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UPTAKE OF 5-HYDROXYTRYPTAMINE BY PLATELETS

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Human blood platelets were incubated with 5-hydroxytryptamine and with tryptamine and the uptake of each amine measured. The uptake of tryptamine, unlike that of 5-hydroxytryptamine, was linearly related to the concentration of the amine in the surrounding fluid, was similar in amount at 0° and 37° C and varied directly with the *p*H of the solution. When both amines were present together the uptake of 5-hydroxytryptamine was depressed. The antagonism of tryptamine was found to be competitive, and the possible site of its action is discussed. The effect of a number of other substances on the uptake of 5-hydroxytryptamine by platelets was examined; of these imipramine, cocaine and chlorpromazine were more potent and dihydroergotamine and lysergic acid diethylamide somewhat less potent than tryptamine in inhibiting uptake.

Blood platelets incubated at 37° C with a solution of 5-hydroxytryptamine take up the amine so that its concentration in the platelets may reach a value several hundred times that of the surrounding solution. The rate of uptake has been shown to be very sensitive to temperature (Hardisty & Stacey, 1955); it is reduced by loss of carbon dioxide from the plasma in which the platelets are suspended (Born & Gillson, 1959), by the presence of various metabolic inhibitors and other substances (Born & Gillson, 1959; Sano, Kakimoto & Taniguchi, 1958; Weissbach & Redfield, 1960); and by pretreatment of the animal with reserpine (Hardisty, Ingram & Stacey, 1956). In human subjects it is diminished in certain diseases of the bone marrow (Hardisty & Stacey, 1957). Some further observations on factors affecting this mechanism are now reported.

METHODS

Platelet-rich plasma was used for these experiments, since the separation and re-suspension of human platelets impair their ability to absorb 5-hydroxytryptamine. Further, Waller, Löhr, Grignani & Gross (1959) have shown that after washing there is a marked reduction in the activity of platelet enzymes. In most of the experiments platelet-rich plasma was prepared from human blood and in a few from dog blood. All glassware with which platelets came in contact was siliconed (I.C.I. silicone fluid M441). Blood was obtained by venepuncture and collected in a tube containing a solution of disodium edetate (1 g/100 ml. 0.7% w/v sodium chloride solution) sufficient to give a final concentration of 1 mg edetate/ml. blood. The blood was cooled in ice and centrifuged at 4° C at 120 *g*, and the platelet-rich plasma pipetted off and pooled after successive periods of 10 to 15 min centrifugation. It was kept at 4° C until used; this was never more than 1 hr after the blood was drawn.

Platelet counts were made in a haemocytometer chamber using a solution of ammonium oxalate (1 g $(COO.NH_4)_2.2H_2O$/100 ml. water) as diluent. The packed platelet volume was determined by centrifuging platelet-rich plasma in the thrombocytocrit described by Hardisty & Stacey (1955) for 90 min at 2,500 *g*.

Absorption of 5-hydroxytryptamine by platelets. Two methods were used. In most experiments 0.5 to 2 ml. platelet-rich plasma was put into a 15 ml. conical centrifuge tube closed by a vaccine cap through which two needles were thrust and gassed with oxygen containing 5% carbon dioxide. The tube was held in a water bath at 37° C in a nearly horizontal position so as to expose a large surface to the gas, and rocked gently to and fro mechanically. Twelve tubes could be incubated simultaneously. After 10 min incubation a solution of 5-hydroxytryptamine in 0.9% sodium chloride solution (0.2 ml. for each 1 ml. platelet-rich plasma) was added by syringe and needle. The concentration of this solution depended on the purpose of the experiment and is given below. After the incubation period the tubes were cooled in ice and the gassing discontinued. They were centrifuged at 2,500 *g* for 30 min at 4° C, the vaccine caps removed, the supernatant poured off from the platelet pellet and the tubes left to drain for a few min, adhering drops of plasma being removed with filter paper.

