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A PHARMACOLOGICAL ANALYSIS OF THE MODE OF ACTION OF SEROTONIN (5-HYDROXYTRYPTAMINE) UPON THE GUINEA-PIG ILEUM

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Serotonin, the substance isolated by Rapport, Green, and Page (1948) from shed blood, was identified with 5-hydroxytryptamine-creatinine sulphate. A close parallelism between the pharmacological properties of serotonin and those of thrombocytin and circulating enteramine was recently established by Reid and Rand (1952) and Erspamer and Asero (1952). Besides the possible intrinsic importance of serotonin as an endogenous principle that may be mobilized in physiological or pathological conditions, serotonin presents interesting problems from a pharmacodynamic point of view the solution of which might help to throw light on questions concerning the mode of action of drugs upon smooth muscle.

When given intravenously, serotonin produces a fall followed by a rise of blood pressure. Atropine appears to abolish the fall and has no effect upon the rise, as shown recently by Page (1952). The action of serotonin upon the smooth muscle of the guinea-pig ileum is strongly influenced by atropine and appears to be parasympathomimetic or cholinergic in nature. Antihistaminic drugs have no action upon this smooth muscle stimulating effect. Tachyphylaxis to serotonin has been noted by Reid and Rand (1952) and by Freyberg *et al.* (1952).

The experiments presented here were undertaken to clarify the mode of action of serotonin upon the guinea-pig gut and especially as an attempt to localize its site of action among the different anatomical structures allegedly responsible for myokinetic drug effects. A preliminary account of this work has already been published (Rocha e Silva, Valle, and Picarelli, 1952).

METHODS AND MATERIALS

The conditions of the assay upon the guinea-pig ileum were the same as described previously (Ambache and Rocha e Silva, 1951). A chamber containing 3.5 ml. of Tyrode solution was used in some preliminary experiments, being replaced by one of 15 ml. capacity in most of the experiments described below. Where not otherwise indicated, the chamber used was the larger one. The drugs were usually added at 1.5 minute intervals; when longer intervals were required these are indicated by a cross (x) in the tracings or in the legends to the figures. After two washings with fresh Tyrode solution, a one minute resting period was allowed for recovery of the muscle. The standard histamine used was a 1 : 2 million solution, calculated as the base, of the dihydrochloride (Eastman Kodak). When not otherwise indicated, the doses of histamine and serotonin were respectively 0.2 of the 1 : 2 million solution and 5 μg. of serotonin. The routine dose of nicotine was 10 μg. of the base. For the sake of brevity, the name of serotonin has been used throughout the paper, although it is well established that the active part of the complex substance is the molecule of the base 5-hydroxytryptamine. All doses indicated in the paper in terms of the creatinine sulphate complex must be divided by 2.3 in order to express them in terms of the base. Acetylcholine was used as a 1 : 1 million solution of the hydrochloride (Roche). Other drugs studied were adrenaline (Parke Davis), pilocarpine hydrochloride, hexamethonium iodide, and *d*-tubocurarine (Intocostrin, Squibb and Sons). The serotonin (creatinine complex with 5-hydroxytryptamine) was obtained through the courtesy of Dr. R. Coghill of Abbott Laboratories, North Chicago, U.S.A.

RESULTS

Tachyphylaxis to Serotonin.—Small doses (4 to 5 μg.) of serotonin added to the bath containing a piece of guinea-pig ileum cause responses that are reproducible without any sign of tachyphylaxis. The responses to doses as low as 10 μg. of serotonin, however, cannot be kept at a constant level if the interval between additions is 1 or 2 minutes. If the interval between additions of 10 μg. of serotonin is increased to 3 minutes, reproducible responses can be obtained, as shown in Fig. 1. By keeping this interval between additions, and by using doses smaller than 20 μg., a dose-response relationship

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may be found and a 2×2 bioassay can be designed (Valle and Picarelli, 1952).

The effect obtained with small doses of serotonin is similar to that observed after small, stimulating doses of nicotine, and is distinguished by minor details from that produced by histamine or acetylcholine. After an almost instantaneous rise of tonus the muscle shows a certain tendency to return to a lower tonus, as soon as the maximum is attained, as contrasted with the sustained tonus observed with histamine, as shown in Figs. 1 and 2.

If the doses of serotonin are increased to 15, 30, or 40 μg. the responses are no longer quantitative and a tendency to partial or total tachyphylaxis is seen, as shown in Fig. 2. With doses of 40 μg. of serotonin, added to a bath of 15 ml. capacity, we can no longer maintain a steady level of responses, as indicated in Fig. 3, although histamine and acetylcholine still elicit reproducible responses. This difference of behaviour of the gut toward serotonin, as compared with histamine and acetylcholine, indicates that serotonin has a different locus of action. It is clear that serotonin acts upon other than histamine receptors, since antihistaminic drugs have no effect upon it. But, since atropine blocks serotonin, it is possible that serotonin acts primarily upon acetylcholine receptors. The experiments presented in Fig. 3, however, which show a tachyphylactic effect to serotonin but not to acetylcholine, indicate that serotonin affects distinct receptor structures.

Large doses of serotonin (50 to 200 μg. or more) produce a quick and powerful contraction followed by a sharp fall of tonus. Repeating the addition of the same large dose will produce no effect and the muscle remains refractory to the addition of a small dose of serotonin for quite a long interval of time, as shown in Fig. 4*a*. Recovery to such

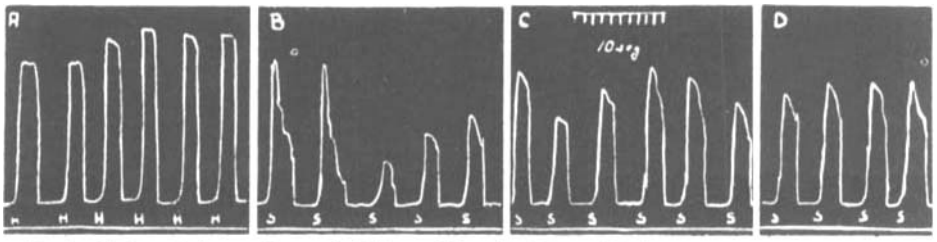

FIG. 1.—Guinea-pig ileum. Repeated additions of histamine 0.1 μg. (H), in panel A; of 10 μg. serotonin at 1 minute intervals in B; the same at 2 minute intervals in C, and at 3 minute intervals in D.

