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Review Article

Release of 5-hydroxytryptamine from blood platelets*

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THE results of Rand & Reid (1951) originally pointed to the high content of 5-hydroxytryptamine (5-HT) in blood platelets. Later Shore, Pletscher, Tomich, Kuntzman & Brodie (1956) demonstrated that reserpine caused a depletion of 5-HT from the platelets. The interest in the relation between blood platelets and 5-HT arises mainly from these two observations. The pharmacological, physiological and biochemical questions concerning 5-HT have been reviewed, for example, by Lewis (1958), Page (1958) and Erspamer (1961). A review by Maupin (1960) was particularly concerned with the role of 5-HT in platelets. The purpose of the present text is to report some of the results obtained while studying this amine in platelets and in particular to discuss its release from platelets.

Although platelets have no nucleus they have typical subcellular structures (cf. Telkkä, Nyholm & Paasonen, 1964) and an active metabolism. Glycolysis is the main source of energy (Waller, Löhr, Grignani & Gross, 1959) and at least 30 enzymes are known to be present in platelets (Zucker & Borrelli, 1958). From the pharmacological point of view it is important that platelets contain, or are able to absorb, adrenaline and noradrenaline *in vitro* (Born, Hornykiewicz & Stafford, 1958). The platelets of some species, especially the rabbit, contain large amounts of histamine (Code, 1952). The high content of adenosine triphosphate (ATP) in the platelets (Born, 1956) has also attracted attention. Although there are great differences in the content of biogenic amines in the platelets, the content of 5-HT in platelets is generally relatively high in all mammalian species. Some of the approximate figures, compiled from the literature and our own results, are presented in Table 1.

The platelets of the rabbit, human carcinoid tumour, the posterior salivary glands of *Eledone moschata* and of *Octopus vulgaris*, and the sting fluid of *Urtica dioica* all contain about the same amount of 5-HT/g. Baker, Blaschko & Born (1959) isolated granules containing 5-HT and ATP from human platelets while Wurzel, Marcus & Zweifach (1965) isolated them from rabbit platelets. It is conceivable that most of the 5-HT in the platelets is bound to the particles since it would otherwise be metabolised by the monoamine oxidase which they also contain (see p. 692). Only traces of 5-HT are present in the platelet-free plasma, and it seems that most of the amine measured outside the platelets may arise from platelet damage.

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TABLE 1. AMOUNTS OF 5-HT IN PLATELETS OF SOME MAMMALIAN SPECIES

Species	µg/10^9 platelets	Whole blood µg/ml	µg/ml platelet substance
Human	0·57	0·16	49
Rabbit	10·0	3·8	1100
Pig	2·2	0·4	
Dog	1·7	0·2	
Cat	0·9	3·8	
Guinea-pig	0·3	0·2	
Rat	0·3	0·4	

Uptake of 5-HT by platelets

Platelets and bone marrow are unable to decarboxylate 5-hydroxytryptophan (5-HTP) to 5-HT *in vitro* (Clark, Weissbach & Udenfriend, 1954; Gaddum & Giarman, 1956) and so 5-HT must originate elsewhere. Portal blood contains more 5-HT than arterial blood (Erspamer & Testini, 1959). It seems probable that the origin of platelet 5-HT is in the enterochromaffin cells of the intestinal mucosa, a view supported by a number of findings. Kärki, Paasonen & Peltola (1960) found that increasing the intraluminal intestinal pressure in rabbits increases the plasma 5-HT 2- to 4-fold in the venous blood coming from this portion of the intestine. The role of other tissues, however, cannot be entirely excluded. Thus the kidney decarboxylates 5-HTP and excretes 5-HT in the urine (Sandler & Spector, 1961) and the amount of 5-HT in blood from the kidneys is higher than that in the arterial blood (Uuspää & Airaksinen, 1963).

It is well established that platelets take up 5-HT from the surrounding fluid *in vitro* and *in vivo* (Humphrey & Toh, 1954; Hardisty & Stacey, 1955; Weissbach, Bogdanski & Udenfriend, 1958; Born & Gillson, 1959) and the concentration of amine in the platelets may reach a value several hundred times that of the surrounding fluid. Absorption continues for 1 to 2 hr. The capacity of uptake is not the same in all instances and it does not occur in the cold (Stacey, 1958). The rate of uptake increases with increasing concentrations of 5-HT up to a concentration of about 0·5 µg/ml (Born & Gillson, 1959), this uptake being facilitated by 2,4-dinitrophenol and potassium and decreased by high concentration of glycolytic inhibitors like cyanide and iodoacetate. These workers also found that the platelet 5-HT can be exchanged completely if the amine is also present outside. This process of uptake is most probably an *active transport* as has been much emphasised by Brodie and his colleagues (Hughes & Brodie, 1959).

At 5-HT concentrations of 50 µg/ml or higher, and especially at higher pH values, another mechanism of uptake exists. This involves a nearly linear dependence of 5-HT accumulation on the amine concentration (Weissbach, Redfield & Titus, 1960). It is unlikely that endogenous 5-HT is absorbed *in vivo* by this mechanism, which is mainly due to *diffusion*.

Reserpine was found by Brodie, Shore & Pletscher (1956), Brodie, Tomich, Kuntzman & Shore (1956) and Born, Ingram & Stacey (1956) to prevent the uptake of 5-HT by platelets *in vitro* and *in vivo*. As was

shown by Stacey (1961), there are a number of other agents which also inhibit this process, although less effectively than reserpine. The release and uptake are closely related phenomena and some questions of the uptake will be dealt with later (for further discussion about uptake, see Born & Gillson, 1959; Stacey, 1961).

The work of Born & others (1956) indicates that the property of platelets to take up 5-HT against a concentration gradient is related to their ATP content. Platelets are rich in this nucleotide and human platelets contain about 22 $\mu g/10^9$ platelets. These authors also found that the amount of ATP in platelets is related to the amount of 5-HT and in *in vitro* experiments the ratio of ATP molecules to 5-HT molecules becomes one. ATP would provide the energy for the uptake process—a situation somewhat comparable to that in the adrenal medulla. According to Carlsson, Hillarp & Waldeck (1963) even the cells of the adrenal medulla take up more 5-HT than catecholamines if equal amounts of these amines are present in the perfusion fluid.

In vivo the platelets hold their 5-HT for a long time. After an intravenous infusion of 20 mg/kg of 5-HT into dogs, elevated values were found for at least 4 days by Weissbach & others (1958). This means that many, if not all, of the platelets retain the amine content, or some portion of it, for as long as they exist. Of course, some of the platelet 5-HT could originate in stores in other tissues after the infusion.