In other experiments platelet-rich plasma was diluted with 9 vol. Krebs Ringer phosphate solution (Umbreit, Burris & Stauffer, 1945) from which the calcium and magnesium salts had been omitted but which contained 0.2 g glucose/100 ml. A large volume of this modified Krebs solution was needed to prevent change in *p*H during the experiment. Incubation was carried out in 25 or 50 ml. conical flasks, closed with rubber bungs to prevent evaporation, but not gassed. A solution of 5-hydroxytryptamine was added to give a final concentration of 1.5 μg/ml. After incubation the flasks were cooled in ice and an aliquot of the solution centrifuged as described above. Whether the plasma was gassed with 5% carbon dioxide or diluted with phosphate buffer, the *p*H at the end of incubation was always between 7.38 and 7.50.

Estimation of 5-hydroxytryptamine uptake

Fluorimetric measurement. The platelet pellet obtained above was mixed thoroughly with 0.5 ml. water and the concentration of 5-hydroxytryptamine in it estimated by the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956). The difference between the concentration in platelets which had and which had not been incubated was then calculated.

Radioactivity measurement. In these experiments 5-hydroxytryptamine creatinine sulphate labelled with ^{14}C at the β-carbon atom and having an activity of 6.87 mc/mM was used (Radiochemical Centre, Amersham). After the platelets had been spun down they were suspended in 0.7 ml. water, frozen and thawed twice to liberate 5-hydroxytryptamine, centrifuged, 0.2 ml. of the clear supernatant added to 2.5 ml. liquid scintillator (Nuclear Enterprises 220) and the radioactivity estimated in a scintillation counter (Nuclear Enterprises No. 5503). In some of the earlier experiments 0.2 ml. supernatant was spread on 1 in. aluminium planchettes and counted in a gas-flow proportional counter. Results of the fluorimetric and radioactive methods are compared below.

Since the platelets were not washed after centrifuging, 5-hydroxytryptamine trapped in the interstices of the platelet pellet was included in the estimation. Born & Bricknell (1959) estimated the volume of trapped solution to be 0.352 μl./10^8 platelets, which was in reasonable agreement with the rough estimate made by Hardisty & Stacey (1955) using Evans blue. In most experiments the concentration of 5-hydroxytryptamine in the platelet was so great compared with that in the solution that the correction for this additional 5-hydroxytryptamine was negligible; however, in those experiments in which high concentrations of 5-hydroxytryptamine were used and in the experiments with tryptamine the estimated concentration of absorbed amine was reduced by the calculated amount in the trapped solution estimated as 0.35 μl./10^8 platelets or 0.25 μl./μl. platelet substance.

Protein estimations were made by the method of Greenberg (1929). The *p*H determinations were all made with a glass electrode. Weights of amines refer to the free bases.

RESULTS

Effect of p*H on the uptake of 5-hydroxytryptamine.* The uptake of 5-hydroxytryptamine was determined by estimating fluorimetrically the amount in the platelets initially and after incubation at 37° C for 90 min with 1.4×10^{-5} M 5-hydroxytryptamine. In Fig. 1 the results of three experiments on platelets obtained from the same individual are shown. In two of these, platelet-rich plasma was gassed