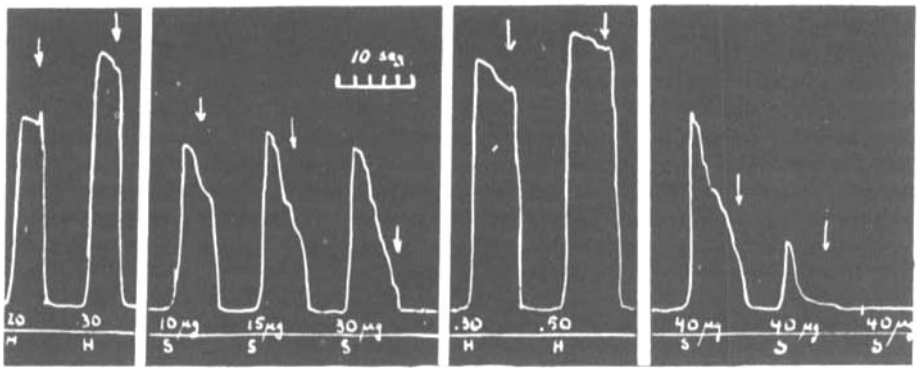

FIG. 2.—Guinea-pig ileum. Comparison between the additions of increasing doses of histamine (H) and serotonin (S). The doses of histamine are indicated as ml. of 10^{-6} solution.

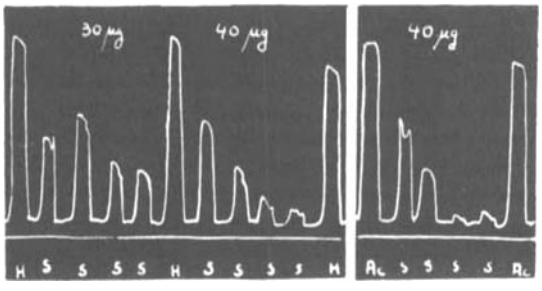

FIG. 3.—Guinea-pig ileum. Decrease in the responses to 30 μg. or 40 μg. of serotonin does not affect the responses to 0.1 μg. of histamine (H) and 0.15 μg. of acetylcholine (Ac).

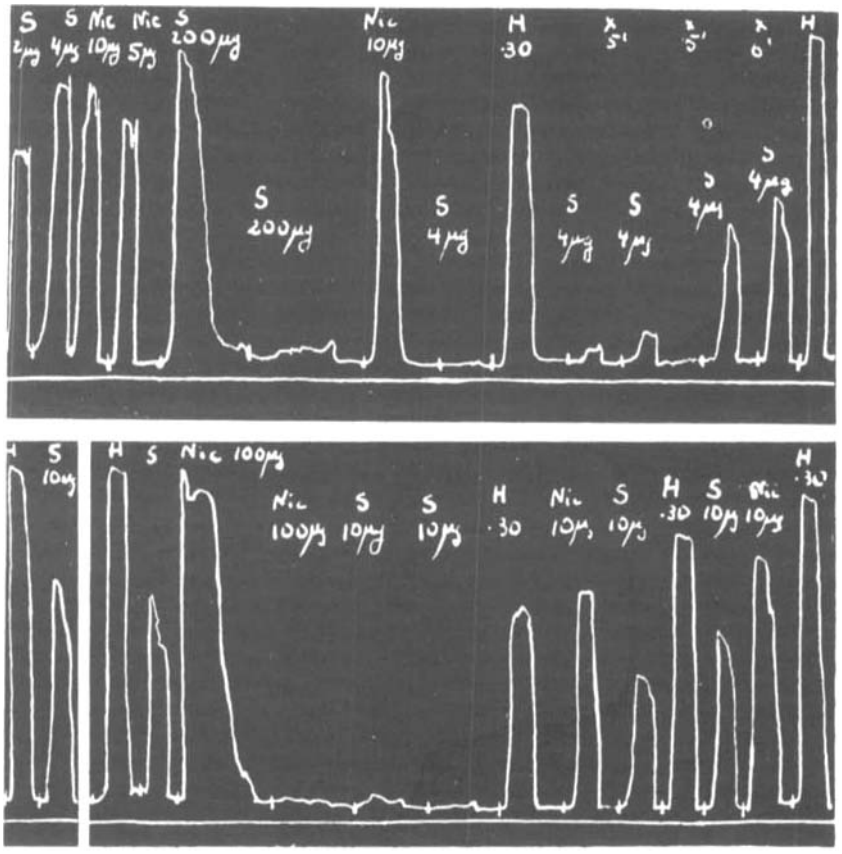

FIG. 4.—(*a*) Addition of 200 μg. of serotonin (S) to the bath produces a quick response followed by an immediate return to the normal level of tonus; the muscle will not react to the same dose or smaller doses of serotonin, although the reactions to nicotine and histamine remain unimpaired. (x) indicates stoppage of the drum for the times indicated. (*b*) Similar experiment with two additions of 100 μg. of nicotine. Note that during refractoriness to smaller doses of nicotine the response to 10 μg. of serotonin is abolished and the response to histamine (H) is strongly depressed. Doses of histamine indicated as ml. of 0.5×10^{-6} solution.

