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METABOLISM, TRANSFER AND STORAGE OF 5-HYDROXYTRYPTAMINE IN BLOOD PLATELETS*

BY

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In recent years, evidence has been obtained that aromatic monoamines—for example, catecholamines and 5-hydroxytryptamine (5HT)—might function as neurohumoral transmitters in the central nervous system, and knowledge of their transfer, storage and metabolism is therefore important. Direct investigations of these processes, for example, in isolated synaptosomes of the brain, are, however, problematic since these elements have to be separated from their natural environment and connexions by rather crude methods (for example, density gradient centrifugation of brain homogenates). Blood platelets which contain substantial amounts of 5HT seem to be more appropriate models in spite of the fact that the function of the amine in this situation is not well understood. Thus, the platelets can be studied in relatively physiological conditions *in vitro*—for example, in plasma or artificial buffer systems—and they also have the capacity to metabolize, transfer and store 5HT.

Some recent findings in this laboratory concerning the metabolism, transfer and storage of 5HT in blood platelets are reported here. Platelets of various species including man were isolated according to the usual procedures (Bartholini, Pletscher & Gey, 1961) and in most of the experiments incubated in buffers such as Tyrode using disodium-ethylenediaminetetraacetate to prevent platelet aggregation. Experiments with plasma as an incubation medium did not differ markedly from those with Tyrode.

METABOLISM

Physiological conditions

In isolated blood platelets incubated for several hours in physiological media such as plasma or Tyrode buffer, the 5HT content is lowered only slightly (by 1–5%) (Fig. 1) (Bartholini & Pletscher, 1964; Paasonen & Pletscher, 1959). The small amounts of 5HT which are lost from the platelets hardly appear as such in the incubation medium, but in the form of two major metabolites. They show the typical activation and fluorescent spectra of 5-hydroxyindole derivatives and have R_F values characteristic of 5-hydroxyindoleacetic acid (5HIAA) and 5-hydroxytryptophol (5HT'ol) on paper and thin-layer chromatography (Fig. 2). Platelets also metabolize exogenous 5HT (Bartholini, Pletscher

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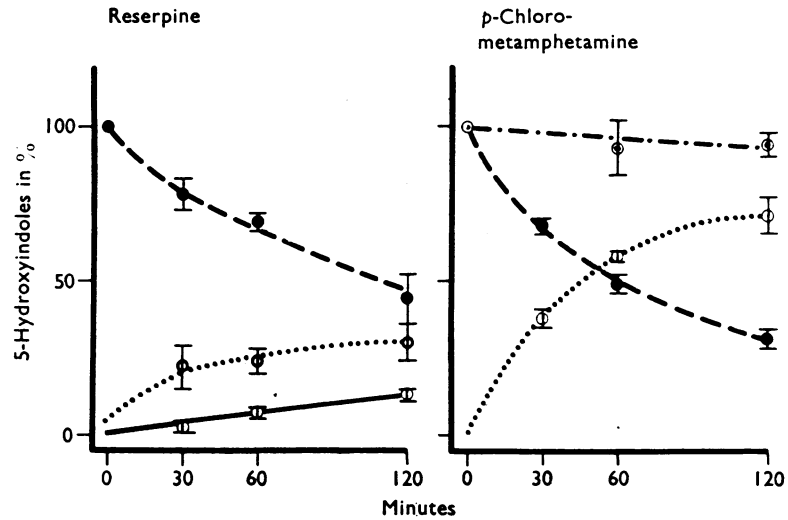

Fig. 1. Effect of reserpine (2.5 μg/ml.) and *p*-chlorometamphetamine-HBr (100 μg/ml.) on the metabolism of 5-hydroxytryptamine (5HT) in rabbit platelets incubated in Tyrode at 37° C. The 5HT and 5-hydroxyindoleacetic acid (5HIAA) are expressed as a percentage of the 5HT content in platelets before incubation with the drugs. – – – –, 5HT of platelets; . . . . , 5HT of incubation medium (Tyrode); ——, 5HIAA of incubation medium; –.–.–., 5HT of platelets incubated without drugs. The points indicate averages ± S.E. of two to seven experiments each. (Bartholini & Pletscher, 1964).

& Bruderer, 1964; Pletscher, Bartholini & Da Prada, 1966a) into 5HIAA and 5HT'ol. The amount of metabolites formed is, however, rather small—for example, about 3% in platelets of guinea-pigs incubated with 10^{-6}M ^{14}C-5HT for 2 hr (Table 1) (Pletscher, Burkhard, Tranzer & Gey, 1967). Inhibitors of monoamine oxidase, such as isocarboxazid, inhibit the metabolism of both endogenous and exogenous 5HT (Bartholini *et al.*, 1964).

The relation between 5HT'ol and 5HIAA formed from 5HT depends on the quantity of erythrocytes present in the incubation medium. Platelet suspensions containing low amounts of erythrocytes produce more 5HT'ol than 5HIAA. Formation of the latter cannot, however, be completely suppressed even after purification of the platelets by repeated washing and recentrifugation. If high amounts of erythrocytes (for example, 1/10 of the original content in blood) are added to the platelet suspensions, the principal metabolite is 5HIAA, and at best little formation of 5HT'ol occurs (Fig. 2). Pure erythrocytes do not seem to transform added 5HT into 5HIAA or 5HT'ol, nor do they metabolize 5HT'ol into 5HIAA. Furthermore, reserpinized platelets (devoid of endogenous 5HT) cause no major reduction of added 5HIAA into 5HT'ol although they still metabolize 5HT into 5HIAA and 5HT'ol (Bartholini *et al.*, 1964; Pletscher *et al.*, 1966a).