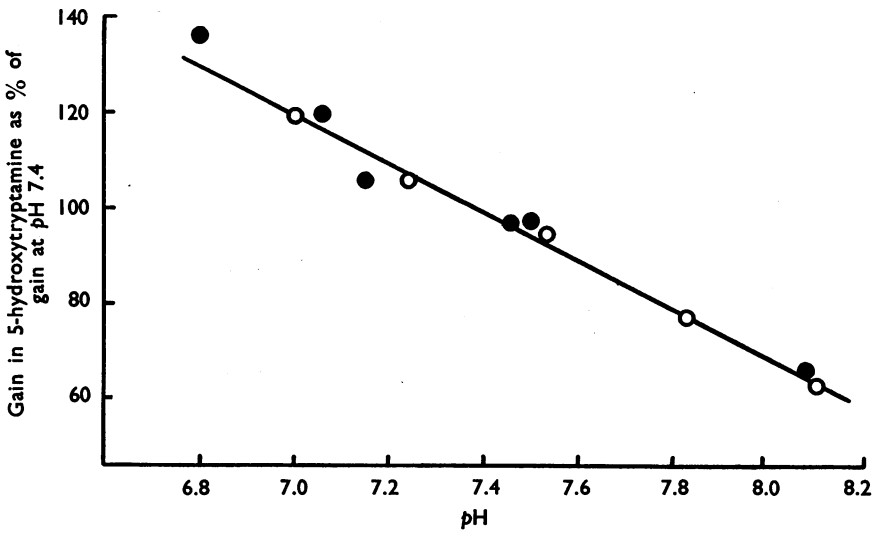

Fig. 1. Gain in 5-hydroxytryptamine by platelets after 90 min incubation at 37° C with $1{\cdot}4 \times 10^{-5}$ M 5-hydroxytryptamine at different *p*H's. In two experiments (●) *p*H was adjusted with carbon dioxide; in one experiment (○) *p*H was adjusted by adding hydrochloric acid or sodium hydroxide to the Krebs solution. Ordinate: Gain of 5-hydroxytryptamine as a % of gain at *p*H 7·4. Abscissa: *p*H of incubation mixture at the end of the experiment.

with oxygen or with oxygen containing various proportions of carbon dioxide up to 30% ; in one experiment, plasma was diluted with Krebs solution the *p*H of which had been adjusted to 5 different values by the addition of hydrochloric acid or sodium hydroxide before the addition of the platelet-rich plasma. In all cases the *p*H of the solution was determined again at the end of the experiment, and it is these values which are given in Fig. 1. The gain in 5-hydroxytryptamine is plotted as a % of the gain at *p*H 7.4 and is seen to vary inversely with the *p*H ; thus the uptake at *p*H 6.8 is nearly double that at *p*H 8.0. The results were similar whether phosphate buffer or carbon dioxide was used, but uptake was often rather less with phosphate. In an experiment with dog platelets the slope of the line was the same.

Variation in uptake with *p*H might occur if 5-hydroxytryptamine were bound to plasma protein to a degree varying with *p*H. Evidence of this was sought by dialysing plasma against buffered solutions of 5-hydroxytryptamine. Dialysis sacs made from " Visking " cellulose ester tubing and containing platelet-free plasma were suspended in solutions of 5-hydroxytryptamine in Krebs solution the *p*H of which had been previously adjusted to values ranging from 6.5 to 8.5. The con-

centration of 5-hydroxytryptamine and of protein inside and outside the sac was subsequently estimated and the *p*H of the solution inside the sac determined. The results of such an experiment in which dog plasma was dialysed against 2×10^{-5} M solutions of 5-hydroxytryptamine for 60 hr at 4° C are given in Table 1. The ratio of the concentration of 5-hydroxytryptamine inside to that outside the sac did not appear to be dependent on the *p*H of the plasma in any experiment.

TABLE 1

DISTRIBUTION OF 5-HYDROXYTRYPTAMINE BETWEEN PLASMA (INSIDE SAC) AND KREBS SOLUTION (OUTSIDE SAC) IN A DIALYSIS EXPERIMENT

*p*H of plasma	5-Hydroxytryptamine (μg/ml.)		Ratio	Protein (mg/ml.)	
	Inside sac (a)	Outside sac (b)	(a)/(b)	Inside sac	Outside sac
6·6	4·23	2·93	1·4	69	0·5
7·3	4·00	3·04	1·3	71	0·5
8·3	4·12	3·02	1·4	72	0·5

Comparison of fluorimetric and radioactive tracer measurements of uptake. Platelet-rich plasma gassed with 5% carbon dioxide and containing 1.3×10^{-5} M ^{14}C-labelled 5-hydroxytryptamine was incubated for 20, 40 and 60 min. The amount of 5-hydroxytryptamine in the platelets was estimated fluorimetrically before and after incubation and by radioactivity measurements. The uptake was calculated and found to be similar by the two methods. The results of an experiment are given in Table 2.

TABLE 2

UPTAKE OF 5-HYDROXYTRYPTAMINE BY PLATELETS AFTER INCUBATION WITH ^{14}C-LABELLED 5-HYDROXYTRYPTAMINE ($1{\cdot}3 \times 10^{-5}$ M) FOR VARIOUS PERIODS

Comparison of fluorimetric and radioactivity estimation

Method of estimation	Uptake of 5-hydroxytryptamine (ng/10^8 platelets)		
	20 min	40 min	60 min
Fluorimetry	169	227	259
Radioactivity	175	237	266

The effect of oxygen, nitrogen and glucose on uptake of 5-hydroxytryptamine. Platelet-rich plasma was incubated in closed flasks with ^{14}C-labelled 5-hydroxytryptamine in Krebs solution either with glucose (0.2 g/l.) or without glucose, through which either oxygen or nitrogen had been bubbled previously for 15 min. Aerobic or anaerobic conditions were maintained during incubation by the passage of either oxygen or nitrogen through the incubation flask. The results of two experiments are given in Table 3. Uptake was unaffected by oxygen lack or by glucose lack either separately or together.