Release of 5-HT from platelets

5-HT is known to be released from platelets in a number of different ways *in vitro*, *in vivo* or both. These include: 1, mechanical trauma; 2, factors present normally or in pathological conditions in tissue fluids; 3, drugs or other foreign agents.

MECHANICAL TRAUMA

As discussed by Stacey (1958), mechanical trauma of any kind, such as contact with unsiliconed glass, frothing of plasma or freezing and thawing, will break up the platelets and release 5-HT as well as other constituents. Ultrasonic waves also cause platelets to lose their vasoactive material (Cole, Livingston, Loughry & Holden, 1953), including 5-HT (Buckingham & Maynert, 1964). For these reasons platelet damage must be considered as a factor in some observed complications in extracorporeal circulation of blood, such as the heart-lung apparatus (Sarajas, Kristoffersson & Frick, 1959).

The best way to minimise platelet damage during the handling of samples *in vitro* is to use plastic vessels and pipettes. Inadequate siliconising may cause much release of 5-HT especially during the incubation of samples. In this connection, it is surprising that platelets retain their 5-HT in plasma made hypotonic by adding water (Ahtee & Paasonen, unpublished). The liberation of the amine begins at a concentration of about 0·5% and is parallel to the haemolysis of red cells. Addition of sodium chloride to give a final salinity equivalent to 1·3% causes no release of 5-HT within a one hr period of incubation.

FACTORS PRESENT IN TISSUE FLUIDS

Clotting of blood. Several authors (Zucker & Borrelli, 1955a,b; Humphrey & Jaques, 1955; Grette, 1959) have shown that during clotting about one half of the 5-HT from platelets is released into the serum. Clotting of plasma by recalcification releases all 5-HT from previously washed platelets (Zucker & Borrelli, 1955b). The reason for the incomplete release or recovery or both, of 5-HT from serum is not clear. Shaking of blood during clotting decreases the amount of the amine in serum (Sharman & Sullivan, 1956).

The release itself is known to be due to the action of *thrombin* (Zucker & Borrelli, 1955a,b; Grette, 1962). It does not arise from the mechanical effects of clot formation, as was demonstrated by the use of a fibrinogenic blood containing no fibrinogen to form fibrin and thus to clot (Hardisty & Pinniger, 1956). The release of 5-HT from platelets by a sufficient amount of thrombin occurs in a few seconds (Zucker & Borrelli, 1956b). Binding of the intracellular calcium by ethylenediaminetetra-acetate (EDTA) decreases the release of 5-HT, and the subsequent addition of calcium liberates the rest of the amine (Markwardt & Barthel, 1964). During the release by thrombin the platelets remain microscopically intact in calcium-free medium (Zucker & Borrelli, 1955b) and viscous metamorphosis occurs only if calcium is present (Markwardt & Barthel, 1964). Grette (1962) has shown that as well as releasing 5-HT, thrombin also releases adenine nucleotides, inorganic phosphate, free amino-acids, but only a small amount of cellular protein.

The release by thrombin is dose dependent from about 0·01 to 1·0 or 10·0 u/ml (Gaintner, Jackson & Maynert, 1962). This means that thrombin may release 5-HT at a concentration of about 10^{-10} M. It is possible that thrombin is the only coagulation factor necessary for the 5-HT release during coagulation.

Trypsin. Trypsin and thrombin react in a similar manner with fibrinogen, and fibrinogen is present on the surface of platelets. Gaintner & others (1962) demonstrated that trypsin at a concentration of 0·1 μg/ml or more releases 5-HT from platelets *in vitro* and that the time-response curves are identical in both cases. Heparin, however, blocks the action of thrombin but not the action of trypsin. Trypsin also releases potassium from platelets but this release is less complete than that of 5-HT (Buckingham & Maynert, 1964). In spite of the presence of trypsin and thrombin in the platelet suspension, the concentration of 5-HT in the platelets begins to rise after the fast initial depletion (Gaintner & others, 1962; Buckingham & Maynert, 1964).

Bacterial endotoxins. Administration of bacterial endotoxin to the rabbit increases 5-HT in the plasma (Davis, Meeker & Bailey, 1961). Incubation of platelet-rich plasma (Des Prez, Horowitz & Hook, 1961) or whole blood of the rabbit (Davis, Meeker & McQuarrie, 1960) with endotoxin also causes an increase of 5-HT in the plasma. This release also concerns histamine. Davis, Bailey & Hanson (1963) demonstrated that warfarin or heparin prevented the release *in vivo*, but these agents did not prevent the transient thrombocytopenia caused by

endotoxin. The release of amines by bacterial endotoxin is almost instantaneous both *in vivo* and *in vitro* and there is a possibility that thrombin is involved in the mechanism of action. Des Prez (1964), however, has pointed to certain differences between the action of thrombin and endotoxin.

Antigen-antibody reaction. Humphrey & Jaques (1955) showed that the addition of purified antigen and antibody to platelet-rich plasma of normal rabbits released 5-HT and histamine. The release also occurs *in vivo* and a maximal depletion is reached within 2 min (Waalkes & Coburn, 1959). Calcium is necessary for the release of 5-HT induced by antigen. Heparin does not inhibit the release but it is prevented by tosylarginine and *p*-tosylarginine methyl ester (TAME), which are substrates for thrombin and thromboplastin. The release of amines from blood cells by antigen has been shown to be associated with the activation of proteolytic enzymes of plasma (Ungar, Yamura, Isola & Kobrin, 1961). The results of Shore & Alpers (1963a) suggested that a direct platelet damaging factor was associated with the early stages of blood coagulation and that antigen may activate this mechanism. It is also possible that the same heat-stable platelet-damaging factor in plasma and serum of the rabbit is responsible both for the *spontaneous* release of 5-HT and histamine as well as for the antigen-induced amine release (Shore & Alpers, 1963a).

Glycogen. Rocha e Silva (1950) showed that intravenous administration of glycogen causes a rapid clumping and disappearance of platelets and leucocytes from the circulation in rabbits and dogs. This action is similar to that of antigen-antibody reaction and, accordingly, Waalkes & Coburn (1959) found that glycogen released 5-HT and histamine from platelets of rabbit both *in vivo* and *in vitro*. In both instances the lung content of the amines rose many times above the control values, obviously as a result of the occlusion of platelet-leucocyte clumps or emboli in the lung (Waalkes & Coburn, 1959).