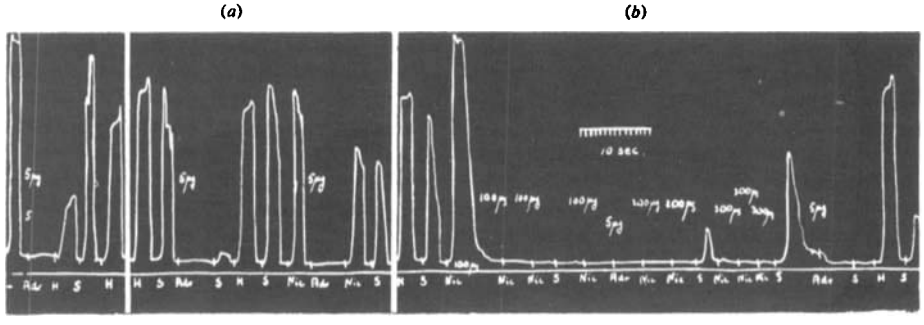

FIG. 5.—(*a*) The responses of the guinea-pig gut to histamine or serotonin are strongly depressed by previous treatment with 5 μg. of adrenaline. Note that 0.1 μg. histamine (H) was less affected than 5 μg. of serotonin (S) by the previous treatment of the gut with adrenaline; nicotine (Nic) was even less affected. (*b*) After three additions of 100 μg. of nicotine the response to serotonin is abolished; after further treatment with 100 μg. to 200 μg. of nicotine, the muscle begins to respond to serotonin, while completely refractory to nicotine itself; this "escape" from the inhibitory effect of nicotine is better seen after three further additions of 300 μg. of nicotine; it is interesting to note that adrenaline (5 μg.) still depressed the response to serotonin.

doses of serotonin takes a longer or shorter time to be complete, depending upon the size of the paralysing dose applied at the beginning. Tachyphylaxis toward serotonin is, therefore, temporary and can be distinguished from other types of tachyphylaxis, such as that observed after successive additions of certain bacterial toxins or animal venoms, in which the muscle remains indefinitely refractory after a first of two additions of any dose of the stimulating material. It can also be distinguished from the tachyphylaxis to antigen in sensitized guinea-pig gut (*in vitro* anaphylaxis), where also, after a first contact with the antigen, the muscle remains indefinitely refractory to the addition of the same dose.

Tachyphylaxis to paralysing doses of serotonin has some features similar to that obtained with large doses of nicotine (as shown in Fig. 4*b*) in that it is very quick to develop (while the drug is still in the bath), is reversible, and is not so unspecific as that seen after large doses of other spasmogenic agents, such as histamine, acetylcholine, or pilocarpine (Cantoni and Eastmann's effect). It can, however, be distinguished from the similar nicotinic effect by not altering appreciably the responses to other drugs such as histamine or acetylcholine. This difference, though slight, appeared of importance in interpreting some results that will be presented hereafter.

Effect of Nicotine upon Serotonin Action.—Small to moderate doses of nicotine (20 to 40 μg.) can depress the gut to serotonin. A full depression of serotonin by nicotine is observed with moderate paralysing doses (50 to 200 μg.), as shown in Figs. 4*b*, 5, and 6. When such doses are added to the chamber, a powerful contraction followed by a quick return to normal or subnormal tonus is seen. If the muscle is then tested with: (*a*) serotonin (4 to 10 μg.); (*b*) histamine; (*c*) acetylcholine; (*d*) nicotine (10 to 15 μg.); (*e*) bradykinin; (*f*) pilocarpine and (*g*) eserine, it is shown to be completely blocked toward *a* and *d*, moderately blocked to *b*, *c*, *e*, and *f*, and still reactive to *g*.

The complete block toward stimulating doses of serotonin produced by moderate paralysing doses of nicotine might induce one to assume that serotonin also acts upon the ganglion cells. Nevertheless, its site of action must be somewhat different from that of nicotine, since a paralysing dose of serotonin does not affect the responses to stimulating doses of nicotine.

The idea that serotonin acts upon the ganglion cells was, however, abandoned when we verified that the inhibitory effect produced by moderate paralysing doses of nicotine could not be obtained after applying large paralysing doses (300 to 400 μg.), even when these were repeatedly applied

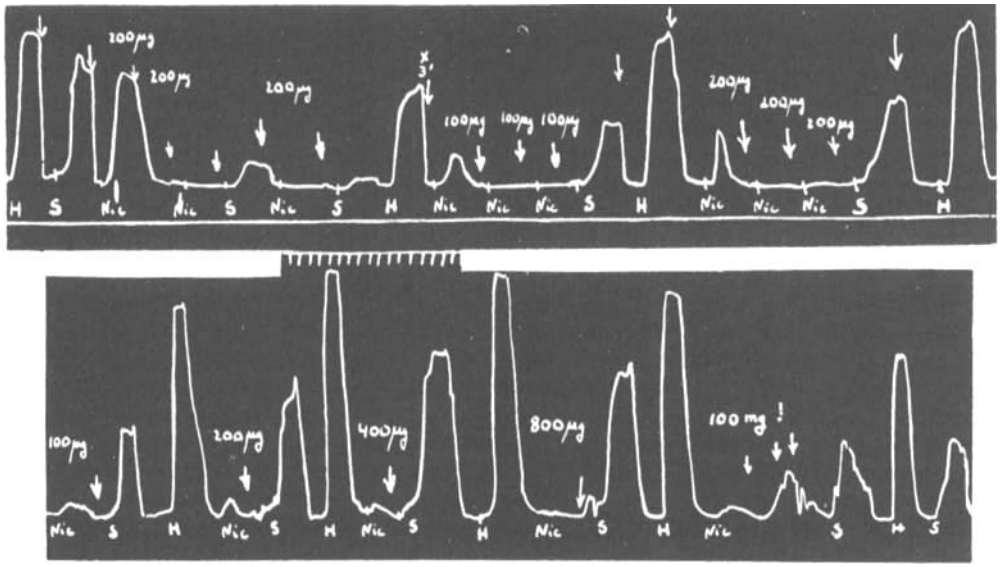

FIG. 6.—(*a*) "Escape" from inhibition by nicotine (Nic). After a strong inhibition of 5 μg. of serotonin (S) and 0.1 μg. of histamine (H) produced by 2 or 3 additions of 200 μg. of nicotine, the muscle recovers its full sensitivity to both agents after the further addition of increasing doses of nicotine. (*b*) Same piece of ileum. The responses to histamine or serotonin become progressively stronger as the dose of nicotine increases. The arrows indicate washings with fresh Tyrode solution.