The uptake of tryptamine by platelets. Varying amounts of ^{14}C-labelled tryptamine were added to platelet-rich plasma gassed with 5% carbon dioxide in oxygen and shaken gently either at 0° or at 37° C for 30 min. The radioactivity in the platelets was then estimated. The results of such an experiment are shown

TABLE 3
EFFECT OF GLUCOSE AND OXYGEN ON THE UPTAKE OF 5-HYDROXYTRYPTAMINE BY PLATELETS

Conditions	Glucose added (mg/ml.)	Uptake of ^{14}C-labelled 5-hydroxytryptamine (μg/ml. platelet substance) Experiment 1	Experiment 2
Aerobic	2	185	170
Aerobic	0	181	176
Anaerobic	2	192	181
Anaerobic	0	182	179

in Fig. 2. The uptake of tryptamine is seen to vary linearly with the concentration of the surrounding solution and to be independent of temperature. This contrasts with the behaviour of 5-hydroxytryptamine, the uptake of which is strongly influenced by temperature. The slope of the line is 2.6, and this gives the ratio of the concentration of tryptamine within and without the platelets.

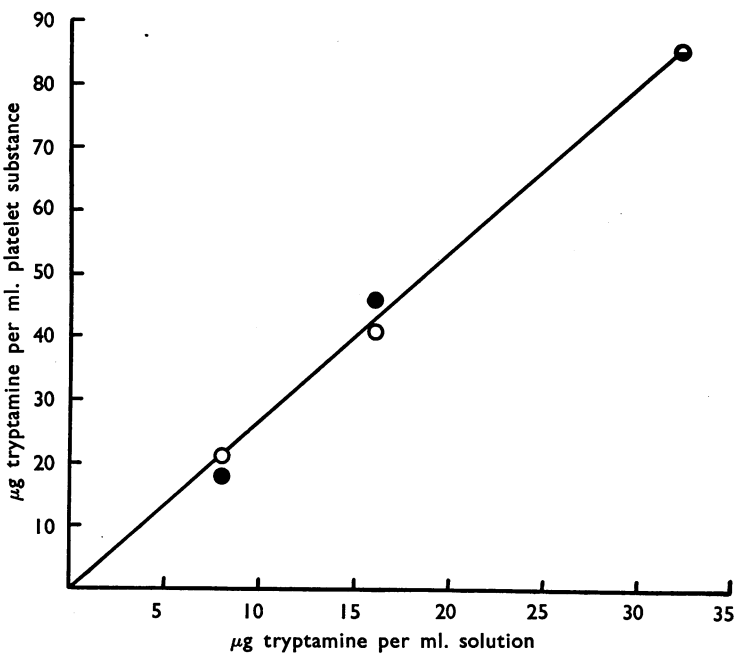

Fig. 2. Uptake of ^{14}C-labelled tryptamine by platelets in 30 min at 0°: ●; at 37° C: ○. Ordinate: μg tryptamine/ml. platelet substance. Abscissa: μg tryptamine/ml. incubation mixture.

The effect of *p*H on the uptake of tryptamine was determined by adding ^{14}C-labelled tryptamine to platelet-rich plasma which had been gassed with oxygen containing 0, 5 and 30% carbon dioxide. After 30 min the *p*H of the plasma and the radioactivity of the platelets were measured. The results are given in Table 4 and show that uptake varied directly with *p*H.