Tissue extracts. According to Toh (1956, 1957) alkaline tissue extracts from some mammalian tissues, particularly from the kidney, stomach mucosa and submucosa, accelerate the release of 5-HT and histamine from platelets *in vitro*. When given intraperitoneally for three days, the extracts depleted the 5-HT content of the spleen of rats (Toh, 1957). The release of amines from platelets is inhibited by EDTA, oxalate or citrate, but this inhibition is not due to the binding of calcium since it also happens in calcium-free salt solution (Toh, 1956). This releasing action of tissue extracts has been confirmed by using another model system, in the shape of the neoplastic mast cells of mouse, as a source of 5-HT and histamine (Giarman, Potter & Day, 1960). The release is time and dose dependent and the active principle is stable to heat, acid or alkali and cannot be separated from protein. Although the extracts caused some damage to the cells, these authors believe that lysis of the cells is not in itself sufficient to explain the activity observed. The release of 5-HT by tissue extracts points to the possibility of a selective local release in tissues *in vivo*.

Fatty acids. Several long chain saturated fatty acids will, at 37° and in the presence of plasma and calcium, release both 5-HT and histamine

from platelets of rabbit *in vitro* (Shore & Alpers, 1963b). The most active are stearic, arachidic and behenic acids, which, in concentrations of 1 to 25 μg/ml, release most of the amines within 30 min. The platelet damage occurs in the presence of heparin used to block the action of thrombin. It is of interest that these saturated long chain fatty acids greatly accelerate thrombus formation in an *in vitro* thrombus-producing system (Connor, 1962). It is suggested that in both instances the mechanism involves the activation of the Hageman factor.

DRUGS

Reserpine and related agents. As for other tissues, reserpine releases 5-HT from platelets *in vivo* (Shore & others, 1956) and *in vitro* (Carlsson, Shore & Brodie, 1957). This action is common to those rauwolfia alkaloids that release 5-HT from the brain (Brodie, 1958). There is a dose-response relationship when the reserpine concentration ranges from about 10^{-7} to 10^{-6} M but it is difficult to effect the release *in vitro* of more than about half of the platelet 5-HT by reserpine-like drugs. After reserpine administration, rabbit platelets *in vivo* lose most, if not all, of their 5-HT within a day. It is possible that this would happen also *in vitro* if such extended incubations were possible. In any case the rate of release *in vitro* becomes very much slower after about 50% of 5-HT is liberated.

Reserpine prevents the uptake of catecholamines (Born & others, 1958; Hughes & Brodie, 1959), and it is therefore likely that these amines, although less concentrated in platelets, are also released by reserpine. Since one molecule of reserpine releases several hundred 5-HT molecules and since the rate or degree of depletion is not related to the 5-HT content of platelets, the mechanism of reserpine action cannot be one of simple displacement. Experiments on lysed platelets have failed to show any binding of 5-HT with platelet components (Hughes, Shore & Brodie, 1958; Sano, Kakimoto & Taniguchi, 1958). Electron microscopy reveals no obvious change in the structure of platelets (Telkkä & others, 1964). Impairment of the active transfer mechanism has been emphasised by Brodie and his colleagues and this would mean that reserpine acts by blocking this carrier mechanism at the platelet membrane (Hughes & Brodie, 1959). As a consequence, the amine could diffuse out passively. Although the best explanation available, it is not compatible with the findings that reserpine-induced release is prevented by lowering the temperature. Bartholini, Da Prada & Pletscher (1965) have recently demonstrated that reserpine prevents spontaneous 5-HT release in a glucose-free potassium phosphate medium probably through an action on the membrane of platelets, but accelerates the release when glucose is supplied. The utilisation of glucose in the cold is inhibited and, as a consequence, this membrane-stabilising action of reserpine might be dominant at low temperatures. Active transport as a mechanism in the outward flux of amines from nerve granules, has been suggested by von Euler, Stjärne & Lishajko (1964). If active outward transport occurs in platelets it could explain the failure of reserpine to act in the cold.

Reserpine does not influence the amount of ATP in platelets. It is also without effect *in vitro* on the intra-platelet content of potassium and causes no change in the protein content or packed volume of platelets (Paasonen, 1964; Paasonen & Solatunturi, 1965). In man, about 1 mg/day is sufficient to maintain platelet 5-HT depletion (Sjoerdsma, Weissbach, Terry & Udenfriend, 1957).

Tetrabenazine is a benzoquinolizine derivative which releases 5-HT from platelets *in vitro* (Paasonen & Pletscher, 1959). In concentrations that *per se* cause some 5-HT depletion it causes inhibition of the reserpine-induced 5-HT release *in vivo* from brain (Quinn, Shore & Brodie, 1959) and from platelets *in vitro* (Paasonen, 1964). This is taken as an indication of the same point of action for tetrabenazine and reserpine. Chlorpromazine, on the other hand, potentiates the 5-HT releasing action of reserpine (Paasonen, 1964). Monoamine oxidase inhibitors also antagonise the 5-HT depletion from platelets (Paasonen & Pletscher, 1960; Paasonen, 1961b). Although reserpine liberates histamine from rabbit platelets *in vivo* (Waalkes, Coburn & Terry, 1959) it is not known why this does not occur *in vitro* (Burkhalter, Cohn & Shore, 1960).

Phenothiazines and allied drugs. It was reported by Marshall, Stirling, Tait & Todrick (1960) that, during imipramine treatment, the 5-HT content of platelets decreases in three weeks to about one-sixth of the original level. These authors also demonstrated that imipramine *in vitro* prevents the uptake of 5-HT by human platelets. Of a number of substances studied by Stacey (1961), imipramine causes 50% inhibition of the 5-HT uptake at a concentration of 5×10^{-7} M. The next most active agents, cocaine and chlorpromazine, are equipotent at concentrations of $2{\cdot}5 \times 10^{-5}$ M and $3{\cdot}5 \times 10^{-5}$ M respectively. *In vitro*, chlorpromazine, imipramine and related agents also cause 5-HT release from platelets of man and rabbit (Bartholini, Pletscher & Gey, 1961). The release by chlorpromazine is dose dependent (Fig. 1) and a high enough concentration causes a total 5-HT depletion. The release is fast with high concentrations and at 10^{-3} M, chlorpromazine releases more than 90% of the amine within 15 min. This would mean that the depletion cannot be due to a simple inhibition of the uptake mechanism. It has been suggested by Paasonen (1964) that chlorpromazine facilitates the permeation of 5-HT through the limiting membranes. Proof of a morphological change in the platelets is given by the finding that chlorpromazine, unlike reserpine, decreases the packed platelet volume (Paasonen, 1964). The size of the platelets is also reduced when the platelet-rich plasma is observed under the microscope. Therefore, the result cannot be due merely to centrifugation. Electron microscopical studies by Telkkä & others (1964) show that incubation of platelet-rich plasma with chlorpromazine damaged the limiting membranes, allowing part of the cytoplasm to leak out (Fig. 2).

