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THE FATE OF 5-HYDROXYTRYPTAMINE IN A SMOOTH MUSCLE AND IN CONNECTIVE TISSUE

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Earlier work (Born & Bricknell, 1959*a*) showed that 5-hydroxytryptamine (5-HT) is taken up by blood platelets even when the temperature is so low that the mechanism responsible for concentrating 5-HT in platelets is abolished. Under these conditions the amounts taken up are very small. It seemed, therefore, that the method used to demonstrate this uptake might show whether smooth-muscle cells which, unlike platelets, contract in the presence of 5-HT also take it up.

The principle of the method is as follows. The volume of extracellular fluid (the 'extracellular space') is measured as accurately as possible in an isolated piece of tissue. This is then immersed in a solution containing 5-HT until its concentration in the tissue increases no more. If this concentration turns out to be significantly greater than that which can be accounted for by the presence of 5-HT in the extracellular fluid, the tissue has taken up 5-HT in some way.

The experiments reported in this paper show what happens to 5-HT in the guinea-pig taenia coli; this tissue is largely made up of smooth-muscle cells which contract in the presence of 5-HT.

Inulin was used to measure the volume of extracellular fluid, and radioactive 5-HT to measure uptake. The 5-HT was labelled with ^{14}C in position 1 on the side chain. The amounts of *intact* 5-HT in the tissues were determined by bioassay. Measurement of the radioactivity of the tissue, together with a knowledge of the specific activity of the 5-HT in the medium, gave values for the amounts of 5-HT that had been broken down in the tissue. As this paper suggests, it is very likely that the break-down of 5-HT occurred only inside the smooth-muscle cells, and the concentration of the break-down product, therefore, provided information about the rate and extent to which 5-HT penetrated into the smooth-muscle cells.

Taenia coli also contains connective tissue and a few nerve cells. Since it was possible that 5-HT might be taken up by connective tissue as well

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as by smooth muscle, similar experiments were made with guinea-pig connective tissue that was free of smooth muscle; for this purpose Achilles tendon was used.

Some of the results have been communicated to the British Pharmacological Society (Born & Bricknell, 1959*b*).

METHODS

Guinea-pigs were anaesthetized with ether and then killed by bleeding. Their taenia coli muscles were dissected free as described by Bülbring (1953). Pieces of the Achilles tendon which contained no visible muscle were cut out.

Inulin was purified by two precipitations in 80% (v/v) ethanol followed by washing in distilled water.

5-Hydroxytryptamine labelled with ^{14}C (5-HT-^{14}C) (5-hydroxy-3-indolyl-(ethyl-2-amine-1-^{14}C) creatine sulphate monohydrate) was obtained from the Radiochemical Centre at Amersham; it had a specific activity of 6·87 mc/m-mole.

Uptake by taenia coli. The uptakes of inulin and labelled 5-HT were determined as follows. As soon as the two taeniae had been removed from an animal each taenia was cut into five pieces. The pieces were weighed and immersed in Krebs's bicarbonate solution as modified by Bülbring (1953). The solution was gassed with a mixture of 95% O_2 and 5% CO_2 and maintained either at 0 or at 37° C. The solution contained inulin and radioactive 5-HT; the concentrations varied in different experiments, as stated in Results. At different times a piece of taenia was taken out of the solution and was lightly rolled on filter paper till no more moisture appeared in the paper; this took about 5 sec. The piece was then weighed and dispersed in water with a glass homogenizer (Potter & Elvehjem, 1936). Water was added until the volume was 1·5 ml. This tissue suspension was used for determining inulin and 5-HT.

Inulin was determined as follows. 0·5 ml. suspension was mixed with 0·2 ml. of 0·5N-NaOH and 0·2 ml. of $ZnSO_4$ solution 10 g/100 ml. Water was added until the volume was 2·0 ml. The precipitate that formed was removed by centrifugation and 0·2 ml. of the clear supernatant solution was analysed for inulin by the method of Kulka (1956). A piece of taenia which had not been exposed to inulin was treated in the same way; the colorimetric reading obtained with it was used to correct the other readings for colour not due to inulin.

Intact 5-HT was determined by bioassay using strips of isolated rat stomach (Vane, 1957).