TABLE 4

UPTAKE OF 5-HYDROXYTRYPTAMINE AND TRYPTAMINE BY PLATELETS FROM SOLUTIONS OF THE AMINES IN KREBS SOLUTION

Results are expressed as a percentage of the uptake at *p*H 7·4. Ionization of the amino group was calculated from the data of Vane (1959); pK_a of 5-hydroxytryptamine 10·2, of tryptamine 10·0

	5-Hydroxytryptamine		Tryptamine	
*p*H	% uptake	% unionized	% uptake	% unionized
6·8	130	0·06	64	0·04
7·4	100	0·25	100	0·14
8·0	68	0·99	120	0·63

The effect of tryptamine on the uptake of 5-hydroxytryptamine by platelets. Platelet-rich plasma was gassed with 5% carbon dioxide in oxygen and incubated with each of six different concentrations of tryptamine. A control tube containing 0.9% sodium chloride solution was treated similarly. After 10 min a solution of ^{14}C-labelled 5-hydroxytryptamine in 0.9% sodium chloride solution was added to each tube to give a 10^{-5} M concentration and incubation continued for a further 20 min. In the tubes containing tryptamine the platelets were found to have taken up less 5-hydroxytryptamine than in the tubes containing no tryptamine. In Fig. 3 the uptake is plotted against the molar concentration of tryptamine in the tube. Over a large part of the range the uptake was proportional to the log. of the concentration of tryptamine. From this and from similar experiments it was found that

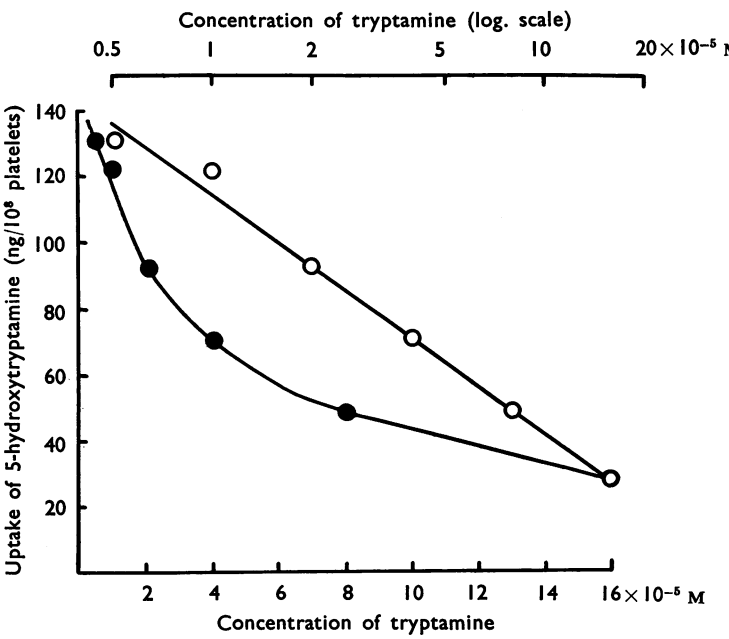

Fig. 3. Effect of various concentrations of tryptamine on the uptake of ^{14}C-labelled 5-hydroxytryptamine by platelets from a 10^{-5} M solution in 20 min. Arithmetical scale ●——●; log scale ○——○. Ordinate: Uptake of 5-hydroxytryptamine ng/10^8 platelets. Abscissa: Concentration of tryptamine.

under the conditions described 50% inhibition of uptake was caused by a concentration of 4×10^{-5} M tryptamine.

In other experiments the concentration of 5-hydroxytryptamine was varied in a series of tubes and the concentration of tryptamine kept the same. The resulting uptake was compared with that in a second series of tubes in which the concentration of 5-hydroxytryptamine was similarly varied but 0.9% sodium chloride solution