Consistent with the structural change are the findings that, *in vitro*, other components—histamine, ATP and potassium—are liberated from platelets by chlorpromazine (Fig. 1) (McLean, Nicholson & Hertler, 1963; Paasonen 1964; Paasonen & Solatunturi, 1965b). The liberation

of histamine proceeds parallel to that of 5-HT but the potassium liberation does not (Fig. 1). Potassium has been demonstrated by Buckingham & Maynert (1964) to exist largely in the free state in platelets, unlike 5-HT, and the faster initial release of potassium may be because of this fact.

Lowering the temperature antagonises the release of 5-HT caused by chlorpromazine but to a less extent than that caused by reserpine; also monoamine oxidase inhibition has hardly any effect (Paasonen, 1964). These findings are also compatible with the increased membrane permeability.

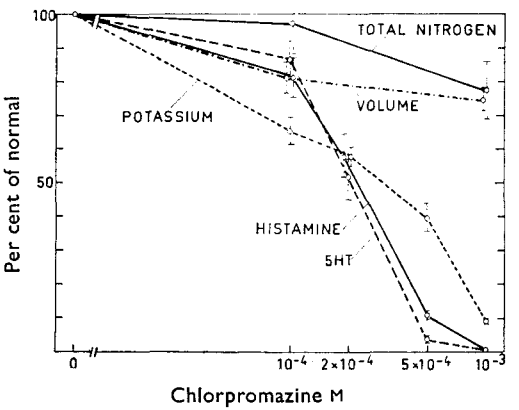

FIG. 1. The effect of chlorpromazine on the amount of various components and packed volume of platelets of rabbit. The platelet-rich plasma was incubated at 37° for 3 hr with the concentrations of chlorpromazine indicated (Paasonen & Solatunturi, 1965b). By permission of Editors of *Ann. Med. exp. Fenn.*

The following results of Ahtee & Paasonen (unpublished) point to a relationship between the 5-HT depletion and the content of the releasing agent in question inside, or in the membrane of, platelets. Chlorpromazine causes haemolysis *in vitro* and *in vivo* and red blood cells are able to concentrate this agent. Similarly the chlorpromazine content is considerably higher in the platelets than in the plasma. *N*-Hydroxyethylpromethazine (Aprobit), which is a quaternary phenothiazine compound,

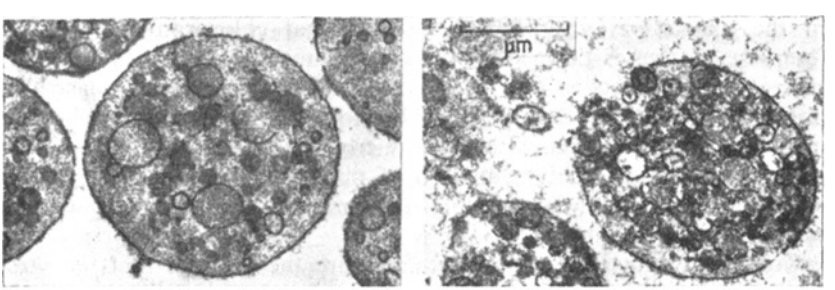

FIG. 2. Electronmicrograph of platelets of rabbit after 1 hr incubation at 37° with (on the right) and without (on the left) chlorpromazine (10^{-3} M). Chlorpromazine ruptures the cell membrane and part of the cytoplasmic material leaks out.

causes no haemolysis of red blood cells and no 5-HT release from platelets. Neither do red blood cells nor platelets absorb this substance as they absorb other phenothiazines.

In vivo, chlorpromazine does not lower the 5-HT content of platelets in acute experiments on rabbits or rats (Paasonen, unpublished). In chronic experiments on psychiatric patients it may cause some decrease in the platelet 5-HT but this effect is not consistent. The reason for this is probably the inadequate amount of chlorpromazine taken up by platelets *in vivo*. Preliminary experiments by Ahtee & Paasonen indicate that, in acute experiments, chlorpromazine decreases the content of 5-HT in the lungs of rabbits. It is known that the concentration of chlorpromazine is higher in this tissue than in others.

Nathan & Friedman (1962) have found that chlorpromazine increases the permeability of *Tetrahymena pyriformis*, a ciliate protozoan. On the other hand, phenothiazines also have a permeability decreasing action on membranes. Freeman & Spirtes (1963) have shown that chlorpromazine prevents the swelling of mitochondria and the haemolysis of red cells in hypotonic solutions. The membrane stabilising actions may well be operating when chlorpromazine acts in therapeutic concentrations *in vivo*. These actions may explain why, for example, chlorpromazine counteracts the reserpine-induced decrease of the monoamines in some experiments (Gey & Pletscher, 1961; Costa, Garattini & Valzelli, 1960).

Guanethidine and prenylamine. Guanethidine lowers the content of catecholamines in the heart and spleen (Sheppard & Zimmerman, 1959.) Unlike reserpine, guanethidine does not reduce the catecholamine content of brain and adrenals (Cass, Kuntzman & Brodie, 1960). Guanethidine lowers the platelet 5-HT *in vitro* but again a concentration of 10^{-3} M or more is needed in platelet-rich plasma (Paasonen, unpublished).

Prenylamine (*N*-3′-phenylpropyl-2′-)1-diphenylpropyl-3-amine is a vasodilating agent which reduces 5-HT and noradrenaline levels both centrally and peripherally (Schöne & Lindner, 1960). Carlsson & others (1963) found it to be a very active inhibitor of the catecholamine uptake by granules of the adrenal medulla. Prenylamine depletes 5-HT from platelets too, and its activity *in vitro* is of the same order of magnitude as that of reserpine (Paasonen, unpublished). The following results (Paasonen, unpublished) indicate a certain difference in the action of guanethidine and prenylamine on the one hand and reserpine on the other. Addition of tetrabenazine to platelet-rich plasma does not clearly inhibit the action of guanethidine and prenylamine in the same way as it inhibits the action of reserpine. Monoamine oxidase inhibitors inhibit the effect of reserpine but less than that of these two agents. The action of guanethidine and prenylamine in these three tests lies somewhere between the action of reserpine and that of chlorpromazine. The failure of guanethidine and prenylamine to change the packed platelet volume indicates that they do not cause structural changes *in vitro* like chlorpromazine.