Intact 5-HT plus its break-down product were determined from measurements of radioactivity, as follows: 0·5 ml. of the tissue suspension was mixed with 2·0 ml. acetone and the mixture was left at −16° C overnight. It was centrifuged and some of the clear supernatant was dried on planchettes in duplicate. The volume dried on planchettes varied from 0·3 to 0·9 ml., depending on the radioactivity. When the volume was greater than 0·3 ml. the amount of solid matter on the planchette was large enough to decrease the counting rates. When necessary, therefore, control experiments were made to establish the magnitude of this decrease and corrections were made for it. The radioactivity on the planchette was determined in a helium gas-flow counter which had an apparent counting efficiency of about 50%. A planchette with glycine-1-^{14}C was used as standard source of radioactivity, which was counted whenever radioactive 5-HT was counted, and these counts were corrected for small variations in counts from the standard source. Whenever possible counting was continued until the statistical error was less than 3%.

In each experiment a sample of Krebs's solution bathing the tissue was used for determining both intact 5-HT (by bioassay) and radioactivity; from these values the *specific activity* of the 5-HT in the solution was calculated. By knowing the specific activity and by determining the intact 5-HT in smooth muscle, the concentration of oxidation product of

5-HT could be calculated, making the assumption that the molecular weight of the oxidation product was the same as that of 5-HT itself.

Loss from taenia coli and from Achilles tendon. The losses of inulin and 5-HT were determined as follows. First, weighed pieces of tissue were immersed at 37° C in modified Krebs's solution containing inulin and radioactive 5-HT. The volume of the solution was about fifty times greater than the volume of the tissue. When, as shown by preceding experiments, no more inulin or radioactive 5-HT entered the tissue, it was removed from the solution, briefly rolled on filter paper as already described, and weighed. The tissue was then immersed in 0·2 ml. of Krebs's solution in the glass tube shown in Fig. 1. The tube was kept in a water-bath either at 0 or at 37° C. After 1 min the tube was lifted out of the water-bath; the upper end was closed with a finger, the bulldog clip on the outflow was released and, by blowing through the rubber tube on the side arm, the solution bathing the tissue was rapidly ejected on to a planchette for counting radioactivity or into a test-tube for determining inulin or intact 5-HT. The clip was closed and 0·2 ml. of fresh solution was pipetted into the tube, which was then replaced in the water-bath.

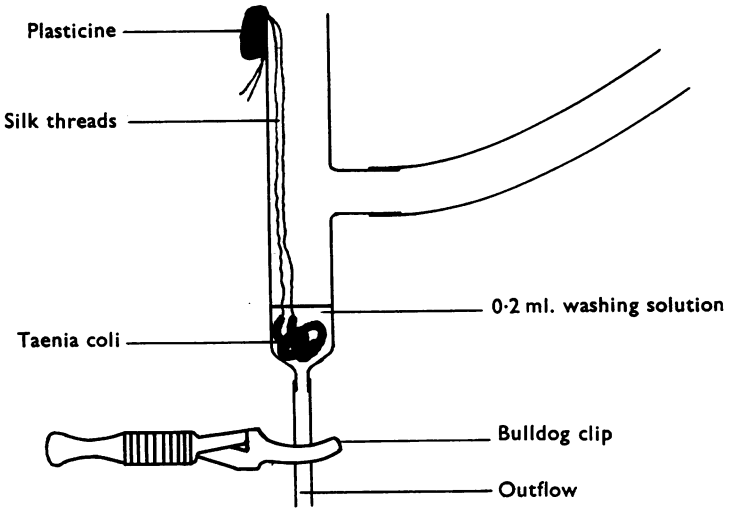

Fig. 1. Method of collecting washings from isolated taenia coli to measure the losses of inulin and of radioactive 5-HT. For description see text.

In this way all the inulin and radioactive 5-HT were collected as they were lost from the tissue. Bathing solution was collected at intervals which varied from 1 or 2 min at the beginning of an experiment to 2, 5 or 10 min at the end. Up to 27 samples were collected in any one experiment. Samples collected in test-tubes were used for determining intact 5-HT and inulin as already described, except that it was unnecessary to go through the procedure for precipitating proteins. The samples collected on planchettes were dried and their radioactivities were determined for calculating intact 5-HT plus oxidation product.

RESULTS

Time course of the uptake of inulin and of 5-HT *by taenia coli*

In two experiments pieces of taenia coli were incubated in modified Krebs's solution containing inulin and labelled 5-HT at 37° C, and in another experiment at 0° C. At different times pieces of taenia were

analysed for inulin and for radioactivity. Results of one of the experiments done at 37° C are shown in Fig. 2. The uptake of inulin stopped after 20 min; in the other two experiments it stopped after about 60 min. In all three experiments the radioactivity of the taenia increased for about 120 min. The rates of uptake of radioactivity at 0 and at 37° C were not significantly different.