These findings suggest the following metabolic pathways for 5HT in platelets. The liberated amine is probably transformed into 5-hydroxyindoleacetaldehyde by monoamine oxidase. In the absence of relevant amounts of erythrocytes, the majority of the aldehyde is reduced to 5HT'ol, whereas in the presence of large amounts of erythrocytes almost

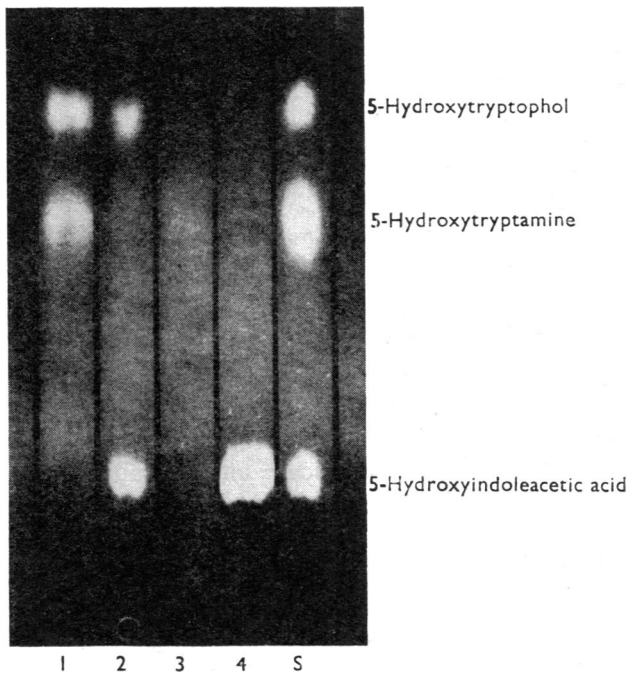

Fig. 2. 5-Hydroxyindolyl compounds in the buffer (calcium-free Tyrode *p*H 7.4 + ethylene diamine tetraacetate) 2 hr after incubation of isolated platelets of rabbits at 37° C. In order to increase the formation of metabolites, reserpine (2.5 μg/ml.) was added to the incubation medium. The buffer was extracted at pH 10 with ethyl acetate (basic extract) and at *p*H 1 with ethyl ether (acid extract). S, standards; 1, basic extract without erythrocytes; 2, acid extract without erythrocytes; 3, basic extract with added erythrocytes; 4, acid extract with added erythrocytes. Schleicher & Schuell No. 2043; solvent system: *n*-propanol/ammonia 1 N (5:1 v/v). The fluorescence of the spots was developed by acidifying the paper with 4 N-HCl and subsequent exposure to ultra-violet light (254 mμ). (Bartholini, Pletscher & Bruderer, 1964.)

TABLE 1

FORMATION OF ^{14}C-5-HYDROXYTRYPTAMINE (5HT) METABOLITES BY ISOLATED PLATELETS OF NORMAL AND RESERPINIZED GUINEA-PIGS INCUBATED IN TYRODE AT 37° C FOR 2 HR WITH OR WITHOUT IMIPRAMINE

The initial concentration of ^{14}C-5HT in the incubation medium was 10^{-6} M. Reserpine (5 mg/kg) was administered i.p. 16 hr before the isolation of platelets. Each result represents an average ±S.E. of three to four experiments. (Pletscher, Burkard, Tranzer & Gey, 1967)

	^{14}C-5HT-metabolites	
	% of added 5HT	% of controls
Normal		
Controls	3·1±0·3	
Imipramine	1·3±0·1	45±7
Reserpinized		
Controls	27·0±3·5	
Imipramine	3·7±0·3	15±2

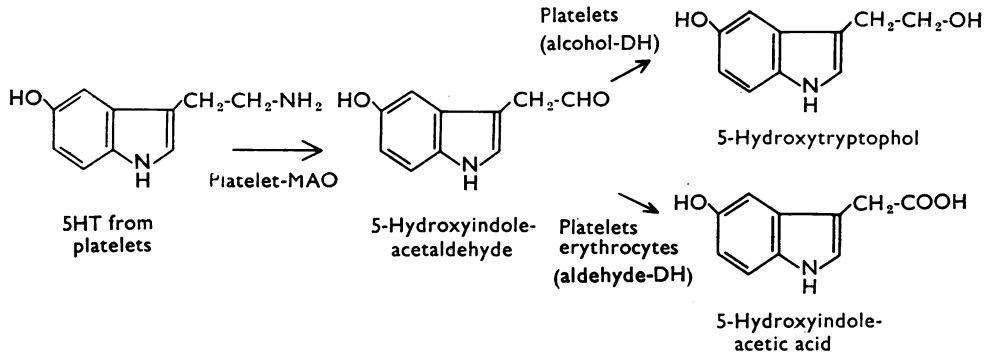

Fig. 3. Metabolism of platelet 5-hydroxytryptamine (5HT).

exclusive oxidation into 5HIAA takes place (Fig. 4). The platelets in contrast to erythrocytes thus contain monoamine oxidase and probably alcohol dehydrogenase as well, whereas aldehyde dehydrogenase is present in both platelets and erythrocytes.