Sympathomimetic amines and related agents. Stacey (1961) showed that amphetamine, tyramine, cocaine and various other agents prevent the uptake of 5-HT by human platelets *in vitro*. As 5-HT releasers these

compounds seem to be less effective. Buckingham & Maynert (1964) found that 1 mg/ml of amphetamine releases 79% of 5-HT from human platelets in 60 min. The same concentration of adrenaline releases 24% and tryptamine 87%. A concentration of about 10^{-3} M of tyramine, cocaine and α-methyldopa is needed to lower the platelet 5-HT *in vitro* (McLean & others, 1963; Bartholini & others, 1961). According to Bartholini & Pletscher (1964) the *p*-chloro derivative of *N*-methylamphetamine (Ro 4–6861) also liberates 5-HT from platelets *in vitro*.

Nicotine. According to Schievelbein & Werle (1962) and Schievelbein (1963), nicotine causes depletion of 5-HT from rabbit platelets *in vitro* at concentrations higher than 4×10^{-4} M. Much more of the alkaloid seems to be necessary for a pronounced release and the concentrations employed by the authors usually seem to be ten times higher than the figure mentioned above. The depletion is not influenced by changes of temperature between 20 and 37°, but the amount of 5-HT released is proportional to the nicotine concentration used. Histamine and catecholamines are also released by nicotine (Schievelbein & Zitzelsberger, 1964). Monoamine oxidase inhibitors inhibit the release and they also antagonise the effect of nicotine in depressing the uptake of 5-HT. The authors conclude that the site of action of nicotine is in the activation of monoamine oxidase in the platelet.

Other agents. Various haemolytic agents, like desoxycholic acid, digitonin, lysolecithin or bee venom, cause 5-HT liberation from platelets (Habermann & Springer, 1958). Some of the agents mentioned above, for example phenothiazines, also induce haemolysis, and the 5-HT release and haemolysis have similarities (Ahtee & Paasonen, 1965). Although the monoamine oxidase inhibitors pheniprazine and iproniazid antagonise the reserpine-induced 5-HT liberation from platelets *in vitro*, they themselves cause 5-HT release at a concentration of about 10^{-3} M or higher.

High concentrations of ouabain, sodium fluoride, iodoacetic acid or *p*-chloromercuribenzoic acid release 5-HT as well as potassium and amino-acids from platelets *in vitro* (Buckingham & Maynert, 1964).

Daily doses of cortisone cause some depletion of 5-HT as well as of histamine from tissues not containing mast cells (platelets, outer skin, intestine) in rats after 3 to 7 days (Cass & Marshall, 1962). Large doses of ACTH have a similar though less marked effect.

COMMENT

The results obtained by different workers *in vitro* are seldom comparable, particularly since the composition of the suspension fluid differs. When platelet-rich plasma is employed, the anticoagulants like EDTA may influence the results by removing calcium ions. As was mentioned earlier, calcium is necessary for the 5-HT releasing action of thrombin, trypsin, bacterial endotoxin, antigen-antibody reaction, glycogen or fatty acids. Heparin, on the other hand, prevents the action of thrombin, but the action of other factors of endogenous origin is not prevented at all or only by high concentrations. According to Zucker & Borrelli (1955b) platelets retain an abnormal spiney appearance during incubation in saline.

Incubation in plasma or in saline with 6% albumin promotes the disc shape again. The anticoagulants seem to have no fundamental effect on the 5-HT release induced by drugs from platelets in platelet-rich plasma. According to Born & Gillson (1959), the uptake of 5-HT is quicker in citrated plasma than in plasma treated with EDTA. Inhibition of the uptake by any agent does not necessarily mean that it also causes a release. However, any drug causing a release of a subcellular constituent will prevent the uptake of that substance.

From the work of Grette (1962) it can be concluded that thrombin acts on the surface membrane of the platelet and increases the permeability to calcium ions. Subsequently the intracellular calcium ions initiate contraction and the quick release of 5-HT and some additional components of the platelets. The release takes place without lysis of the cells but viscous metamorphosis is a parallel process at least with thrombin and trypsin.

There are certain similarities in the 5-HT release caused by endogenous factors. The involvement of proteolytic activation has been suggested as a common mechanism in most instances. The work done to explain the histamine liberation from mast cells (Uvnäs, 1958; West, 1959) is likely to be of value in studying the release of 5-HT. Some results indicate that histamine and 5-HT are localised in the same storage sites and that both amines are released *in vitro* by 48/80 (Schievelbein & Zitzelsberger, 1964) and by chlorpromazine.

Although reserpine is the best established 5-HT releaser, its mode of action is not yet clear. The only way to induce a pronounced depletion of 5-HT from platelets *in vivo* within a day is to give certain rauwolfia alkaloids. The mechanism of action of tetrabenazine is most probably similar, and a lack of effect by this drug *in vivo* is a question of dose and time of action.

The platelet damaging action of phenothiazines *in vitro* has similarities with the action of proteolytic enzymes (Paasonen & Solatunturi, 1965b). *In vivo* the lack of effect of chlorpromazine is probably again a matter of local concentration, and the membrane stabilising action of small concentrations as is suggested to occur in the brain by Gey & Pletscher (1961) may well be operating *in vivo* in platelets too. In the rat brain, however, Giarman & Schanberg (1962) found that chlorpromazine decreased the ratio of 5-HT present in the particles and in the supernatant fluid without decreasing the total amine. This finding is in keeping with the experiments showing *in vitro* platelet damage previously discussed in detail.

Not much is known about the 5-HT releasing action of the other drugs mentioned. Perhaps, with the exception of prenylamine, their influence on the platelets resembles that of chlorpromazine rather than reserpine. They act quicker than reserpine and at least some of them are known to liberate potassium, amino-acids and ATP from platelets. According to Buckingham & Maynert (1964), reserpine-treated platelets fail to accumulate 5-HT even after washing, whereas the effect of amphetamine can be washed out. The list of 5-HT releasers is growing but the list of mechanisms involved need not similarly increase.

There may be more than one storage site for noradrenaline at the nerve endings (Kopin, 1964), but there is no clear evidence that 5-HT is stored in more than one type of particle in platelets. Practically nothing is known about the release of 5-HT from the intra-platelet store(s). It is therefore impossible to discuss release at different cellular levels. An extensive discussion about the possible cellular mechanisms of amine binding has been presented by Green (1962).