to the intestine, as shown in Fig. 6*a* and *b*. This "escape" from inhibition by nicotine could be observed by repeatedly adding such moderate paralysing doses as 100 to 200 μg. of nicotine, as shown in Fig. 7. A speedier return of the full responsiveness to serotonin could be seen after the addition of a few large doses of nicotine (300 to 400 μg.). It is interesting to note that large doses of nicotine may produce a similar "escape" from the partial inhibition, by smaller doses of nicotine, the depression produced by nicotine could not result from any unspecific depressant effect upon the muscle itself, as suggested by Emmelin and Feldberg (1947) and Feldberg (1950), since this kind of depression too would obviously be greater with the larger doses. It is true that massive doses of nicotine, such as 800 μg., can sometimes depress the effect of serotonin or histamine, as shown in Fig. 6*b*, but this effect is slight compared with the strong depression produced by moderate (50 to 100 μg.) doses of nicotine.

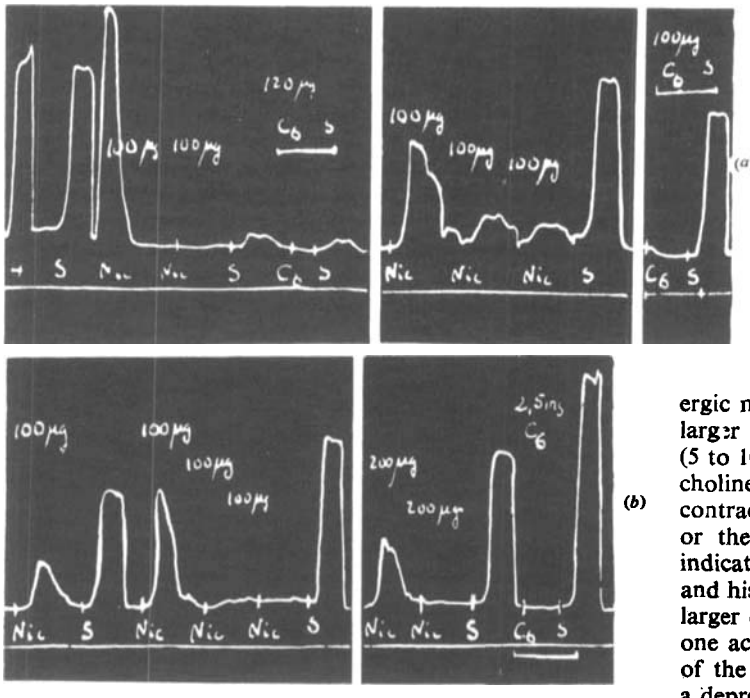

FIG. 7.—(*a*) Hexamethonium (C_6) added during refractoriness to 5 μg. of serotonin (S) produced by nicotine (Nic) will not change the sensitivity of the muscle to serotonin. After repeating the additions of nicotine, the muscle "escapes" from inhibition and responds fully to serotonin; hexamethonium added again before serotonin and kept in the bath does not affect the size of the response. (*b*) After repeated treatments with 100 to 200 μg. of nicotine the full sensitivity to serotonin is restored and hexamethonium potentiates serotonin action.

Instead of assuming a direct depression of the contractility of the muscle by such moderate doses of nicotine, we are more prone to accept the possibility that the inhibitory effect is due to the release of minute amounts of a mediator from adrenergic fibres. As with the parasympathetic ganglion apparatus, larger doses of nicotine (300–400 μg.) would exhaust the system. According to the experiments presented above, the adrenergic mechanism would require somewhat larger doses of nicotine, since a few μg. (5 to 10 μg.) apparently act only upon the cholinergic ganglion system, producing a contraction without affecting the histamine or the serotonin added afterwards. As indicated above, a depression of serotonin and histamine is seen only after somewhat larger doses of nicotine (30 to 50 μg.). If one accepts the hypothesis of a discharge of the adrenergic system to explain such a depression, these doses are probably the threshold ones for nicotine to affect the inhibitory fibres of the intestinal wall. Abolition of the depression of serotonin or histamine ("escape from nicotine") can be obtained by adding doses of nicotine that are twice or three times larger than those necessary to paralyse the parasympathetic ganglion system. We may therefore assume that the concentrations of nicotine which affect the intramural adrenergic system are two to three times higher than those which act upon the parasympathetic ganglion cells.

of the effects of histamine, acetylcholine, or bradykinin (Ambache and Rocha e Silva, 1951; Rocha e Silva, 1952). In Fig. 6*a* and *b*, a partial inhibition of the histamine effect by repeated addition of 200 μg. of nicotine was followed by a clear-cut "escape" after further treatment with 100 to 800 μg. of nicotine.

The foregoing results indicate that the depression caused by moderate paralysing doses of nicotine is not due to the ganglionic paralysis itself, since this would be even greater with the larger doses of nicotine. They also clearly demonstrate that

Effect of Adrenaline upon Serotonin Action.—In order to substantiate the hypothesis of a discharge of the adrenergic system by moderately paralysing doses (50 to 100 μg.) of nicotine, to explain the depression of the responses to serotonin and other

drugs such as histamine, acetylcholine, and bradykinin, we have tried to correlate the depression produced by nicotine with that observed by adding adrenaline. A perfect parallelism would not, of course, be expected, since we do not know whether adrenaline or noradrenaline, or a mixture of both, would be released by the stimulating effect of nicotine. Thus a commercial adrenaline preparation "Adrenalin," Parke Davis) was judged likely to give as good a result as the use of pure adrenaline or noradrenaline.