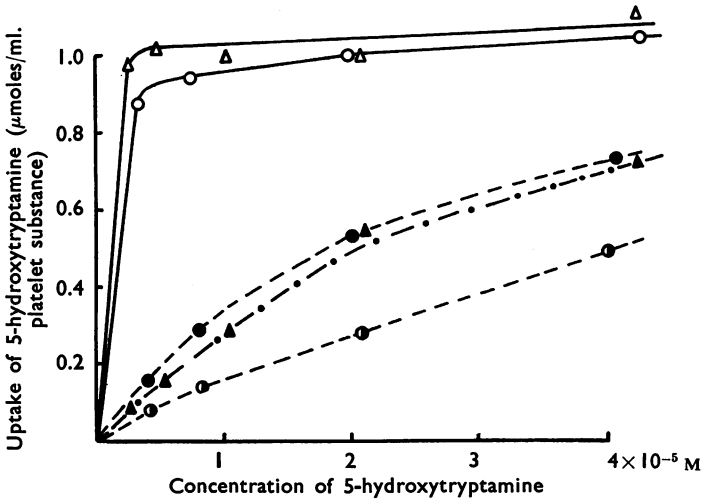

Fig. 4. Uptake of 5-hydroxytryptamine by platelets in 20 min at various concentrations of ^{14}C-labelled 5-hydroxytryptamine in the absence of an inhibitor (continuous lines) and in the presence of tryptamine (5×10^{-5} M) ▲- . -▲; cocaine ($2{\cdot}4 \times 10^{-5}$ M) ●- - -● and imipramine ($1{\cdot}9 \times 10^{-6}$ M) ◑- - -◑. Expt. 1: triangles; expt. 2: circles. Ordinate: Uptake of 5-hydroxytryptamine μmoles/ml. platelet substance. Abscissa: Concentration of 5-hydroxytryptamine in the incubation mixture.

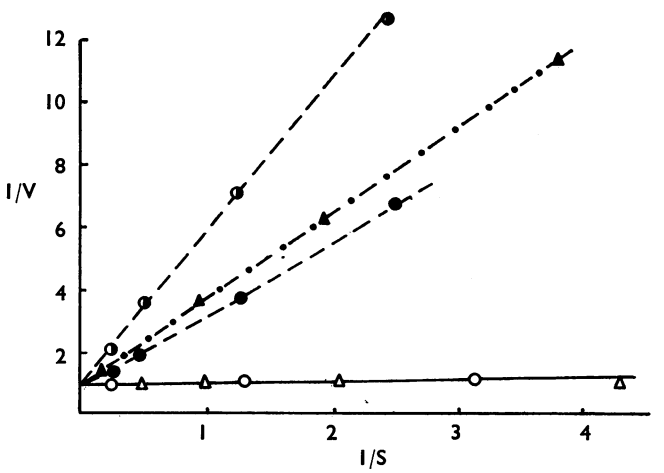

Fig. 5. Ordinate: the reciprocal of the uptake of 5-hydroxytryptamine during the first 20 min incubation (1/V). Abscissa: the reciprocal of the concentration of 5-hydroxytryptamine (1/S) in the absence of an inhibitor and in the presence of tryptamine, cocaine, and imipramine. Concentrations and symbols as in Fig. 4.

was added instead of tryptamine. The results of such an experiment are shown in Fig. 4. In the tubes which contained no tryptamine, uptake changed little over the range 0.25×10^{-5} M to 10^{-4} M 5-hydroxytryptamine, whereas in the presence of 5×10^{-5} M tryptamine it increased considerably with increasing concentration of 5-hydroxytryptamine. If the uptake during the first 20 min is taken as the initial velocity of uptake and the data treated by the method of Lineweaver & Burk (1934) by plotting the reciprocal of the velocity (1/V) against the reciprocal of the concentration of 5-hydroxytryptamine (1/S), Fig. 5 is obtained. From this it is found that both regression lines have the same ordinate intercept; that is, the uptake at infinite 5-hydroxytryptamine concentration is the same whether tryptamine is present or not. This is in agreement with inhibition being competitive.

The effect of various other substances on the uptake of 5-hydroxytryptamine by platelets. The substances listed in Table 5 were tested for inhibitory action by the method described for tryptamine, and the concentration giving 50% inhibition

TABLE 5

CONCENTRATION CAUSING 50% INHIBITION OF 5-HYDROXYTRYPTAMINE UPTAKE BY PLATELETS

Uptake was measured after 20 min incubation at 37° C with 0·7 to $0{\cdot}9 \times 10^{-5}$ M 5-hydroxytryptamine. The following compounds produced inappreciable inhibition at 5×10^{-4} M: histamine, hyoscine, morphine, L-tryptophan, D,L-5-hydroxytryptophan, D,L-dihydroxyphenylalanine, iproniazid, chlordiazepoxide, ouabain