Metabolism of 5-HT by platelets

In spite of the lack of synthesis, the platelets also possess the ability to metabolise 5-HT. When the platelet-rich plasma of rabbit is incubated *in vitro* with reserpine, about half of the platelet-amine will be liberated within 3 hr. Paasonen & Pletscher (1959) found no concomitant 5-HT increase in the plasma outside the platelets when the incubation was in air. Pretreatment of rabbits with a monoamine oxidase inhibitor (Paasonen & Pletscher, 1960), addition of such an inhibitor to the incubation material, or incubation in nitrogen (Paasonen, 1961a) all prevented the metabolism of the liberated amine. A minute amount of oxygen in the atmosphere is sufficient to metabolise the released 5-HT. Although the platelet-free plasma of rabbit has a weak 5-HT-metabolising activity *in vitro* (Paasonen & Airaksinen, 1965b), the plasma is not, or only to a small extent, responsible for the metabolism of the 5-HT liberated from the platelets. A large amount of 5-HT released from human platelets *in vitro* is also metabolised by the platelets, but only a small amount by platelets of the rat (Paasonen, 1961a).

Incubation of whole blood of the rabbit with reserpine causes a 5-hydroxyindoleacetic acid (5-HIAA)-like material to appear in plasma (Waalkes & Coburn, 1958). This requires the presence of the red blood cells, but these cells themselves are not able to metabolise 5-HT. A summary of the metabolism of the 5-HT released from platelets is presented in Fig. 3, taken from a report by Paasonen & Airaksinen (1965a). During

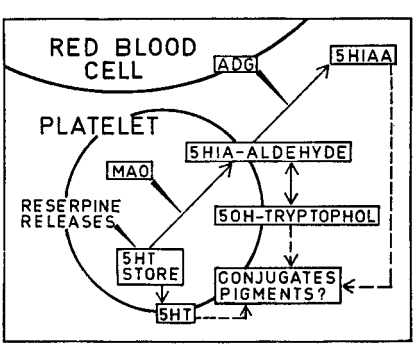

FIG. 3. The fate of 5-HT released from platelets by reserpine-like drugs. Mono amine oxidase converts 5-HT to 5-hydroxyindoleacet(5-HIA)-aldehyde which is then oxidised in the plasma to 5-hydroxyindoleacetic acid (5-HIAA) in the presence of red blood cells or their aldehyde dehydrogenase (ADG). For further explanation see text (Paasonen & Airaksinen, 1965a). By permission of Editors of *Ann. Med. exp. Fenn.*

the liberation from the platelets of 5-HT by reserpine, most of the amine is oxidized to 5-hydroxyindoleacetaldehyde and then reduced to 5-hydroxytryptophol [2-(5-hydroxyindol-3-yl)ethanol]. *O*-Sulphate conjugates of 5-hydroxytryptophol are probably also formed. If a small amount of blood or aldehyde dehydrogenase prepared from red blood cells is present in the platelet-rich plasma during the incubation, about 75% of the 5-HT liberated by reserpine is oxidised to 5-HIAA. Its phenolic sulphate is also formed. Bartholini, Pletscher & Bruderer (1964) have also reported the formation of 5-hydroxytryptophol by platelets. Monoamine oxidase inhibitors prevent this metabolism of 5-HT by platelets. According to Horita (1963), red blood cells prevent this action of hydrazine monoamine oxidase inhibitors like iproniazid and pheniprazine. Therefore, in experiments involving red blood cells and monoamine oxidase inhibitors, it is advisable to use non-hydrazine inhibitors, as for example pargyline, tranylcypromine or harmine.

The presence of monoamine oxidase in platelets is now well established (Paasonen, Solatunturi & Kivalo, 1964) and the activity of the enzyme per unit weight of homogenised platelets is about the same as that in the sympathetic ganglia. The monoamine oxidase activity of homogenised platelets of some mammalian species is listed in Table 2. Bone marrow

TABLE 2. THE MONOAMINE OXIDASE ACTIVITY IN PLATELETS OF SOME MAMMALIAN SPECIES EXPRESSED AS THE AMOUNT OF INDOLEACETIC ACID FORMED FROM TRYPTAMINE *in vitro* (Paasonen & Solatunturi, 1965a)

Species	nmol indoleacetic acid/mg/hr ± s.e.
Rabbit	1·250 ± 0·066 (9)
Human	0·700 ± 0·094 (5)
Cattle	0·157 ± 0·025 (4)
Dog	0·102 ± 0·002 (3)
Cat	0 (10)
Horse	0 (5)
Rat	0 (4)

No. of animals in parentheses.

of rabbit formed 1·000 ± 0·110(4) and that of cat 0·367 ± 0·092(3) nmol of indoleacetic acid/mg/hr. Because only a small part of bone marrow consists of megakaryocytes, the relatively high monoamine oxidase values obtained indicate high enzyme activity in these parent cells of platelets (or in other cells present). In our experiments where 5-HT was liberated by chlorpromazine from platelets in platelet-rich plasma, rabbit platelets metabolised their whole 5-HT content if the concentration of chlorpromazine was such that the time of total release was about one hr or more. The amine release during clotting occurs too quickly to be influenced by the monoamine oxidase of platelets.

It is likely that other pharmacologically active amines are also metabolised by the platelets. At least rabbit intact platelets *in vitro* oxidase adrenaline (Paasonen & Lahovaara, unpublished). The physiological and pharmacological significance of monoamine oxidase in platelets is unknown as also is the question of the extent to which the oxidative deamination occurs in these cells *in vivo*.

Platelet 5-HT and diseases

It is well known that an abnormally high amount of tryptophan is oxidized to 5-HTP and subsequently to 5-HT and 5-HIAA by malignant *carcinoid tumours*. Accordingly, relatively high values for 5-HT have been reported by several workers in the blood of these patients, sometimes associated with thrombocytosis. The increased level of 5-HT in platelets is probably due to an increased amount of free 5-HT in plasma available for absorption (Sjoerdsma & others, 1957).

In some *mentally defective patients*, for example those with cerebral palsy (with I.Q. <50) or with maternal rubella, Pare, Sandler & Stacey (1960) observed high levels of 5-HT in the serum and platelets. According to these workers the reason is not to be found in an increased uptake of 5-HT by platelets as studied *in vitro*, or in an increased ATP content in platelets. The volume and monoamine oxidase content of the platelets in these patients are normal (Paasonen & Kivalo, 1962; Paasonen & others, 1964). In this context it is interesting to note that lowered amounts of 5-HT were found by Krieger, Kolodny & Warner (1964) in the serum of patients suffering from certain brain tumours, especially those located in the hypothalamus. This might indicate that the function of certain brain areas may influence the structure of platelets, making them less capable of absorbing 5-HT *in vivo*, or may decrease the amount of 5-HT available in plasma for absorption, or alternatively impair the uptake and storage of 5-HT in some other way. It could be suggested that in mental deficiency with an elevated platelet 5-HT there is a hyperfunction of those brain areas whose injury leads to lowered platelet 5-HT values.

