiments, little suppression was observed. Ambache(17) has recorded similar observations with rabbit ileum. It would appear that either the toxin penetrates to the histamine receptor sites to a different extent in different tissue samples, or that histamine stimulates at more than one kind of site. Our results as a whole seem to indicate that the most likely mechanism for the Schultz-Dale reaction involves stimulation of postganglionic nerve fibers by antigen in the presence of antibody, followed by muscular contraction. Our experiments thus support the theoretical contentions of Campbell(6).

Summary. A study has been made of the effects of a number of substances upon the Schultz-Dale reaction. For the most part, results in the literature concerning the effects of these substances were confirmed. The Schultz-Dale reaction, but not the responses due to histamine and acetylcholine, was blocked by urethane, Nupercaine, lower aliphatic alcohols, and type D botulinum toxin. The results are interpreted to indicate that the Schultz-Dale reaction involves postganglionic cholinergic nerve.

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