This hypothesis is confirmed by experiments with indole-3-acetaldehyde which is more stable than 5-hydroxyindoleacetaldehyde. Thus pure platelets metabolize indoleacetaldehyde into tryptophol (indole-3-ethanol) as well as into indoleacetic acid, whereas in the presence of erythrocytes indoleacetic acid is formed predominantly (Pletscher *et al.*, 1966a). Isolated platelets metabolize exogenous dopamine in a way analogous to 5HT—that is, into 3,4-dihydroxyphenylethanol and 3,4-dihydroxyphenylacetic acid (Pletscher *et al.*, 1966a).

Thrombin

In isolated platelets incubated with thrombin (especially in the presence of calcium) more than 90% of the 5HT is liberated within a few minutes. Practically no metabolites can be detected initially in the incubation medium; only after 15–30 min do they appear, and their amount increases on further incubation. After 2 hr, 10–15% of the liberated 5HT is transformed into 5HT'ol and 1–2% into 5HIAA (Table 2) (Da Prada, Bartholini & Pletscher, 1965a; Pletscher *et al.*, 1966a). These findings indicate that during the thrombin-induced rapid liberation of 5HT from platelets, virtually no 5HT metabolites are formed, whereas on further contact with the agglutinated platelets part of the extracellular 5HT is metabolized.

TABLE 2

FORMATION OF 5-HYDROXYTRYPTOPHOL BY ISOLATED PLATELETS OF RABBITS INCUBATED IN TYRODE AT 37° C WITH RESERPINE 10 μg/ML. OR THROMBIN 1 NIH UNIT/ML.

The figures represent averages ± S.E. of four to seven experiments each. (Pletscher, Bartholini & Da Prada, 1966a). Absolute amounts of 5-hydroxytryptamine (5HT) liberated after 0·5 and 2 hr respectively (μg/10^{10} platelets): Reserpine: 41±5, 104±8; thrombin: 175±1, 174±1

	5-Hydroxytryptophol in per cent of liberated 5HT	
	0·5 h	2 h
Reserpine	19·9±4·8	21·7±3·2
Thrombin	0·8±0·0	12·1±2·5

Monoamine-liberating drugs

Reserpine-type

Reserpine and similar drugs—for example Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine) and Ro 4-9040 (*bis*-(3,4-dichloro-phenethyl)amine)—cause a liberation of 5HT from platelets *in vitro* which is relatively slow. Reserpine (2.5 μg/c.c.), for instance, lowers the 5HT content of the platelets by 22±5% and 56±8% after 30 and 120 min, respectively (Fig. 1). The metabolism of the 5HT liberated from platelets by these drugs is similar to that of endogenous and exogenous 5HT described earlier. Thus, after incubation of rabbit platelets with small amounts of reserpine, Ro 4-1284 or Ro 4-9040, part of the liberated 5HT is transformed into 5HT'ol and 5HIAA (Figs. 1 and 2) and Table 2. Formation of these metabolites also occurs if platelets are incubated with exogenous 5HT in the presence of one of the drugs (Bartholini & Pletscher, 1964 ; Pletscher & Bartholini, 1964 ; Bartholini *et al.*, 1964 ; Pletscher, Da Prada & Bartholini, 1965a ; Pletscher, Da Prada, Bartholini & Burkard, 1965c ; Pletscher, Da Prada, Bartholini, Burkard & Bruderer, 1965d ; Pletscher *et al.*, 1966a ; Pletscher, Da Prada, Burkard, Bartholini, Steiner, Bruderer & Bigler, 1966c ; Da Prada, Bartholini & Pletscher, 1965a, 1965b).

The 5HT liberated by reserpine is only partially transformed into metabolites, a considerable part of the liberated amine (about 60–80%) appears unchanged in the incubation medium (Fig. 1) (Bartholini & Pletscher, 1964 ; Da Prada *et al.*, 1965a ; Pletscher *et al.*, 1966a). In contrast to what is seen with thrombin, the metabolism probably takes place during or immediately after the liberation of 5HT because the relative amount of metabolites formed (as compared with the total of liberated 5HT) does not change with the time of incubation (Table 2) (Da Prada *et al.*, 1965a ; Pletscher *et al.*, 1966a). This difference of the metabolic pattern in the experiments with reserpine and thrombin might be connected with the different speed of 5HT liberation.

Sympathomimetic amines

Some sympathomimetic amines—for example, amphetamine, *p*-chloro-metamphetamine and tyramine, also liberate 5HT from platelets *in vitro*. Practically all the liberated 5HT is found in the incubation medium ; no metabolites are formed (Fig. 1). Furthermore, the metabolism of exogenous 5HT by isolated platelets is abolished by amphetamine and *p*-chloro-metamphetamine, although the penetration of 5HT into the cells does not seem to be inhibited by the drugs (Bartholini & Pletscher, 1964 ; Pletscher & Bartholini, 1964 ; Pletscher *et al.*, 1965a, 1965c, 1965d ; Da Prada *et al.*, 1965b).

Amphetamine and its derivatives might therefore cause an inhibition (probably competitive) of the oxidative deamination of the liberated 5HT. This assumption is confirmed by comparative studies with the two chemically related chlorinated phenylalkylamines Ro 4-9040 (see above) and *p*-chloro-metamphetamine. Ro 4-9040 liberates 5HT from isolated platelets in concentrations which do not interfere with mitochondrial monoamine oxidase of rat liver *in vitro*. As mentioned, part of the liberated 5HT is transformed into 5HT metabolites. On the other hand, *p*-chloro-metamphetamine liberates platelet 5HT only in concentrations in which it markedly inhibits mitochondrial monoamine oxidase, and therefore no formation of metabolites occurs (Fig. 1) (Pletscher *et al.*, 1966c).