Extremely small concentrations of adrenaline (0.07 μg./ml.) in the bath depressed the effect of histamine and serotonin. The latter was found to be more influenced than the former by such small concentrations. With bradykinin and histamine we have found, in some preparations, that both are influenced in the same way by adrenaline and by nicotine. It is of interest to note that on adding increasing doses of nicotine, the muscle "escaped" from inhibition toward bradykinin as it usually does toward serotonin and histamine.

The "escape" to nicotine appears to be a general phenomenon and can probably be explained by a diphasic effect upon the adrenergic system. In a few preparations the inhibition toward histamine or serotonin can be obtained when small doses of nicotine are added, but a definite depression is usually seen after doses around 50 to 100 μg. of nicotine. Even with such moderate doses of nicotine, if repeatedly added to the bath, the "escape" to the inhibitory effect of nicotine may be noticeable; but a quicker "escape" can only be seen if the doses of nicotine are increased beyond the 300 or 400 μg. level. A direct "toxic" effect of the alkaloid upon the muscle fibres is only obtained with much higher doses, far above the mg. level.

Effect of Decamethonium, Hexamethonium, and d-Tubocurarine on Serotonin Action.—The peristaltic reflex is abolished by hexamethonium (C6) as shown by Paton and Zaimis (1949), and this effect is due to a paralysing action of hexamethonium upon the parasympathetic ganglion cells of the mammalian intestine. Feldberg (1951) considered hexamethonium the most specific ganglion inhibitor, as far as the guinea-pig gut is concerned.

Decamethonium (C10) displays very slight or no effect as a ganglion blocking agent when assayed upon the guinea-pig gut. According to Feldberg (1951), *d*-tubocurarine has strong atropine-like activity, since it depresses the response to acetylcholine as well as that to nicotine.

Hexamethonium has no inhibitory effect upon the contraction elicited by serotonin upon the guinea-pig ileum, as is shown in Fig. 8*a* and *b*. In most instances the addition of 50 to 100 μg. of hexamethonium tended to enhance the effect produced by 10 μg. of serotonin. The results obtained with C6 indicate very clearly that serotonin does not act through stimulation of the parasympathetic ganglion cells.. This agrees with the fact, presented above, that very large doses of nicotine, such as completely block the parasympathetic ganglion system, have no action upon serotonin. The enhancement of the serotonin effect by C6 makes it interesting to recall that ganglion blocking

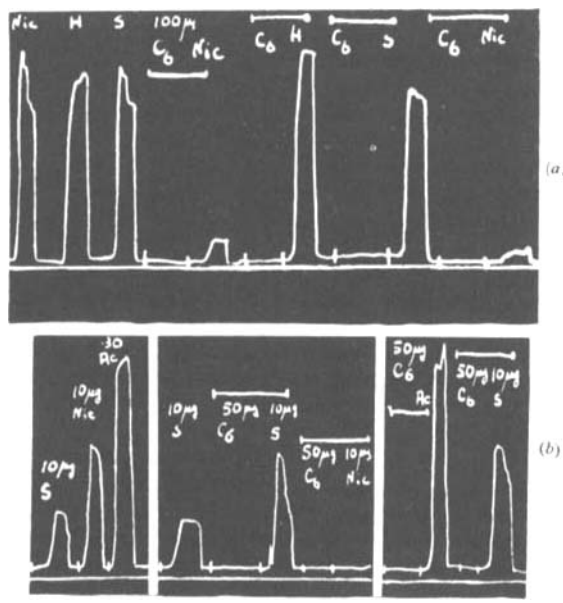

FIG. 8.—Hexamethonium (C_6) in concentrations that almost abolished the nicotine responses had no effect upon 0.1 μg. histamine (H) or 5 μg. serotonin (S) or 0.3 ml. 10^{-6} acetylcholine (Ac), though it had sometimes a potentiating effect upon serotonin (middle panel of *b*). The white horizontal lines indicate that both drugs were kept in the bath.

agents have been described as potentiating the effects of other spasmogenic drugs. Thus hexamethonium and tetraethylammonium sometimes potentiate the effect of histamine (Feldberg, 1951, and Collins, 1948), while *d*-tubocurarine has a potentiating effect upon histamine (Rocha e Silva and Schild, 1949, and Feldberg, 1951). This potentiating effect was interpreted by Feldberg (1951) as due to an increase of excitability of the muscle fibres after blockade of the ganglion cells. The effect of C6 upon serotonin, as shown in Fig. 7*b* and Fig. 8*b*, can likewise be interpreted in the same way.

Decamethonium (C10), when added in doses as high as 100 μg., had no effect upon the serotonin induced contraction (Fig. 9*a*). The inhibition by