	$\times 10^{-5}$ M		$\times 10^{-5}$ M
Imipramine	0·05	Lysergic acid diethylamide	13
Cocaine	2·5	2-Bromolysergic acid diethylamide	13
Chlorpromazine	3·5	Dopamine	15
Tryptamine	4·0	Methysergide	15
Chlorprothixene	5·0	Atropine	40
Dihydroergotamine	7·5	Trifluoperazine	50
Benzyloxygramine	9·0	Mescaline	50
Promethazine	11·0		
D,L-Amphetamine	11·0		
Tyramine	11·0		

determined graphically from curves such as those in Fig. 3. The final concentration of 5-hydroxytryptamine in the platelet-rich plasma at the end of incubation was not exactly the same in all experiments, because the amount removed by the platelets varied and could not be foretold. It varied from 0.7 to 0.9×10^{-5} M. In the absence of an inhibitor, uptake over this range is unaffected by concentration, but in the presence of an inhibitor the concentration of 5-hydroxytryptamine influences uptake (Fig. 5). The error introduced is, however, small, and the values given in Table 1 indicate the approximate relative potency of the inhibitors.

The uptake at various concentrations of 5-hydroxytryptamine in the presence of 2.4×10^{-5} M cocaine and 1.9×10^{-6} M imipramine is shown in Fig. 4, and the relationship between the reciprocal of the uptake during the first 20 min to the reciprocal of the concentration in Fig. 5. These results are similar to those with tryptamine.

The inhibitory effect of tryptamine and cocaine was also determined in experiments in which the gain in 5-hydroxytryptamine by platelets was measured fluorimetrically. The results of an experiment in which both methods were used

TABLE 6

UPTAKE OF 5-HYDROXYTRYPTAMINE BY PLATELETS AFTER INCUBATION FOR 20 MIN WITH ^{14}C-LABELLED 5-HYDROXYTRYPTAMINE (10^{-5} M) ALONE AND IN THE PRESENCE OF TRYPTAMINE AND COCAINE

Method of estimation	Uptake in ng by 10^8 platelets incubated with 5-hydroxytryptamine		
		In the presence of	
	Alone	Tryptamine 4×10^{-5} M	Cocaine $2{\cdot}5\times10^{-5}$ M
Fluorimetry	194	99	70
Radioactivity	187	97	75

are given in Table 6. The results with the two methods are the same; hence tryptamine and cocaine in the conditions of these experiments do not cause an appreciable efflux of 5-hydroxytryptamine from platelets.

DISCUSSION

The uptake of 5-hydroxytryptamine by platelets varies inversely with the *p*H of the solution, whether the *p*H is changed by variation in the amount of carbon dioxide in solution or by changing the reaction of phosphate buffer. The decrease in uptake at alkaline *p*H values might be due to a change in the amount of free 5-hydroxytryptamine in solution or to a change in its ionization. The proportion of diffusible 5-hydroxytryptamine has been found to be unaffected by change in *p*H over the range 6.6 to 8.3. Changes in ionization can be calculated from the data provided by Vane (1959). The ionization of the amino group is nearly complete at *p*H 8 and increases by $<1\%$ when the *p*H is reduced to 6.8. The hydroxyl group is 99.9% unionized at *p*H 8, and this is increased by $<0.1\%$ when the *p*H is changed to 6.8. In neither case does change in ionization seem adequate to explain the change in uptake. It seems therefore likely that the platelets themselves are sensitive to change of *p*H.

The radioactivity found in platelets after they had been incubated with the ^{14}C-labelled compound was found to be equivalent to their increased 5-hydroxytryptamine content. Since they are unable to synthesize 5-hydroxytryptamine, this indicates that there was no appreciable loss, during the period of incubation, of the 5-hydroxytryptamine originally contained in the platelets, nor was there any loss when tryptamine and cocaine were also present. The possibility of an escape of some of the 5-hydroxytryptamine absorbed during incubation is not excluded.

The uptake of 5-hydroxytryptamine has two striking features: it takes place rapidly even at very low plasma concentrations and continues against a concentration gradient of several hundred to one; secondly, it is very sensitive to temperature change (Hardisty & Stacey, 1955). The optimum temperature for this process was found to be 40° C and the temperature coefficient to be about 3. This suggests that uptake is dependent on the metabolism of platelets, probably for the supply of energy for transport against the concentration gradient. This is supported by the finding that uptake is inhibited by various metabolic inhibitors (Born & Gillson, 1959; Weissbach & Redfield, 1960). In anaerobic conditions uptake continues

unaffected. This is to be expected from the investigations of Waller, Löhr, Grignani & Gross (1959) on platelet metabolism. They found that glycolysis preponderates over the oxidative metabolism of glucose and that only 1/5 of the metabolized glucose could be accounted for by the production of respiratory carbon dioxide. The observation that uptake was unaffected by a 10-fold dilution of plasma with glucose-free Krebs solution does not conflict with the view that the requisite energy comes from glycolysis because the remaining glucose might well have been adequate to maintain metabolism for 20 min incubation. According to Waller *et al.* (1959), the glucose consumption of human platelets is only 75×10^{-6} moles/10^{11} platelets/hr. However, platelets also contain a large amount of adenosine triphosphate which may act as the immediate source of energy.