TRANSFER

The transfer of 5HT into the platelets seems to involve active, that is, energy-requiring, mechanisms, but in addition passive processes not dependent on specific metabolic requirements participate. In the following, the characteristics of the active 5HT transfer in platelets will be summarized but briefly, because they have already been extensively discussed in the literature. More emphasis will be put on some recent findings concerning the properties of the passive transfer as well as regarding to subcellular sites at which the 5HT transfer takes place.

Active transfer

The uptake of small amounts of 5HT by platelets shows the following characteristics:

(*a*) It occurs against a considerable concentration gradient between platelet and medium 5HT (for example, 1000:1) and reaches a saturation level. The relation between the initial rate of 5HT uptake and the 5HT concentration in the incubation fluid can be described by the Michaelis-Menten equation (Humphrey & Toh, 1954; Hardisty & Stacey, 1955; Zucker & Borelli, 1956; Brodie, Tomich, Kuntzman & Shore, 1957; Born & Gillson, 1959; Hughes & Brodie, 1959).

(*b*) It shows structural specificity. Thus the uptake of tryptamine and bufotenine by platelets is less pronounced than that of 5HT and does not reach a saturation level, but increases linearly to the concentration in the incubation fluid (Hughes & Brodie, 1959; Stacey, 1961).

(*c*) It is decreased by metabolic inhibitors (for example, cyanide, fluoride, dinitrophenol, monoiodoacetate) as well as by ouabaine in concentrations which do not seem to cause structural damage to the platelets (Sano, Kakimoto & Taniguchi, 1958; Born & Gillson, 1959; Weissbach & Redfield, 1960; Pletscher *et al.*, 1967).

From these and other findings, it has ben concluded that carrier mechanisms which seem to depend on metabolic energy are probably involved in the transfer of small amounts of 5HT into the platelets. It has been calculated that the hypothetical carrier might bind about 10^4 molecules 5HT per human platelet (Born & Bricknell, 1959).

Passive transfer

The 5HT of the platelets is also transferred by passive diffusion—that is, by a process not linked to an energy-requiring carrier mechanism. Thus, in platelets incubated with low concentrations of 5HT in the cold small amounts of the amine still enter the platelets, but the uptake is proportional to the 5HT concentration in the medium (Born & Bricknell, 1959). A passive transfer of 5HT also becomes evident if platelets are incubated in media containing much higher 5HT concentrations (for example, 20–300 γ/c.c.) than those at which the active 5HT transfer operates (0.1–10.0 γ/c.c.). Under these conditions the accumulation of 5HT in the platelets parallels the concentration of the amine in the medium, and no saturation levei s reached (Fig. 4). Similarly, the endogenous 5HT liberated from storage depots, for example, by drugs such as reserpine and phenylalkylamines, seems to leave the platelets by diffusion (Hughes & Brodie, 1959). According to experiments carried out in our laboratories, this 5HT outflow is not inhibited by metabolic inhibitors (for example, KCN) and ouabaine.

Effect of temperature

The passive transfer of 5HT, like the active transfer (Hardisty & Stacey, 1955; Born & Bricknell, 1959), depends on the incubation temperature. The intracellular accumulation of 5HT by normal platelets (for example, of guinea-pigs) incubated with high amounts (300 γ/c.c.) of 5HT is decreased by more than 90% at 4° C (Fig. 4). Lowering of the incubation temperature similarly diminishes the outflow of endogenous 5HT

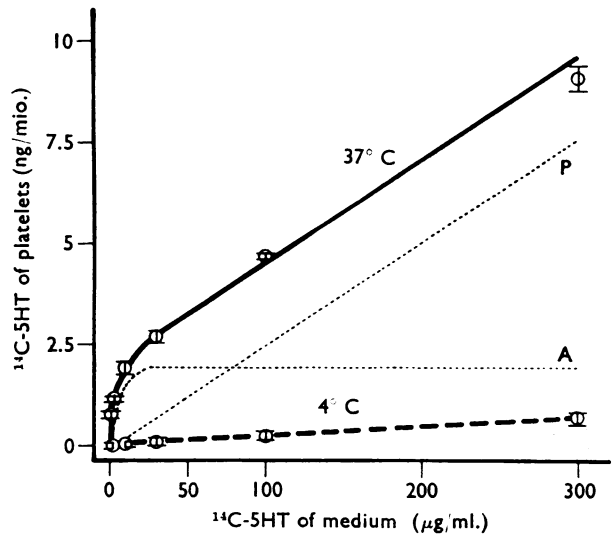

Fig. 4. Effect of temperature on the transfer of ^{14}C-5-hydroxytryptamine (5HT) into isolated platelets of rabbits incubated in Tyrode at 37° and 4° C respectively for 1 hr. Each point indicates an average ±S.E. of four to twelve experiments. The dotted lines represent the active (A) and passive (P) component of the transfer at 37° C.

liberated by drugs like reserpine and phenylalkylamines (diminution to about 50% at 30° C). Thereby, as demonstrated for *p*-chloro-metamphetamine, the penetration of this drug into the platelets does not show marked dependence on temperature. In addition, the in- and outflow of platelet 5HT occurring in unphysiological media (glucose-free potassium-phosphate) is markedly modified by small alterations of the incubation temperature (inhibition by about 50% at 32°–33° C, enhancement to 120–130% at 40° C) (Fig. 5). In glucose-free potassium phosphate, active transfer mechanisms are unlikely to exist because the platelets, although showing an intact membrane, have lost most of their ultrastructural elements, for example, α-granules, mitochondria, glycogen granules, 5HT organelles (" platelet ghosts ") (Fig. 6) (Da Prada, Pletscher & Bartholini, 1965c; Pletscher, Da Prada & Bartholini, 1966b; Da Prada, Tranzer & Pletscher, 1967).