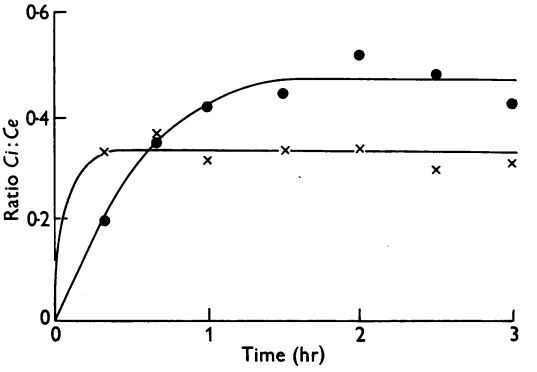

Fig. 2. Rate of uptake by isolated taenia coli at 37° C of inulin (×) and of radioactive 5-HT (●) from a solution containing 40 mg inulin/ml. and 0·1 μg 5-HT-^{14}C/ml. Ratio *Ci*:*Ce* = inulin or radioactivity/mg. taenia: inulin or radioactivity/μl. medium. Note that for convenience the curve for the radioactivity shows the ratio divided by 10.

TABLE 1. The inulin space in isolated taenia coli

Expt. no.	Temperature (° C)	Concn. of inulin in fluid bathing the taenia (mg/ml.)	Inulin space, i.e. ratio inulin/mg taenia: inulin/μl. fluid		
			No. of estimations	Range	Mean
1	0	8	3	0·228–0·232	0·230
2	0	8	9	0·193–0·217	0·211
3	37	8	9	0·279–0·312	0·297
4	37	8	7	0·255–0·287	0·270
5	37	8	4	0·275–0·324	0·308
		40	5	0·269–0·332	0·312
6	37	40	4	0·291–0·337	0·317
7	37	40	7	0·296–0·364	0·325

Determination of the inulin space of taenia coli

In these experiments taeniae were left in solution containing inulin until no more was taken up. The inulin space was defined as the ratio, inulin/mg taenia: inulin/μl. bathing fluid (Creese, 1954). Table 1 shows that the inulin space was about 0·30 at 37° C and about 0·22 at 0° C. A possible explanation for this difference is suggested in the discussion. Bozler & Levine (1958) found that in the smooth muscle of frog's stomach the

average inulin space at 25–27° C was 0·28, a result which agrees well with ours. It may, therefore, be assumed that in taenia coli, as in other tissues, the inulin space measures the extracellular space (Boyle, Conway, Kane & O'Reilly, 1941; Creese, 1954).

Determination of the '5-HT space' of taenia coli

'5-HT space' was defined similarly as the ratio, 5-HT/mg taenia: 5-HT/μl. bathing fluid. To measure this space isolated taeniae were left in solutions containing labelled 5-HT until no more radioactivity was taken up. Table 2 shows that at 0° C the '5-HT space', as calculated from the radioactivity of the tissue, was 0·80–0·98 and that it did not depend on the concentration of radioactive 5-HT in the medium. This space was thus about 3 times greater than the inulin space. At 37° C the '5-HT space' was 4·68, i.e. about 15 times greater than the inulin space. The table suggests,

TABLE 2. The '5-HT space' in isolated taenia coli

Expt. no.	Temperature (° C)	Concn. of 5-HT-^{14}C in fluid bathing the taenia (μg/ml.)	'5-HT space', i.e. ratio 5-HT-^{14}C/mg taenia: 5-HT-^{14}C/μl. fluid
1	0	0·1	0·98
2	0	10	0·80
		100	0·83
3	37	0·1	4·65
4	37	0·1	4·68
5	37	0·01	4·47
		0·1	3·62
		1·0	4·13
6	37	1·0	4·45
		10	2·80
		100	2·82
7	37	10	3·13
		100	2·46

moreover, that at 37° C the size of the '5-HT space' depended on the concentration of 5-HT in the medium: the higher the concentration the smaller the 'space'.

The results show that the '5-HT space' at both temperatures measured something different from the inulin or true extracellular space. The size of the '5-HT space' at 0° C could be explained by assuming that radioactive 5-HT entered the cells of the taenia and distributed itself throughout the intracellular as well as throughout the extracellular water, being also very slightly concentrated in the tissue. Clearly, however, something more was happening at 37° C, since the radioactivity was much more concentrated in the tissue than outside.