The evidence that 5-hydroxytryptamine is associated in platelets with adenosine triphosphate which provides the negative charges of a cation exchange system has been presented by Born & Gillson (1959). This has been further strengthened by the presence of 5-hydroxytryptamine and adenosine triphosphate in the same fraction when platelet homogenates are centrifuged through a sucrose density gradient (Baker, Blaschko & Born, 1959). That the combination of these two substances is the sole reason for the concentration of 5-hydroxytryptamine in platelets is made unlikely by the high temperature coefficient, and an active transport system at the platelet surface, as suggested by Hughes & Brodie (1959), is probable. There are thus three stages at which the uptake of 5-hydroxytryptamine might be blocked: (*a*) by inhibition of platelet metabolism, (*b*) at the transport mechanism at the platelet surface, (*c*) at the storage sites.

The uptake of tryptamine by platelets is very different from that of 5-hydroxytryptamine: the concentration gradient is low and does not vary with the concentration of tryptamine, and there is no difference in the rate of uptake at 0° and 37° C. These features suggest a physical process not dependent on platelet metabolism. Moreover, uptake *increases* with rising *p*H, and it is of interest that there is 15 times as much of the unionized form at *p*H 8 as at *p*H 6.8.

Low concentrations of tryptamine depress the uptake of 5-hydroxytryptamine by platelets, and it is now necessary to consider at which of the three sites mentioned above it acts. A consideration of the quantities of the amines involved throws some light on this. Platelets are very nearly saturated with 5-hydroxytryptamine after 90 min incubation, and Pare, Sandler & Stacey (1960) found that human platelets after this period contained 1.3 μmoles/ml. platelet substance. This gives an estimate of the total storage capacity of platelets. In experiments described above it was found that tryptamine reaches a concentration in platelet substance 2.6 times that in the surrounding fluid. When the latter is 4×10^{-5} M, the concentration of tryptamine in platelet substance would be 0.10 μmoles/ml. If all this tryptamine were used in blocking storage sites, only 1/12 of the sites would be blocked. Nevertheless, this concentration of tryptamine causes a 50% inhibition of 5-hydroxytryptamine uptake. From this calculation it appears unlikely that the inhibitory action of tryptamine is exerted on the storage mechanism. Nor is it likely that tryptamine will depress glycolysis or any other energy-producing mechanism. It therefore appears most likely that the inhibitory action of tryptamine

is exerted by blocking the transport sites. This is supported by the kinetics of the reaction, which give a classical picture of competitive inhibition when the results are plotted according to Lineweaver & Burk (1934); however, this may be a deceptive simplification of the overall process.

Of the other substances found to have an inhibitory action, some have structural similarities to tryptamine (tyramine and dopamine) and others antagonize the actions of 5-hydroxytryptamine by competitive inhibition elsewhere in the body (dihydroergotamine, lysergic acid diethylamide, 2-bromolysergic acid diethylamide and benzyloxygramine). Recently evidence has been produced by Trendelenburg (1959), Muscholl (1960) and Whitby, Hertting & Axelrod (1960) that cocaine depresses the uptake of noradrenaline, injected intravenously, in cats and rats. The inhibition of 5-hydroxytryptamine uptake by platelets might thus be another manifestation of this action. Imipramine was by far the most potent of all the substances investigated, and its action has already been reported by Marshall, Stirling, Tait & Todrick (1960). Structurally it is close to the phenothiazines, of which chlorpromazine was the most effective, and to chlorprothixene. All three substances have a dimethylpropylamine chain attached to a condensed three-ring system and all have effects on the central nervous system. However, a number of other compounds need testing before the relationship of action to structure can be profitably discussed. It is of interest that the amino-acids, tryptophan, 5-hydroxytryptophan and dopa, were without action.

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