These experiments indicate that the passive transfer of 5HT in platelets is sensitive to temperature changes which may also occur *in vivo* (for example in hyper- or hypothermia). Alterations of the physico-chemical properties of certain cell membrane constituents modifying, for instance, the size of pores, might thereby be involved. It has indeed been shown that minor temperature changes may considerably influence the surface area of lipid monolayers *in vitro* (Adam, 1941).

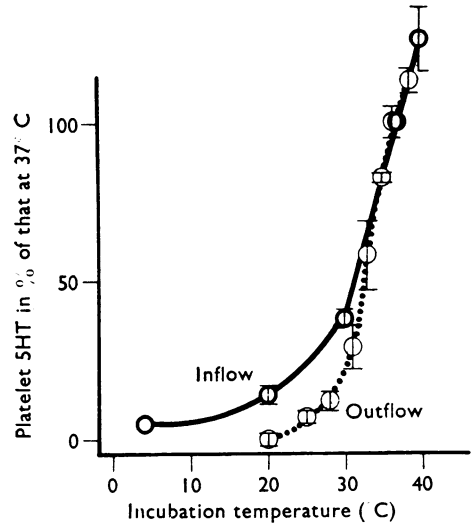

Fig. 5. Effect of temperature on the passive in- and outflow of 5-hydroxytryptamine (5HT) in platelets of rabbits. The platelets were preincubated for 60 min in glucose-free isotonic potassium phosphate at 37° C and then re-incubated in the same buffer at various temperatures. In the outflow experiments, platelets of normal animals were used and the reincubation period was 60 min. In the inflow experiments, platelets of reserpinized animals (16 hr after reserpine 5 mg/kg i.p.) were used, the platelets were reincubated for 15 min in buffer containing 5HT 600 μg/ml. Before the 5HT determination in platelets, these were washed twice with ice-cold potassium phosphate. Each point represents an average ±S.E. of three to six experiments. (Pletscher, Da Prada & Bartholini, 1966b.)

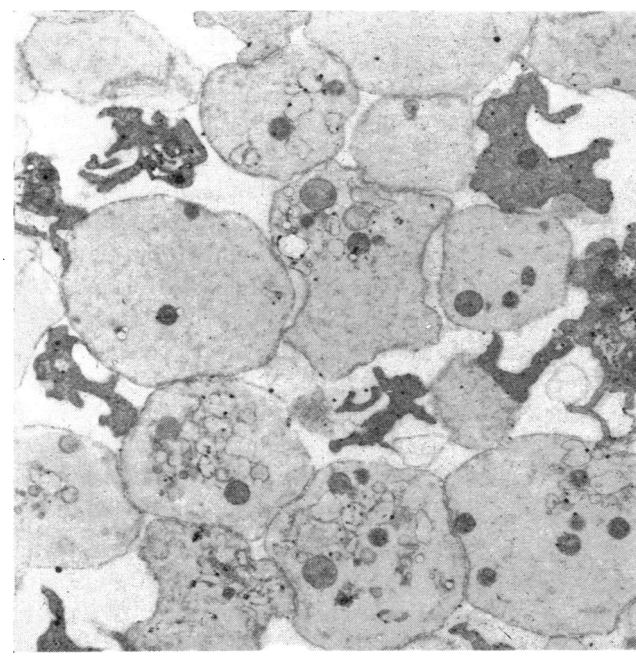

Fig. 6. Electron micrograph of isolated rabbit platelets incubated in isotonic potassium phosphate at 37° C for 2 hr (×10,000) (made by Dr. J. P. Tranzer, F. Hoffmann-La Roche & Co. Ltd., Basle).

Effect of drugs

The outflow of 5HT from rabbit platelets incubated in glucose-free potassium phosphate is moderately decreased by reserpine and the benzoquinolizine derivative Ro 4-1284 (for example, from about 80% in controls to about 60% in platelets from reserpinized animals; ($P<0.01$). These drugs therefore not only seem to impair the intracellular storage of 5HT, but in addition they possibly exert some effect on the platelet membrane, for example, by diminishing its permeability for 5HT. This action on the membrane might not be detected in normal circumstances, but might be disclosed under conditions in which the intracellular storage mechanism is abolished (for instance, in glucose-free potassium phosphate) (Bartholini, Da Prada & Pletscher, 1965; Pletscher, Da Prada & Bartholini, 1965b).

Subcellular sites

Experiments with drugs, as well as electron microscopy indicate that the transfer of platelet 5HT takes place at at least two subcellular sites—the platelet membrane and the intracellular storage organelles. These two sites and the possible mechanisms involved in the transfer of 5HT will be briefly discussed.

Cell membrane

It is generally assumed that imipramine and similar compounds inhibit the transfer of norepinephrine through the membrane of sympathetic nerve endings (Carlsson, 1966). Furthermore, imipramine has been shown to be a very potent (probably competitive) inhibitor of the 5HT uptake by human platelets (Marshall, Stirling, Tait & Todrick, 1960; Stacey, 1961; Fuks, Lanman & Schanker, 1964). Attempts were therefore made to localize the site of action of this drug in the platelets.