Relation between the concentration of radioactive 5-HT *in the medium and the concentration of radioactivity in taenia coli*

Pieces of taenia were immersed in solutions containing increasing concentrations of radioactive 5-HT until no more radioactivity entered the tissue. Figure 3 shows that, with increasing concentrations of radioactive 5-HT in the medium, the radioactivity of the tissue increased. However, there was a difference between 0 and 37° C. At 0° C the radioactivity in the taenia was directly proportional to the concentration in the medium, the

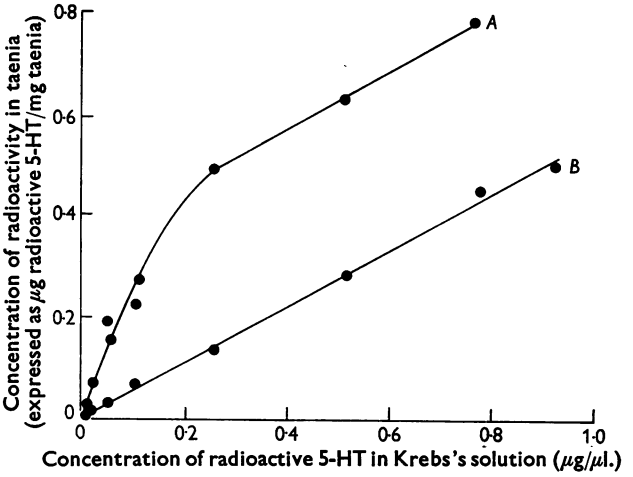

Fig. 3. Relation between the concentration of radioactive 5-HT in the modified Krebs's bicarbonate solution and the amount of radioactivity taken up by isolated taenia coli. Curve *A*, experiments at 37° C; curve *B*, at 0° C.

proportionality constant being 0·55. At 37° C, as the concentration in the solution increased from 0 to 0·2 μg 5-HT/μl., the radioactivity in the taenia increased more than at 0° C; with higher concentrations the radioactivity increased once more in proportion to the concentration of radioactive 5-HT in the medium, the proportionality constant being 0·57. The figure shows also that, with concentrations up to almost 1 μg radioactive 5-HT/μl. medium, i.e. 1 in 1000, there was no evidence of a limit to the increase of radioactivity in the taenia. The results show that in some way radioactive 5-HT became associated with taenia coli at 0 and 37° C, and that at 37° C the radioactivity became concentrated in the tissue up to fivefold.

Loss of radioactivity from taenia coli

From the experiments done so far it was not possible to tell whether the radioactivity recovered from taenia represented intact 5-HT or a

break-down product still bearing the radioactive label. Pieces of taenia were, therefore, soaked at 37° C in Krebs's solution containing radioactive 5-HT until the radioactivity of the taenia increased no longer. Then the rates were determined at which not only radioactivity but also intact 5-HT (assayed biologically) were lost into inactive Krebs's solution, as described under Methods.

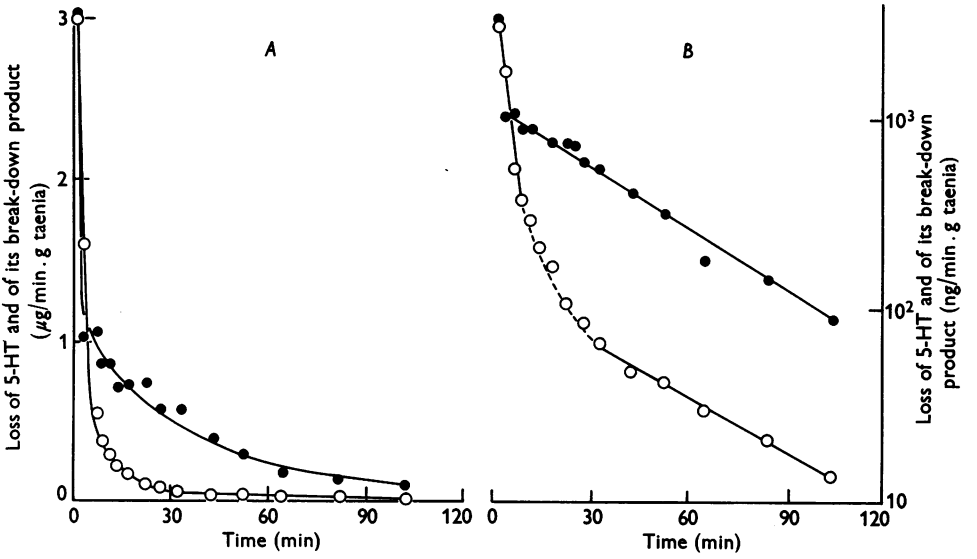

Fig. 4. *A*, Loss of radioactive 5-HT (○) and of its radioactive break-down product (●) from isolated taenia coli into Krebs's solution. *B*, the same results plotted semi-logarithmically.