In *blood diseases* the most common finding is that platelets contain less 5-HT than normal. Hardisty & Stacey (1957) measured low values in myeloid and lymphatic leukaemia and pernicious anaemia as well as in iron deficiency, Hodgkin's disease and polycythaemia vera. The absorption of 5-HT *in vitro* was subnormal in many of these cases. In thrombocythaemias the 5-HT content of platelets can be normal, subnormal or above normal. The reason for the decrease may be connected with structural defects known to occur at least in some of these dyscrasias (Schulz, Jürgens & Hiepler, 1958).

Decreased amounts of 5-HT in platelets have been demonstrated in *rheumatoid arthritis* and other *inflammatory states* by Kerby & Taylor (1959a), who also noted (1959b) a decreased uptake of 5-HT by platelets of these patients *in vitro*.

Platelet 5-HT as a model in pharmacology

The concentration of 5-HT and other pharmacologically active substances in platelets as in some tissues of particular pharmacological interest such as nerve endings, brain cells and adrenal medulla, points to certain similarities between platelets and the other tissues. The release and uptake experiments are comparable in many respects. Only those rauwolfia alkaloids which have a sedative action and which cause amine depletion in the brain, cause a liberation of 5-HT from platelets (Brodie,

Shore & Pletscher, 1956). Of the phenothiazines, *N*-hydroxyethylpromethazine, a non-sedative agent which does not enter the brain, is not absorbed by the platelets and causes no 5-HT release (Ahtee & Paasonen, 1965 & unpublished). It differs in these respects from chlorpromazine. Thrombin, which releases 5-HT from platelets, has been shown by Markwardt & Nuernbergk (1965) to liberate catecholamines from perfused suprarenals.

It is conceivable that the absorption of 5-HT and other compounds from plasma by platelets is used as a means of detoxication. As has been discussed, it is likely that the mammalian body has facilities of its own for releasing 5-HT, and other amines, from the platelets and to metabolise the amine(s). Modification of either the uptake, the release, or the metabolism of the amine is expected to cause reactions whose nature remains undiscovered. In 5-HT research, platelets serve mainly as a model system for solving problems of general interest. The results obtained are also useful if they throw some light on the rôle of platelets themselves in health and disease.

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References

Ahtee, L. & Paasonen, M. K. (1965). *Ann. Med. exp. Fenn.*, **43**, 101–105.
Baker, R. V., Blaschko, H. & Born, G. V. R. (1959). *J. Physiol.*, **149**, 55P–56P.
Bartholini, G., Da Prada, M. & Pletscher, A. (1965). *Nature, Lond.*, **206**, 440–401.
Bartholini, G. & Pletscher, A. (1964). *Experientia*, **20**, 376–378.
Bartholini, G., Pletscher, A. & Bruderer, H. (1964). *Nature, Lond.*, **203**, 1281–1283.
Bartholini, G., Pletscher, A. & Gey, K. F. (1961). *Experientia*, **17**, 541–542.
Born, G. V. R. (1956). *Biochem. J.*, **62**, 339P.
Born, G. V. R. & Gillson, R. E. (1959). *J. Physiol.*, **146**, 472–491.
Born, G. V. R., Ingram, G. I. C. & Stacey, R. S. (1956). *Ibid.*, **135**, 63P–65P.
Born, G. V. R., Hornykiewicz & Stafford, A. (1958). *Brit. J. Pharmacol.*, **13**, 411–414.
Brodie, B. B. (1958). In *5-Hydroxytryptamine*, Editor, Lewis, G. P. pp. 64–83, London: Pergamon Press.
Brodie, B. B., Shore, P. A. & Pletscher, A. (1956). *Science*, **122**, 992–993.
Brodie, B. B., Tomich, E. G., Kuntzman, R. & Shore, P. A. (1956). *J. Pharmacol.*, **118**, 461–467.
Buckingham, A. & Maynert, E. W. (1964). *J. Pharmacol.*, **143**, 332–339.
Burkhalter, A., Cohn, V. H. Jr. & Shore, P. A. (1960). *Biochem. Pharmacol.*, **3**, 328–331.
Carlsson, A., Hillarp, N.-Å. & Waldeck, B. (1963). *Acta physiol. scand.*, **59**, Suppl. 215, 1–38.
Carlsson, A., Shore, P. A. & Brodie, B. B. (1957). *J. Pharmacol.*, **120**, 334–339.
Cass, R., Kuntzman, R. & Brodie, B. B. (1960). *Proc. Soc. exp. Biol. N.Y.*, **103**, 871–872.
Cass, R. & Marshall, P. B. (1962). *Arch. int. Pharmacodyn.*, **136**, 311–332.
Clark, C. T., Weisbach, H. & Udenfriend, S. (1954). *J. biol. Chem.*, **210**, 139–148.
Code, C. F. (1952). *Physiol. Rev.*, **32**, 47–65.
Cole, J. W., Livingston, H., Loughry, C. W. & Holden, W. D. (1953). *Surgery*, **34**, 482–490.
Connor, W. E. (1962). *J. clin. Invest.*, **41**, 1199–1205.
Costa, E., Garattini, S. & Valzelli, L. (1960). *Experientia*, **16**, 461–463.
Davis, R. B., Bailey, W. L. & Hanson, N. P. (1963). *J. Physiol.*, **205**, 560–566.
Davis, R. B., Meeker, W. R. Jr. & Bailey, W. L. (1961). *Proc. Soc. exp. Biol., N.Y.*, **108**, 774–776.