In isolated platelets of guinea-pigs incubated with small amounts of ^{14}C-5HT (for example, 10^{-6}M), imipramine also markedly inhibits the intracellular accumulation of the labelled amine. Simultaneously, the formation of ^{14}C-5HT metabolites is decreased (Table 1). Concentrations as low as 10^{-7}M of imipramine are effective. The action of imipramine is probably not caused by primary interference with the intracellular storage of 5HT. Thus the drug, even in relatively high concentrations (3.5×10^{-5}M), does not significantly decrease the endogenous platelet 5HT or the number of 5HT organelles (see under INTRACELLULAR STORAGE). Furthermore, in reserpinized platelets in which the intracellular storage mechanism is abolished, imipramine has an effect similar to that in normal platelets: it diminishes the formation of ^{14}C-5HT metabolites (Table 1) (Pletscher *et al.*, 1967).

Imipramine probably does not interfere with platelet monoamine oxidase, because the drug, even in concentrations of 10^{-5}M, causes no inhibition of mitochondrial monoamine oxidase of guinea-pig liver using 10^{-6}M-5HT as substrate. Unspecific platelet damage could be excluded by electron microscopy (Pletscher *et al.*, 1967). It may therefore be concluded that the decreased intracellular accumulation and metabolism of 5HT induced by imipramine is the result of inhibition of the 5HT penetration into the cell. This indicates the existence of an imipramine-sensitive transfer mechanism for 5HT, probably at the level of the cell membrane. Thus the membranes of the platelets and of the sympathetic nerve endings behave in a similar way with regard to the transfer of 5HT and norepinephrine respectively.

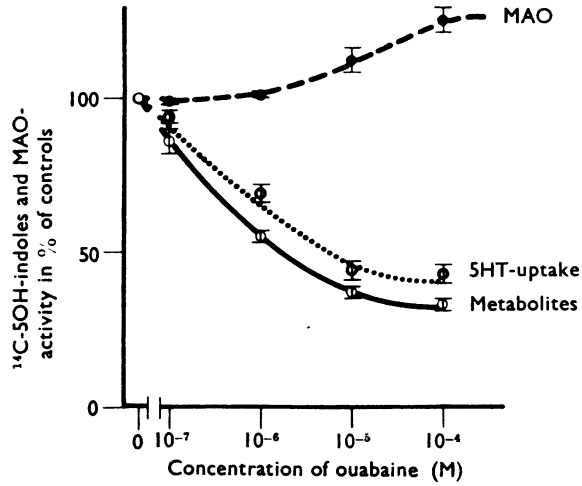

Fig. 7. Effect of various concentrations of ouabaine on the formation of ^{14}C-5-hydroxytryptamine (5HT) metabolites by isolated platelets of reserpinized guinea-pigs and on the ^{14}C-5HT uptake by isolated normal platelets and on the monoamine oxidase (MAO) activity of liver mitochondria of guinea-pigs. Reserpine 5 mg/kg was administered i.p. 16 hr before isolation of the platelets. Initial concentration of ^{14}C-5HT in the incubation media is 10^{-6}M. Each value represents an average ±S.E. of three to four experiments. (Pletscher, Burkard, Tranzer & Gey, 1967.)

The nature of the transfer mechanism at the membrane level is not yet fully understood. Experiments with ouabaine and KCN indicate that metabolic processes might be involved. Thus these inhibitors, in concentrations which do not interfere with the ultramorphological structure of the platelets and which do not inhibit mitochondrial monoamine oxidase (10^{-7}–10^{-5}M and 10^{-3}M, respectively), decrease the formation of ^{14}C-5HT metabolites in isolated reserpinized platelets (Fig. 7) (Pletscher *et al.*, 1967). In other systems—for example, of electrolytes, sugars and amino-acids ouabain and metabolic inhibitors like KCN are thought to interfere with an active transfer. In analogy to this interpretation, the results reported might indicate that the 5HT transfer through the platelet membrane is an active process, dependent in a yet unknown manner on metabolic energy.

Intracellular sites

The existence of intracellular storage sites, where 5HT might be taken up and liberated, has been clarified by electron microscopic findings. These are discussed in the following section.

INTRACELLULAR STORAGE

The hypothesis of an intracellular storage mechanism for 5HT in platelets has been put forward on the basis of experiments with reserpine. This monoamine releaser markedly inhibits the accumulation of exogenous 5HT in platelets incubated with small concentrations of the amine (Brodie *et al.*, 1957 ; Hughes & Brodie, 1959) and, thereby, the formation of 5HT metabolites (for example, 5HIAA and 5HT'ol) is decidedly enhanced (Table 1) (Pletscher *et al.*, 1967). This finding indicates that the reserpine-

induced diminution of the intracellular accumulation of 5HT is not or is not caused only by an impaired transfer of the amine through the platelet membrane. A special intracellular storage mechanism has been postulated which under normal conditions seems to protect the major part of the 5HT which has penetrated into the cell from being metabolized by mitochondrial monoamine oxidase. After abolition of this mechanism, most of the intracellular 5HT is probably exposed to monoamine oxidase which would explain the enhanced formation of 5HT metabolites. In recent experiments, which will be described, special intracellular organelles containing 5HT have indeed been demonstrated.