Figure 4*A* shows the results of a typical experiment. The open circles represent the intact 5-HT; almost all of this was lost from taenia within the first 10 min; a small proportion was lost at a very slow rate which was still measurable after 105 min. The closed circles represent '5-HT' as calculated from the loss of radioactivity; these values did not agree at all with those for intact 5-HT. Therefore, a large proportion of the radioactivity lost from taenia was in the form of a break-down product of 5-HT. The same results are plotted semi-logarithmically in Fig. 4*B*. It shows that intact 5-HT was lost at at least two exponential rates, the first with a half-time of about 4 min, and the second of 32 min. The break-down product was lost at one exponential rate with a half-time of 29 min.

Loss of inulin from taenia coli

It seemed likely that the intact 5-HT which was lost at the faster of the two rates came from the extracellular space. If this was so, inulin should

be lost at a similar rate. Figure 5 shows that after taenia had been soaked in Krebs's solution containing inulin it was lost into inulin-free solution at two exponential rates. Both were rapid: the first had a half-time of about 3 min; the second began after about 8 min, and had a half-time of about 6·4 min. With the same taenia we also determined the first exponential rate of loss of 5-HT; it had a half-time of about 3·5 min, very similar to the first rate for inulin. This supported the contention that this 5-HT was in the extracellular space.

Loss of radioactive 5-HT *from taenia treated with isopropyl-phenylhydrazine*

The next step was to determine the nature of the radioactive break-down product. A piece of taenia was soaked at 37° C in Krebs's solution containing isopropyl-phenylhydrazine at a concentration of 0·2 μg/ml.; this substance inhibits amine oxidase. After 30 min radioactive 5-HT was added and the tissue was left in the solution for another 2 hr. The taenia was transferred to inactive Krebs's solution and the losses of radioactivity and intact 5-HT were determined as before. Figure 6*A* shows that the loss of 5-HT as determined by bioassay was the same as that calculated from radioactivity measurements. Thus, the isopropyl-phenylhydrazine had completely prevented the break-down of 5-HT. It was concluded that the break-down product was formed through the oxidation of 5-HT by amine oxidase and that when amine oxidase was inhibited only intact 5-HT was lost from taenia. Figure 6*A* shows that this loss occurred at two exponential rates the half-times of which were about 4 min and 33 min respectively.

The concentration of 5-HT *and of its break-down product in taenia coli*

It was now clear that when the amine oxidase of taenia was not inhibited the considerable concentration of radioactivity in the tissue represented a break-down product of 5-HT, whereas when amine oxidase *was* inhibited the radioactivity represented only intact 5-HT. The extents to which intact 5-HT and break-down product became concentrated in taenia were compared in the following experiment. Pieces of taenia were incubated at 0 and 37° C for 30 min without and with isopropyl-phenylhydrazine (0·2 μg/ml.). Radioactive 5-HT was then added to give a concentration of 20 μg/ml. and incubation was continued for 90 min.

The concentrations of intact 5-HT and break-down product that were then found in the pieces of taenia are set out in Table 3. At 0° C the concentration of intact 5-HT plus break-down product was the same as the concentration of 5-HT in the medium (i.e. *ca.* 20 μg/g and 20 μg/ml.

respectively), whether isopropyl-phenylhydrazine was present or not. However, in its presence *intact* 5-HT accounted for 87 %, but in its absence for only 40 %, of the 5-HT that had entered the tissue.

At 37° C the results were different. In the presence of isopropyl-phenylhydrazine all the 5-HT in the taenia was intact and at a concentration 2·5 times higher than that in the medium. In the absence of isopropyl-phenylhydrazine, almost all (96 %) the 5-HT in the taenia had been broken down, and the concentration of break-down product was 4·2 times higher than that of 5-HT in the medium. Moreover, what little 5-HT had

