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.

References

Ambache, N. (1946). *J. Physiol.*, **104**, 266.
—— and Edwards, J. (1951). *Brit. J. Pharmacol.*, **6**, 311.
—— and Rocha e Silva, M. (1951). Ibid., **6**, 68.
Cantoni, G. L., and Eastmann, G. (1946). *J. Pharmacol.*, **87**, 392.
Collins, O. A. (1948). Ibid., **94**. 244.
Emmelin, N., and Feldberg, W. (1947). *J. Physiol.*, **106**, 482.
Erspamer, V., and Asero, B. (1952). *Nature, Lond.*, **169**, 800.
Feldberg, W. (1950). *Proc. XVIII Int. Physiol. Congress*, Copenhagen.
—— (1951). *J. Physiol.*, **113**, 483.
—— and Lin, R. C. Y. (1949). *Brit. J. Pharmacol.*, **4**, 33.
—— —— (1950). *J. Physiol.*, **11**, 96.
Fisher, E. (1944). *Physiol. Rev.*, **24**, 467.
Freyberg, W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. M., Swoap, O. F., and Brook, M. J. V. (1952). *J. Pharmacol.*, **105**, 80.
Page, I. H. (1952). Ibid., **105**, 58.
Paton, W. D. M., and Zaimis, E. J. (1949). *Brit. J. Pharmacol.*, **4**, 381.
Rapport, M. M., Green, A. A., and Page, I. H. (1948). *J. biol. Chem.*, **176**, 1243.
Reid, G., and Rand, M. (1952). *Nature, Lond.*, **169**, 801.
Rocha e Silva, M. (1952). *Reaçoes inesperadas em Farmacologia*, São Paulo.
—— and Schild, H. O. (1949). *J. Physiol.*, **109**, 448.
—— Valle, J. R., and Picarelli, Z. P. (1952). *Ciência e Cultura, São Paulo*, **4**, 130.
Valle, J. R., and Picarelli, Z. P. In the press.

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

(1953) claim that atropine does not influence serotonin, we have made further observations on the action of atropine upon serotonin. The conditions of the experiments were different and were those prevailing at the Department of Physiology of the Caroline Institute in Stockholm (3 ml. intestinal bath and a somewhat more alkaline Tyrode, *p*H 8.2). Doses of atropine as small as 0.1 μg./3 ml. strongly depressed (80 to 90% reduction in the size of the response) the effect of 1 to 5 μg. of serotonin; a dose of 0.2 μg./3 ml. almost completely abolished the response to 1 μg. of serotonin. The different results described by the British authors might be due to the use of a more alkaline Tyrode solution, for Dr. Edlund from Uppsala has found that a more acid Tyrode solution (*p*H 6.8) increases considerably the sensitivity of the gut to the inhibitory action of atropine (personal communication).

In some experiments, especially when the gut was very sensitive to histamine, the effect of the latter was also influenced by such concentrations of atropine; but the depression of the histamine effect was much less (25 to 30% reduction) than was the inhibitory effect upon serotonin (90% reduction). But, even in preparations where the histamine effect was not influenced, serotonin was strongly depressed by 0.2 μg./3 ml. of atropine sulphate. Doses of atropine that reduced to 10% or to 0 the action of serotonin had not the slightest effect upon bradykinin (5 to 10 μg. of a preparation containing 8 units per mg.). The effect of substance P is also not affected by such doses of atropine, as shown recently by Pernow (1953). We cannot therefore ascribe to a direct effect of atropine upon the smooth muscle fibres the depression or abolition of the serotonin response. The doses of atropine required to affect that response to serotonin are at least 10 times larger than the threshold doses to abolish the effect of 0.1 μg. of acetylcholine; but if the comparison is made with nicotine as the spasmogenic agent the differences are much smaller. In one experiment upon the same ileum, 0.1 μg./3 ml. of atropine reduced to 15% the effect of 5 μg. of serotonin and to 7% the effect of nicotine. Half of that dose of atropine (0.05 μg./3 ml.) had practically no effect upon 5 μg. of serotonin or 5 μg. of nicotine, although it abolished completely the effect of 0.1 μg. of acetylcholine.

As regards the specificity of the atropine effect, we have analysed the recovery of the gut toward serotonin and acetylcholine, after a 1 minute contact of 0.1 and 0.2 μg./3 ml. of atropine, testing the muscle every 1.5 minute by alternating the additions of serotonin (5 μg.) and acetylcholine (0.1 μg.). The recovery to both drugs followed a parallel curve, essentially similar to that described by Rocha e Silva (1950) for specific antagonists such as antihistaminic, antispasmodic, and atropine-like substances.

I am much indebted to Professor U. S. von Euler for kind advice and for giving me facilities to make these observations at the Caroline Institute, and to Dr. B. Pernow for help in performing the experiments.

References

Feldberg, W., and Toh, C. C. (1953). *J. Physiol.*, **119**, 352.
Gaddum, J. H. (1953). Ibid., **119**, 363.
Pernow, B. (1953). *Acta physiol. scand.*, **20**, Supp. 105, 1.
Rocha e Silva, M. (1950). *Exp. Med. Surg.*, **8**, 346.