Storage organelles

Experiments with reserpine and similar drugs have led to the conclusion that within the cell the amine is probably localized in a way in which it is protected from metabolizing enzymes (see earlier). Recently, a new type of subcellular platelet organelles has been discovered which seems to contain high amounts of 5HT. These organelles become visible in the electron microscope if platelets have been fixed in glutaraldehyde as well as in osmium tetroxide and if the platelet sections are then contrasted with uranylacetate or lead citrate. The organelles are of spherical shape and smaller (500–1500 Å) than the α-granules. A single membrane surrounds a very dense osmiophilic content which is denser than the α-granules and frequently somewhat detached from the membrane (artifact ?) (Fig. 8). Most of the rabbit platelets contain several of these organelles in each section ; in other species—for example, the cat—the dense bodies are somewhat less numerous, and in guinea-pigs and man they are rather rare (Tranzer, Pletscher & Da Prada, 1966a ; Tranzer, Da Prada & Pletscher, 1966b). The following findings indicate that the dense osmiophilic organelles contain 5HT (Tranzer *et al.*, 1966b).

(a) Reserpine added *in vivo* or *in vitro* significantly diminishes the number of dense bodies as well as the 5HT content of the platelets, while the number of the α-granules remains unchanged (Fig. 8). (*b*) Incubation of platelets from reserpinized rabbits with high amounts of 5HT (for example, 500 γ/c.c.) increases the number of dense bodies as well as the content of platelet 5HT. The organelles which reappear are very similar in shape to those of normal platelets except that the osmiophilic content may be somewhat smaller (Fig. 8). (*c*) Platelets of species rich in 5HT (for example, rabbits) contain more organelles than do platelets poor in 5HT (for example, from humans and guinea-pigs). (*d*) After incubation of normal platelets of guinea-pigs with 5HT, the number of osmiophilic organelles increases together with the 5HT content. (*e*) Histamine and adenosine triphosphate (ATP), which are also present in platelets and may be decreased after reserpine, probably do not give histochemical reactions similar to 5HT. Thus, unlike 5HT they are not precipitated by glutaraldehyde and do not react with osmium tetroxide in aqueous solutions. (*f*) Catecholamines like 5HT react with glutaraldehyde and osmium tetroxide *in vitro*. Their amount in platelets is, however, less than one-tenth that of 5HT.

These findings indicate that the dense osmiophilic organelles may be the intracellular storage sites of 5HT in which the amine is protected from the action of monoamine oxidase. Preliminary experiments indicate that the dense bodies can be separated from the α-granules by density gradient centrifugation. It has, however, not yet

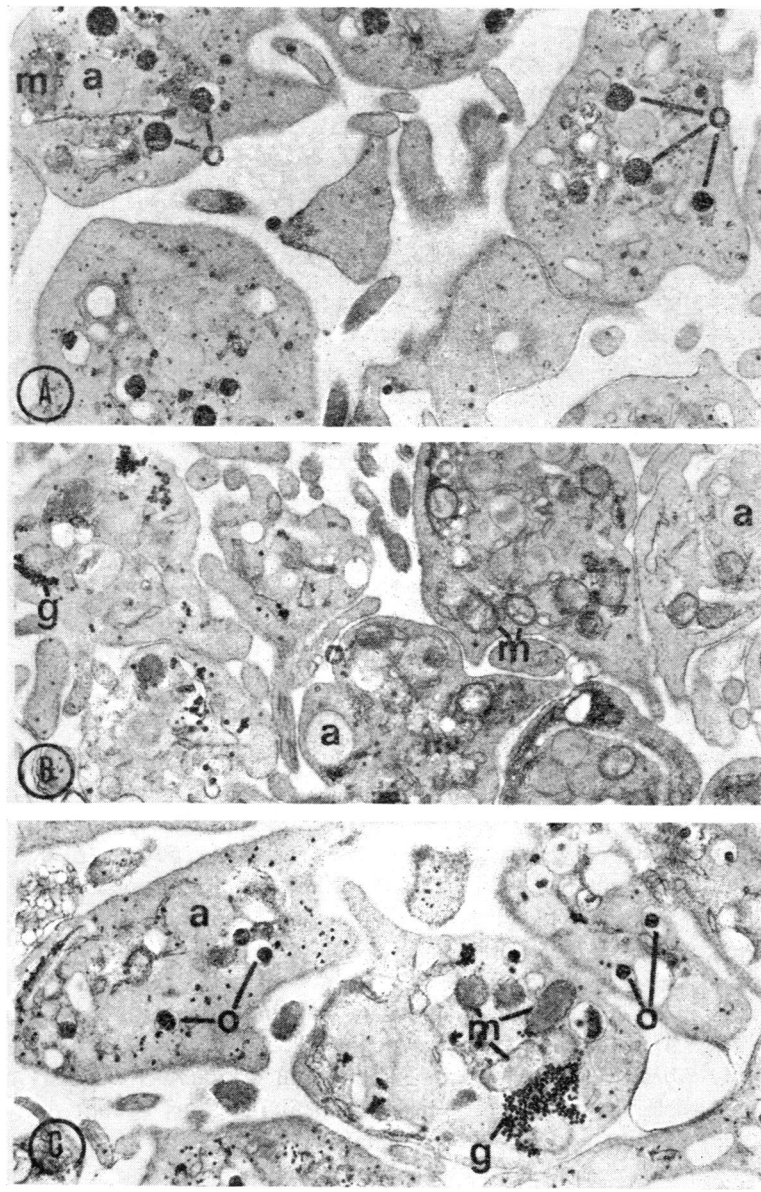

Fig. 8. Electron micrographs of rabbit platelets. A, Normal platelets; B, reserpine 5 mg/kg i.p. 16 hr before isolation of the platelets; C, platelets of reserpinized animals incubated with 5-hydroxytryptamine (5HT) 1,000 μg/ml. for 2 hr at 37° C. a, α-granules; o, osmiophilic 5HT organelles; g, glycogen; m, mitochondria. (Tranzer, Da Prada & Pletscher, 1967.)