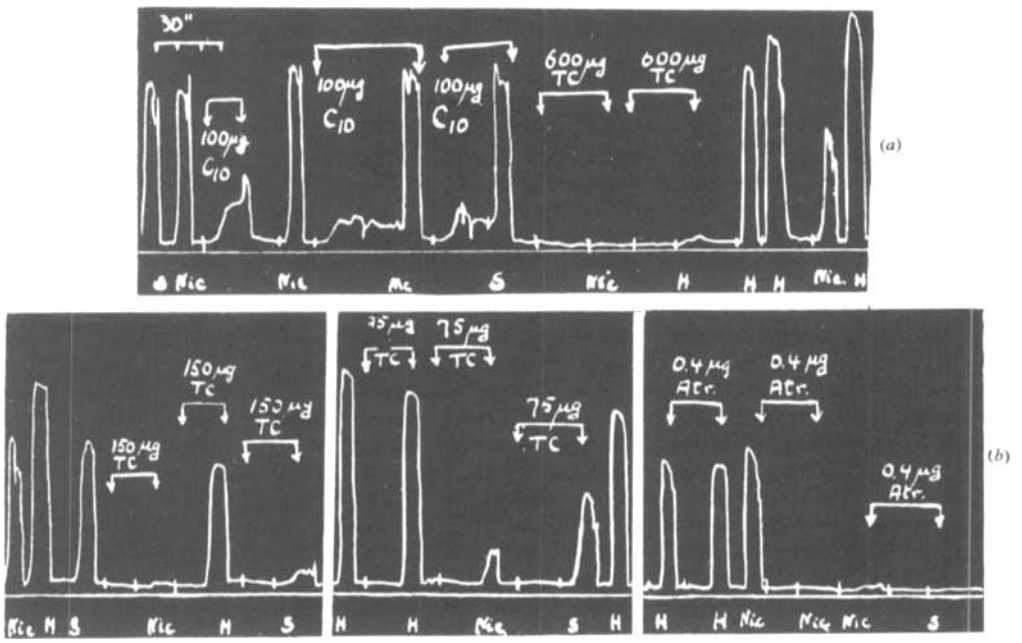

FIG. 9.—(*a*) Decamethonium (C_{10}) in a dose of 100 μg. had no effect upon 10 μg. nicotine (Nic) and 5 μg. serotonin (S). High doses (0.6 mg./15 ml.) of *d*-tubocurarine totally blocked the stimulating effect of nicotine and histamine. (*b*) Smaller doses of *d*-tubocurarine totally (150 μg./15 ml.) or partially (75 μg./15 ml.) inhibited the response to nicotine and serotonin, the blocking effect being greater upon nicotine and much less so upon histamine. A small dose of atropine (0.4 μg.) entirely blocked nicotine and serotonin and had no effect upon histamine.

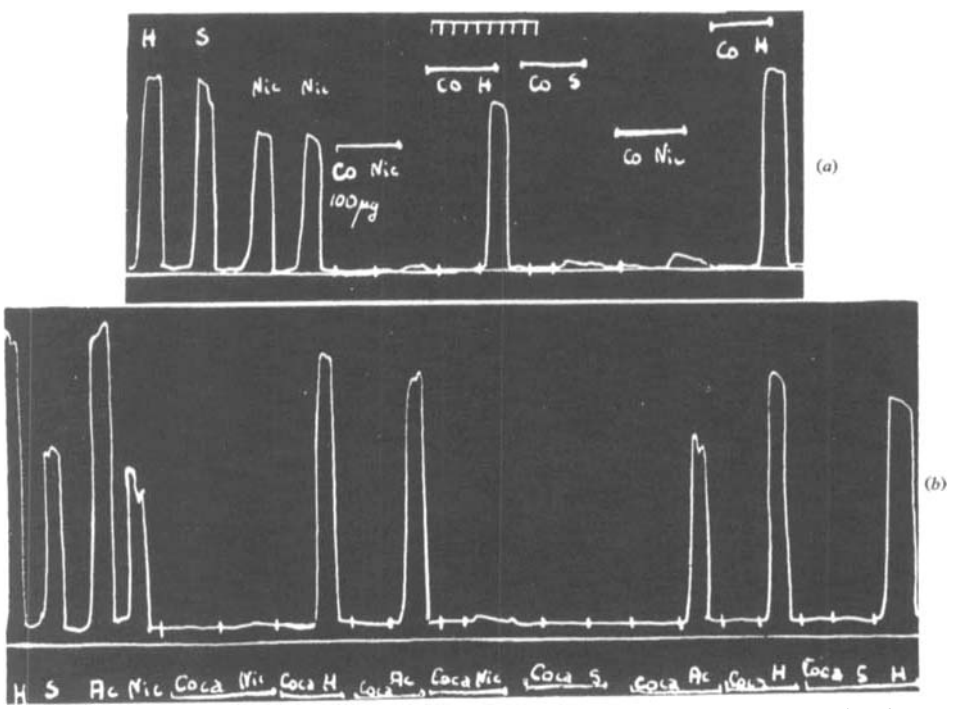

FIG. 10.—(*a*) and (*b*). Cocaine (Co), in concentrations (100 μg./15 ml.) that had no effect upon 0.1 μg. histamine (H) or 0.15 μg. of acetylcholine (Ac), completely abolished the responses to 10 μg. nicotine (Nic) or 5 μg. serotonin (S).

d-tubocurarine, shown in Fig. 9*a* and *b*, might be accounted for by its atropine-like activity, demonstrated by Feldberg (1951).

Effect of Cocaine upon Serotonin Action.—According to Feldberg and Lin (1949, 1950) it is possible, with a suitable dose of cocaine, to paralyse the nicotine stimulating action, without impairing the effect of histamine or acetylcholine. Doses of cocaine below 0.4 mg./ml. are usually able to block completely the stimulating effect produced by 10 μg. of nicotine and have no effect upon other drugs supposed to act directly upon the muscle (Fig. 10*a* and *b*). With serotonin we have found that such a minute dose of cocaine as 10 μg. in a 15 ml. bath will inhibit the spasmogenic effect of 10 μg. of serotonin, as shown in Fig. 11. An inhibition of the histamine or acetylcholine effects can only be obtained with doses 50 to 150 times stronger, and even then the blockade is not complete.