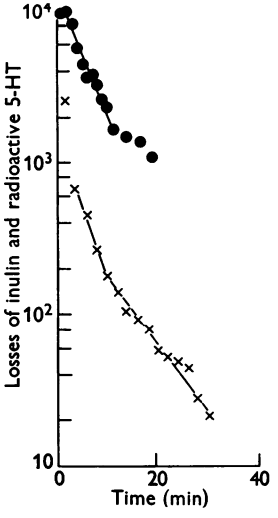

Fig. 5

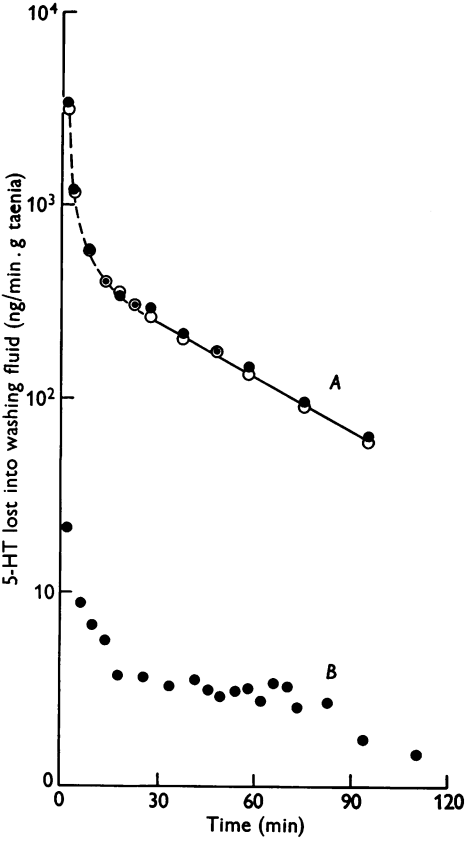

Fig. 6

Fig. 5. Loss of inulin (×) and of radioactive 5-HT (●) from isolated taenia coli. Ordinate: concentrations of inulin (expressed as optical density) and of 5-HT (expressed as counts/min) in washing fluid, plotted semi-logarithmically.

Fig. 6. Loss of 5-HT from taenia coli which had been bathed in Krebs's solutions containing isopropyl-phenylhydrazine (0·2 μg/ml.) and radioactive 5-HT either in a *high* concentration (20 μg/ml.; curve *A*) or in a *low* concentration (0·1 μg/ml.; curve *B*). ○ 5-HT as determined by bio-assay; ● as determined by radioactivity.

remained intact (4%) was presumably all in the extracellular space for, with a concentration of 20 μg 5-HT/ml. in the medium and an extracellular space of about 30%, the space should contain about 7 μg 5-HT/g taenia.

TABLE 3. Concentration of intact 5-HT and of its break-down product in isolated taenia coli

The concentration of radioactive 5-HT in the medium was 20 μg/ml. Concentrations are given as μg 5-HT or break-down product/g fresh weight of taenia. Concentration ratio means, concentration in taenia: concentration in medium.

Incubation temperature (° C)	Isopropyl-phenyl-hydrazine (0·2 μg/ml.)	Intact 5-HT			Break-down product		
		Concn.	Intact (%)	Concn. ratio	Concn.	Broken down (%)	Concn. ratio
0	Absent	8·3	40	0·4	12·2	60	0·6
	Present	17·6	87	0·9	2·7	13	0·1
37	Absent	3·9	4	0·2	83·8	96	4·2
	Present	49	100	2·5	Nil	Nil	Nil

Repetition with lower concentrations of 5-HT

In all the experiments described so far the solution in which taenia were soaked contained radioactive 5-HT in rather high concentrations, i.e. 20 μg/ml. This was to ensure that the amounts of radioactivity and intact 5-HT in taenia were enough for accurate determination. In one experiment the concentration of 5-HT added to the solution was nearer to those used in pharmacological experiments. A piece of taenia was soaked at 37° C in a solution containing isopropyl-phenylhydrazine, and radioactive 5-HT at a concentration of 0·1 μg/ml. Figure 6*B* shows that from this taenia also radioactive 5-HT was lost at two exponential rates with half-times similar to those shown in Fig. 6*A*. The radioactivity was very low and counting had to be continued for long times to obtain values significantly different from the background counts.

Loss of radioactive 5-HT *from Achilles tendon*

Figure 7 shows the results of experiments in which the loss of radioactive 5-HT from isolated Achilles tendon was determined. Curve *A* shows that the values for 5-HT determined by bioassay were, within the experimental error, the same as those determined by radioactivity. Therefore there was no break-down of 5-HT in Achilles tendon and it was unnecessary to add isopropyl-phenylhydrazine. Curve *A* shows that the loss occurred at two successive exponential rates, the first of which had a half-time of 11 min, and the second of 22 min. The change in rates occurred after *ca.* 30 min. Achilles tendon is a dense mass of fibrous tissue and it was possible that 5-HT, without becoming actually attached to the tissue, might simply be hindered in its outward diffusion. Another Achilles tendon was therefore

gently teased apart and the rates of loss of radioactive 5-HT were determined again. Curve *B* in Fig. 7 shows that the loss occurred again at two or even three exponential rates, of which the first two were certainly much faster than those obtained with normal Achilles tendon (Curve *A*); the half-times of these rates were about 4, 8·5 and 36 min, respectively.