been decided whether the storage of 5HT in these organelles involves active intracellular transfer mechanisms (for example, at the level of the membrane of the organelles) or whether physico-chemical processes independent of specific metabolic requirements are responsible. Various findings suggest that 5HT in the platelets might be associated with ATP (Born, 1956; Born & Gillson, 1957; Born, Ingram & Stacey, 1958; Baker, Blaschko & Born, 1959; Weber & Mondt, 1967). In addition, 5HT has been found to form complexes with ATP *in vitro* (Roberts, 1966). It was therefore speculated that 5HT is retained in the platelets by "ionic exchange" (Born & Gillson, 1959) or by complex formation, and that reserpine might consequently interfere with these physico-chemical processes in an as yet unknown way.

The dense osmiophilic granules described have not been found in megakaryocytes of rabbits. This supports the view that platelets do not contain 5HT when they leave their mother cell, but rather pick up the amine in the circulation (Tranzer, Da Prada & Pletscher, 1967).

Alterations of the storage organelles

Drugs

Monoamine liberators. Rauwolfia alkaloids, benzoquinolizine derivatives and phenylethylamines, which are known to liberate 5HT from platelets, decrease the number of 5HT storage organelles *in vitro* in a rather specific way—that is without inducing alterations of other ultramorphological structures of the platelets (Fig. 8). Only in very high concentrations do reserpine, Ro 4-1284 and amphetamine impair the general ultrastructure including α-granules, glycogen granules and mitochondria (Tranzer *et al.*, 1966a, 1966b; Da Prada *et al.*, 1967). It is not yet known whether the 5HT organelles disappear because of a specific mechanism. Reserpine might, for instance, interfere with an active transfer system (for example, at the granular membrane) or change some physico-chemical properties by which 5HT may be bound in the granules (see above). On the other hand, phenylalkylamines like amphetamine may displace the 5HT from the organelles by unspecific mechanisms such as ionic exchange.

Imipramine. Imipramine in concentrations which markedly inhibit the uptake of 5HT (for example, 10^{-5}M) does not diminish the number of dense osmiophilic bodies and at best slightly decreases the endogenous 5HT, such as that in isolated cat platelets after prolonged incubation (12 hr) (Pletscher & Tranzer, 1967). Therefore, the 5HT stored in the osmiophilic bodies of the platelets can hardly be decreased by inhibition of the transfer of the amine at the level of the cell membrane. This possibly indicates that under physiological conditions spontaneous liberation and re-uptake of 5HT occurs only to a minor extent.

Platelet aggregation in vivo

During thrombus formation *in vivo* (for example, by experimentally induced endothelial lesions of ear veins of rabbits) the 5HT storing organelles almost completely disappear. A minor part of osmiophilic organelles persists outside the platelets (Baumgartner, Tranzer & Studer, 1967). It cannot be decided whether the diminution of the 5HT organelles is a consequence of the general ultramorphological changes occurring during platelet aggregation or whether a more specific mechanism is involved.

Incubation medium

Platelets incubated in media devoid of sodium (for example in potassium phosphate) lose a great part of their 5HT and of their osmiophilic organelles within 1–2 hr. Concomitantly, other structural alterations occur, such as the disappearance of α-granules, mitochondria, glycogen granules. However, the platelet membrane remains intact (platelet " ghosts ") (Fig. 6). On addition of sodium to the incubation medium, the ultrastructural changes and the 5HT losses gradually decrease, and in buffers containing 10% potassium and 90% sodium they are minimal. In media containing exclusively sodium as cation, similar changes occur as in potassium phosphate ; they are, however, much less pronounced (Da Prada *et al.*, 1967). These experiments therefore demonstrate that the disappearance of 5HT and 5HT organelles from platelets in media containing insufficient amounts of sodium and potassium is probably the result of non-specific ultrastructural damage to the platelets. Sodium and, to a lesser extent, potassium, seem to be essential for preserving a normal ultrastructure of platelets including the dense osmiophilic bodies.

SUMMARY

1. Isolated platelets of various species metabolize 5-hydroxytryptamine (5HT) mainly into 5-hydroxytryptophol (5HT'ol) and also into 5-hydroxyindoleacetic acid (5HIAA). In the presence of erythrocytes which contain aldehyde-dehydrogenase, 5HIAA is the major metabolite. The endogenous platelet 5HT liberated by drugs like reserpine and by thrombin is also partly converted into 5HT'ol and 5HIAA. In contrast, phenylalkylamines, such as amphetamine, liberate 5HT without formation of metabolites probably due to an inhibition of monoamine oxidase.

2. Experiments with drugs and electron microscopic studies indicate the existence of at least two subcellular transfer sites for 5HT—the platelet membrane and specific intracellular storage organelles which are highly osmiophilic. The 5HT transfer at the level of the cell membrane seems to involve active processes because it is inhibited by KCN and ouabaine. The dense osmiophilic 5HT organelles disappear selectively after reserpine- and amphetamine-like drugs, but not after imipramine. In media deficient in sodium or potassium as well as in experimental thrombosis, the organelles diminish simultaneously with the occurrence of generalized ultramorphological alterations of the platelets.

3. The passive diffusion of platelet 5HT shows a marked dependence on temperature and seems to be inhibited by drugs like reserpine.

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