The inhibitory effect of threshold doses of cocaine upon serotonin gave us the key to explain the locus of action of the latter drug upon the gut. Since cocaine, in concentrations that will not affect histamine or acetylcholine, is supposed to act by blocking the transmitting fibres of the myenteric plexus, only two possibilities are left to explain inhibition of the action of serotonin upon the gut: (*a*) the pre-ganglionic and (*b*) the post-ganglionic parasympathetic fibres. A stimulus upon *a* would need, of course, a free path through the ganglion cells; but, as we have shown in the above paragraphs, serotonin acts upon the gut even when the ganglion cells have been completely blocked either by large doses of nicotine or by hexamethonium. Apparently, therefore, the only indirect point of attack for serotonin would be the post-ganglionic fibres that can be blocked by cocaine (see diagram, Fig. 12).

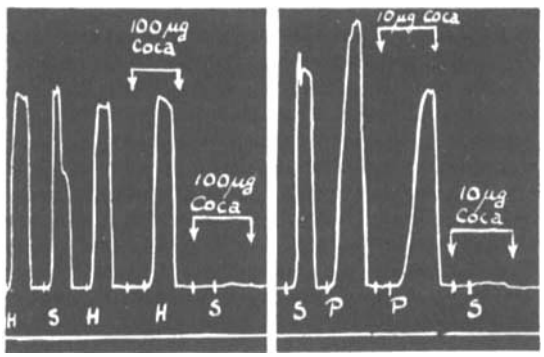

FIG. 11.—100 μg. of cocaine (Coca) had no effect upon 0.1 μg. histamine (H), but completely blocked the effect of 5 μg. serotonin (S). Inhibition of the serotonin action was observed with doses as small as 10 μg. of cocaine per 15 ml. A partial block of pilocarpine (P) was observed with 10 μg. of cocaine added to the bath (15 ml.).

DISCUSSION

In any attempt to localize the site of action of a drug upon such a complicated anatomical structure as the gut of vertebrates, we have to be aware of the fact that all evidence is indirect, since it is impossible to isolate any single motor unit as can be done with striated muscle (Fisher, 1944). There are, however, a few data that can provide solid ground on which to establish a theory to account for the experimental results presented above. There is no question, for example, that the efferent nerve fibres to the mammalian small intestine come from the two main sections of the autonomic nervous system—the parasympathetic and the sympathetic outflow. There is also enough histological evidence to show that most of the ganglion cells of the myenteric plexus of Auerbach and Meissner belong to the parasympathetic system, while the possibility that some of the adrenergic terminal fibres come from ganglion cells of the myenteric plexus is still open. Ambache and Edwards (1951) have recently provided fresh evidence of the presence of synapses along the sympathetic fibres to the cat intestine. But, whatever be the final answer to that question, one has to consider a system of inhibitory fibres in close connection with the smooth muscle effectors. A third class of nerve fibres is constituted by the so-called "terminal reticulum," a tangled system of argyrophil neurofibrils around the ganglion cells and in close association with the so-called Cajal cells. A discussion of the whole problem can be found in Fisher (1944), Feldberg and Lin (1949), Ambache (1946), and Ambache and Edwards (1951). The diagram (Fig. 12) gives a schematic view of the possibilities for the different loci of action of drugs upon the guinea-pig gut. The structures have been drawn separately for the sake of simplicity, but it seems probable that the "degree of entanglement" is higher than is represented in the diagram.

In such conditions, we have to rely upon the indirect information obtained by applying inhibitors of several kinds—antihistaminics, atropine, ganglion blocking agents (decamethonium, hexamethonium, nicotine, and *d*-tubocurarine), local anaesthetics and so forth—to define the mode of action of any substance upon the gut. The evidence presented above strongly indicates that serotonin might be considered a specific stimulant of the post-ganglionic cholinergic fibres. Our knowledge of the physiological significance of the so-called "ter-

minal reticulum" is scanty, but if it be found that such fibres are cholinergic in character they might well constitute a site of action for an indirectly working drug such as serotonin. That serotonin has receptors of its own, distinct from those for histamine, acetylcholine, pilocarpine, bradykinin, or nicotine, is suggested by the following facts: (*a*) antihistaminics will not abolish the effect of serotonin; (*b*) when the muscle is made refractory to a higher dose of nicotine, and its ganglion cells are thus paralysed, serotonin will act upon the gut better than when the latter has been paralysed by a moderate dose of nicotine; (*c*) when the muscle is made refractory to serotonin by applying a large paralysing dose of this substance, it still responds to histamine, nicotine, acetylcholine, and pilocarpine; (*d*) hexamethonium completely blocks the action of nicotine, yet rather potentiates the effect of serotonin; (*e*) cocaine, which in the concentrations used will not affect histamine or acetylcholine, entirely blocks the action of serotonin upon the gut. Since serotonin apparently does not work directly upon the muscle fibres of the intestine, or upon the ganglion cells, its stimulating effect can properly be ascribed to a discharge, followed by temporary exhaustion, of the post-ganglionic cholinergic fibres.

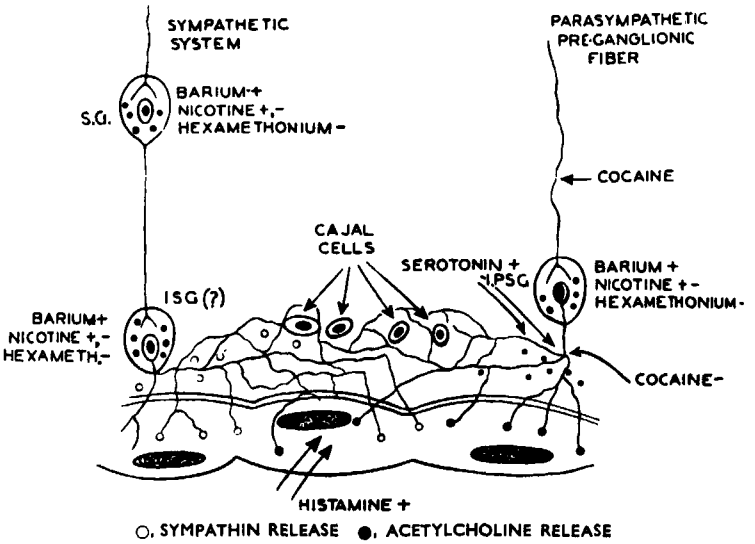

FIG. 12.—Schematic picture of the possible loci of drug action upon the guinea-pig ileum. ISG, intramural sympathetic ganglion (hypothetical); IPSG, intramural parasympathetic ganglion cell; ○, sympathin release; ●, acetylcholine release.