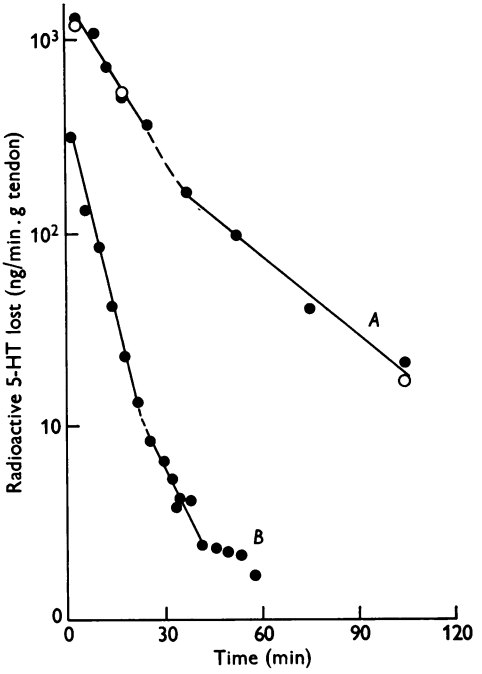

Fig. 7. Loss of radioactive 5-HT from Achilles tendon, ○ as determined by bioassay and ● as determined by radioactivity. Curve *A* represents loss from freshly dissected tendon and curve *B* from tendon which had been gently teased apart.

DISCUSSION

The purpose of these experiments was to see whether something could be discovered about the nature of 'receptors' for 5-HT by following its fate in smooth muscle. It has been generally held that 5-HT, which, like other primary amines, is charged at physiological pH, is able to penetrate into cells either not at all or only very slowly indeed. It seemed possible, therefore, that if in smooth muscle what has been called here the '5-HT space' turned out to be significantly greater than the true extracellular space the excess 5-HT might be bound in some way.

Inulin was used to measure the true extracellular space of the taenia. This was greater at 37° C than at 0° C. The reason may have been that at 0° C the pumping mechanisms which maintained the osmotic steady states

between the intra- and extracellular fluids were inactive; the cells probably swelled so that the space between them was diminished.

At 0° C the inulin space was about 0·22 of the volume of the tissue. The first experiments with 5-HT showed already that the '5-HT space' was about four times larger, i.e. almost as large as the volume of the whole tissue. The simplest way to account for this is that, unlike inulin, 5-HT was able to diffuse into the cells and to distribute itself throughout the intracellular as well as throughout the extracellular water.

At 37° C taenia incubated in a solution containing radioactive 5-HT accumulated radioactivity until its concentration in the tissue was almost 5 times greater than in the medium. However, this radioactivity was due not to 5-HT itself but to a break-down product. Presumably the product accumulated in taenia until the rate at which it was produced was equalled by the rate at which it was lost from the tissue by diffusion. Taenia which had been exposed to phenyl-isopropylhydrazine also took up radioactive 5-HT, but all this remained intact. Phenyl-isopropylhydrazine inhibits amine oxidase and amine oxidase is associated with intracellular particles (Blaschko, 1952). It was concluded that 5-HT was able to penetrate into smooth-muscle cells, in which it was broken down by amine oxidase unless the enzyme was inhibited.

The extent to which intact 5-HT was taken up by taenia was small, because the highest concentration of 5-HT in taenia was only about 2·5 times greater than that in the solution bathing the tissue. This is very much less than the extent to which 5-HT is concentrated by blood platelets (Born & Gillson, 1959) and is presumably due to a different mechanism. The slight concentration in smooth muscle is accounted for most simply by assuming that 5-HT distributes itself according to a Donnan equilibrium. The Krebs's solution bathing the taenia had a pH of about 7·4. The internal pH of smooth-muscle cells is not known, but that of striated muscle is just over 7 (for references see Caldwell, 1958). If the pH in smooth-muscle cells is similar, the concentration of H ions in them would be 2–3 times greater than in the bathing solution. Since 5-HT is almost entirely in the form of a cation at physiological pH (Vane, 1959) the concentration of 5-HT should also be 2–3 times greater in the muscle cells than in the external solution, as was found experimentally. In red blood cells 5-HT (Stacey, 1956) and adrenaline (Bain, Gaunt & Suffolk, 1937) are also 2–3 times more concentrated than in the medium in which the cells are suspended, and this distribution may be explained in the same way.