A point of interest to be discussed is the mechanism of the "escape" to the paralysing dose of nicotine. Small, paralysing doses of nicotine (50 to 100 μg.) strongly depress or abolish the effect of serotonin upon the gut. Other drugs, such as histamine, acetylcholine, and bradykinin, are also depressed by such doses of nicotine, although as with bradykinin this depression is seen irregularly, as indicated by Ambache and Rocha e Silva (1951). Feldberg (1950) assumed that nicotine in such doses would depress unspecifically the smooth muscle fibres to any gut stimulating drug. On the contrary, Ambache (1946) pointed out that this effect of nicotine in depressing histamine or acetylcholine would indicate that such drugs act at least partially through the ganglion systems. That neither view is correct was first shown by Ambache and Rocha e Silva (1951) on the basis of the fact that the depression produced by nicotine is stronger with moderate doses (stimulating and paralysing doses), and that, if the addition of the same dose was many times repeated or higher doses (200 μg. to 1 mg.) were used, the inhibition was considerably reduced or abolished. To explain the depression produced by moderate (stimulating and paralysing) doses of nicotine, Ambache and Rocha e Silva (1951) assumed, in accordance with Emmelin and Feldberg (1947), that partial refractoriness of the muscle cells might be due to Cantoni and Eastmann's effect—that is, to exhaustion of energy-rich metabolites after the maximal contraction produced by such doses of nicotine. The absence of depression after the higher (paralysing only) doses of nicotine might result from the contraction being of such short duration—or being absent altogether—that Cantoni and Eastmann's effect could not come into play. This explanation might also hold for the total inhibition of serotonin by moderate (stimulating and paralysing) doses of nicotine; while its "escape" from nicotine inhibition might be accounted for by the fact that if the muscle is kept for a long time under the paralysing action of nicotine no metabolites are consumed so that, again, Cantoni and Eastmann's effect is absent.

However, an alternative explanation has been presented in this paper, based on the possibility of nicotine discharging and paralysing the adrenergic inhibitory fibres. This requires only the assumption that the range of doses in which nicotine acts upon the parasympathetic ganglion system is lower than that in which it acts upon the inhibitory intramural

system. That nicotine can stimulate the inhibitory system was demonstrated by Ambache and Edwards (1951) in the atropinized intestine of the cat. That this effect might result from a release of adrenaline or noradrenaline is a strong possibility. In the depression produced by nicotine upon the effects of several drugs a similar mechanism might operate. If one assumes that moderate doses (50 to 100 μg.) of nicotine discharge the adrenergic fibres to the smooth muscle with liberation of minute amounts of a sympathomimetic mediator (adrenaline or noradrenaline, or both) the fall in tonus and also the unspecific inhibition toward serotonin, histamine, acetylcholine, pilocarpine, etc., might be due to the effect of the liberated mediator. If it be so that nicotine discharges these fibres, or the ganglion cells interposed in their pathways, one might expect that large doses (up to 200 to 400 μg.) of nicotine would also paralyse this system, in the same way that smaller doses will discharge and paralyse the cholinergic ganglion system. This view agrees with the observation by Feldberg and Lin (1949) that moderately paralysing doses of nicotine produce a fall of tonus of the rabbit intestine, from which inhibition the muscle recovers, even after having been submitted to the continuous action of nicotine.

Summary

1. The mode of action of serotonin (complex of creatinine+5-hydroxytryptamine) was studied upon the isolated guinea-pig ileum. The stimulating effect of serotonin on the gut was blocked by atropine and appeared, therefore, to be cholinergic in nature. Tachyphylaxis could be observed with small doses (10 μg./15 ml.) if they were added at intervals of less than 3 minutes.

2. Total, though transitory, tachyphylaxis was observed after repeated additions of 40 μg./15 ml. of serotonin. With higher doses, a quick contraction followed by spontaneous return to the normal tonus (while the drug still was in the bath) was observed. For a certain interval of time thereafter the muscle remained refractory to smaller doses of serotonin, but still reacted to other drugs, including nicotine. Sensitivity to small doses of serotonin progressively reappeared.

3. Moderate paralysing doses (50 to 100 μg.) of nicotine depressed or abolished the responses to serotonin and also depressed the responses to histamine, acetylcholine, and bradykinin. If much higher doses (200 to 800 μg.) of nicotine were added, the muscle "escaped" from inhibition by nicotine, responding again to serotonin and increasing in sensitivity to the other drugs, although continuing to be irresponsive to nicotine itself.

4. Decamethonium and hexamethonium had no inhibitory effect upon serotonin action. In some experiments, hexamethonium even potentiated serotonin action. *d*-Tubocurarine in concentrations that had no effect upon histamine depressed the effects produced by serotonin or nicotine.

5. The action of serotonin was completely blocked by cocaine in doses (10 to 100 μg.) that did not affect histamine or acetylcholine.

5. From these observations the provisional conclusion is drawn that serotonin acts upon the post-ganglionic cholinergic fibres of the intramural nervous system of the guinea-pig ileum.

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Addendum

As indicated in Fig. 9*b*, a dose of atropine of 0.4 μg. in a 15 ml. bath abolished the effect of 5 μg. of serotonin upon the guinea-pig ileum. Under the conditions of our laboratory (São Paulo), using a Tyrode solution of *p*H 7.4, this dose of atropine strongly depressed or abolished the action of small doses of serotonin. Since, in recent papers, Feldberg and Toh (1953) and Gaddum