The movement of 5-HT into smooth muscle, unlike its movement into platelets, did not depend greatly on temperature. The rate of movement into smooth muscle was similar to that into platelets at 0° C. Therefore

the movement was presumably by diffusion. The diffusion of intact 5-HT had a half-time of 32 min, and that of the break-down product one of 29 min. The similarity of these rates suggests that the primary amino group in the molecule does not affect the diffusion of 5-HT through cell membranes.

When taenia contracts under the influence of 5-HT the tension reaches maximum after about 2 min. It has been shown that this is the time required for 5-HT to diffuse evenly throughout the extracellular space (Born & Vane, 1961); and now it is clear also that, because the rate at which 5-HT crosses cell membranes is so slow, the amount that *enters* the cells while they develop tension is exceedingly small.

In the absence of amine-oxidase inhibitor the loss of intact 5-HT from taenia occurred at two very different rates. Most of the 5-HT was lost at an initial rate which was rapid and similar to that for inulin; presumably it represented the diffusion of 5-HT out of the extracellular space. The small proportion of intact 5-HT which remained was lost at a rate which was very much slower and which continued for as long as it was observed, i.e. for at least 105 min.

It is interesting to consider from where this slowly-moving 5-HT could have come. First, it might have come out of the cells, like the break-down product, which would imply that a small proportion of intracellular 5-HT was not broken down. This seems unlikely in view of the slowness with which 5-HT diffuses into cells and the high activity of amine oxidase in smooth muscle (Vane, 1959). Secondly, the 5-HT might have been held back by a barrier to its free diffusion in the extracellular space. Such a barrier might be the connective tissue in taenia, since, as the experiments with Achilles tendon have shown, connective can slow diffusion considerably. Against this possibility, however, is the observation that the loss of inulin from taenia was complete long before that of 5-HT, although the molecules of inulin are much larger than those of 5-HT. Thirdly, it is possible that the small amount of 5-HT that was lost at the slow rate was rather firmly bound to chemical groupings which are specific for 5-HT and which are on the surface of the smooth-muscle cells. This is the most interesting possibility, since it suggests that the experimental approach described in this paper may lead to information about 'receptor' or 'storage' sites for 5-HT in tissues that react to it.

Apart from this the results may provide an explanation of the tachyphylaxis which is observed when smooth muscle, such as that of taenia coli, responds to repeated doses of 5-HT (Bülbring & Burnstock, 1960): it is found that the greater the dose the smaller the response to a subsequent dose of the same size. This may be explained by the assumption that the continued presence of 5-HT in the tissue brings about a reduction in its

responsiveness. Thus, when a piece of taenia coli is suspended in a bath and 5-HT is added it diffuses rapidly throughout the extracellular space. When the bath is refilled with solution free from 5-HT most of it rapidly diffuses out, but a little remains in the muscle for much longer. If this 5-HT is on or near the 'receptors' it may well make the muscle less sensitive to a subsequent dose.

SUMMARY

1. The fate of radioactive 5-hydroxytryptamine (5-HT) was observed in isolated tissues of guinea-pigs.

2. Radioactive 5-HT taken up by Achilles tendon was lost intact, at rates which depended upon the denseness of the connective tissue.

3. Taenia coli took up radioactive 5-HT from the bathing solution for about 120 min at both 0 and 37° C. The radioactivity was not concentrated at 0° C, but at 37° C its concentration was up to 5 times higher in the taenia than in the bathing fluid.

4. The radioactivity in the tissue increased in proportion to the concentration of 5-HT in the bathing fluid.

5. Radioactivity taken up by taenia coli was lost from it into non-radioactive solution, but only a small proportion of this radioactivity represented intact 5-HT. The intact 5-HT was lost at two successive exponential rates. The half-time of the first rate was *ca.* 4 min. This was similar to the rate at which inulin was lost from taenia; presumably the 5-HT lost at this rate was in the extracellular space. A small proportion of intact 5-HT was lost at the second rate; this was much slower and had a half-time of about 32 min.

6. When the amine oxidase inhibitor isopropyl-phenylhydrazine was present in the fluid bathing the taenia all the radioactivity that was lost could be accounted for as intact 5-HT. Under these conditions the concentration of intact 5-HT in taenia was about 2·5 times greater than in the bathing fluid.

7. It may be concluded that 5-HT is able to diffuse into the smooth-muscle cells of taenia coli, and that in this smooth muscle it is broken down only by amine oxidase.

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