Davis, R. B., Meeker, W. R. Jr. & McQuarrie, D. G. (1960). *Surg. Forum.* **10**, 401–404.
Des Prez, R. M. (1964). *J. exp. Med.*, **120**, 305–313.
Des Prez, R. M., Horowitz, H. I. & Hook, E. W. (1961). *Ibid.*, **114**, 857–873.
Erspamer, V. (1961). *Progress in Drug Research*, **3**, 151–367. Basel: Birkhäuser Verlag.
Erspamer, V. & Testini, A. J. (1959). *J. Pharm. Pharmacol.*, **11**, 618–623.
Euler, U. S. von, Stjärne, L. & Lishajko (1964). *Life Sci.* **3**, 35–40.
Freeman, A. R. & Spirtes, M. A. (1963). *Biochem. Pharmacol.*, **12**, 47–53.
Gaddum, J. H. & Giarman, N. J. (1956). *Brit. J. Pharmacol.*, **11**, 88–92.
Gaintner, J. R., Jackson, D. P. & Maynert, E. W. (1962). *Bull. Johns Hopkins Hosp.*, **111**, 185–197.
Gey, K. F. & Pletscher, A. (1961). *J. Pharmacol.*, **133**, 18–24.
Giarman, N. J., Potter, L. T. & Day, M. (1960). *Experientia*, **16**, 429–494.
Giarman, N. J. & Schanberg, S. M. (1962). *Biochem. Pharmacol.*, **9**, 93–96.
Green, J. P. (1962). *Advances in Pharmacology*, **1**, 349–422. London: Academic Press.
Grette, K. (1959). *Scand. J. clin. Lab. Invest.*, **11**, 50–56.
Grette, K. (1962). *Acta physiol. scand.*, **56**, Suppl. 195, 1–93.
Habermann, E. & Springer, H. (1958). *Naturwiss.* **45**, 133–134.
Hardisty, R. M. & Pinniger, J. L. (1956). *Brit. J. Haemat.*, **2**, 193–152.
Hardisty, R. M. & Stacey, R. S. (1955). *J. Physiol.*, **130**, 711–720.
Hardisty, R. M. & Stacey, R. S. (1957). *Brit. J. Haemat.*, **3**, 292–298.
Horita, A. (1963). *Ann. N.Y. Acad. Sci.*, **107**, 951–956.
Hughes, F. B. & Brodie, B. B. (1959). *J. Pharmacol.*, **127**, 96–102.
Hughes, F. B., Shore, P. A. & Brodie, B. B. (1958). *Experientia*, **14**, 178–180.
Humphrey, J. H. & Jaques, R. (1955). *J. Physiol.*, **128**, 9–27.
Humphrey, J. H. & Toh, C. C. (1954). *Ibid.*, **124**, 300–304.
Kärki, N. T., Paasonen, M. K. & Peltola, P. (1960). *Ann. Med. exp. Fenn.*, **38**, 231–236.
Kerby, G. P. & Taylor, S. M. (1959a). *Proc. Soc. exp. Biol. N.Y.*, **102**, 45–48.
Kerby, G. P. & Taylor, S. M. (1959b). *J. clin. Invest.*, **38**, 1059–1064.
Kopin, I. J. (1964). *Pharmacol., Rev.*, **16**, 179–191.
Krieger, D. T., Kolodny, H. D. & Warner, R. R. P. (1964). *Neurology*, **14**, 578–580.
Lewis, G. P. (1958). *J. Pharm. Pharmacol.*, **10**, 529–540.
Markwardt, F. & Barthel, W. (1964). *Arch. exp. Path. Pharmak.* **249**, 176–184.
Markwardt, E. & Nuernbergk, K. (1965). *Experientia*, **21**, 120–121.
Marshall, E. F., Stirling, G. S., Tait, A. C. & Todrick, A. (1960). *Brit. J. Pharmacol.*, **15**, 35–41.
Maupin, B. (1960). *La Biologie*, **1**, 75–164, English transl. (1961), Psychopharmacology Serv. Centr. Bull., Dec., 15–57.
McLean, J., Nicholson, A. & Hertler, D. (1963). *Life Sci.* **9**, 683–690.
Nathan, H. A. & Friedman, W. (1962). *Science*, **135**, 793–794.
Paasonen, M. K. (1961a). *Biochem. Pharmacol.*, **8**, 241–244.
Paasonen, M. K. (1961b). *Ibid.*, **8**, 301–306.
Paasonen, M. K. (1964). *Arch. exp. Path. Pharmak.*, **248**, 223–230.
Paasonen, M. K. & Airaksinen, M. M. (1965a). *Ann. Med. exp. Fenn.*, **43**, in the press.
Paasonen, M. K. & Airaksinen, M. M. (1965b). *Ibid.*, **43**, in the press.
Paasonen, M. K. & Kivalo, E. (1962). *Psychopharmacologia*, **3**, 188–129.
Paasonen, M. K. & Pletscher, A. (1959). *Experientia*, **15**, 477–479.
Paasonen, M. K. & Pletscher, A. (1960). *Ibid.*, **16**, 30–31.
Paasonen, M. K. & Solatunturi, E. (1965a). *Ann. Med. exp. Fenn.*, **43**, 98–100.
Paasonen, M. K. & Solatunturi, E. (1965b). *Ibid.*, **43**, in the press.
Paasonen, M. K., Solatunturi, E. & Kivalo, E. (1964). *Psychopharmacologia*, **6**, 120–124.
Page, J. H. (1958). *Physiol. Rev.*, **63**, 277–335.
Pare, C. M., Sandler, N. & Stacey, R. S. (1960). *J. Neurol. Neurosurg. Psychiat.*, **23**, 341–346.
Quinn, G. P., Shore, P. A. & Brodie, B. B. (1959). *J. Pharmacol.*, **127**, 103–109.
Rand, M. & Reid, G. (1951). *Nature, Lond.*, **168**, 385.
Rocha e Silva, M. (1950). *Ann. N.Y. Acad. Sci.*, **50**, 1045–1067.
Sandler, M. & Spector, K. G. (1961). *Nature, Lond.*, **189**, 838–840.
Sano, I., Kakimoto, Y. & Taniguchi, K. (1958). *Amer. J. Physiol.*, **195**, 495–498.
Sarajas, H. S. S., Kristoffersson, R. & Frick, M. H. (1959). *Nature, Lond.*, **184**, 1127–1128.

Schievelbein, H. (1963). *Arzneimitt-Forsch.*, **10**, 916–919.
Schievelbein, H. & Werle, E. (1962). *Psychopharmacologia*, **3**, 35–43.
Schievelbein, H. & Zitzelsberger, B. (1964). *Med. exp.*, **11**, 239–246, 247–252.
Schulz, H., Jürgens, R. & Hiepler, E. (1958). *Thromb. Diath. Haem.*, **2**, 300–323.
Schwartz, D. E., Burkard, W. P., Roth, M., Gey, K. F. & Pletscher, A. (1963). *J. Pharmacol.*, **141**, 135–144.
Schöne, H.-H. & Lindner, E. (1960). *Arzneimitt.-Forsch.*, **10**, 583–585.
Sharman, D. F. & Sullivan, F. M. (1956). *Nature, Lond.*, **177**, 332.
Sheppard, H. & Zimmerman, J. H. (1959). *Pharmacologist*, **1**, 69.
Shore, P. A. & Alpers, H. S. (1963a). *Amer. J. Physiol.*, **205**, 348–350.
Shore, P. A. & Alpers, H. S. (1963b). *Nature, Lond.*, **200**, 1331–1332.
Shore, P. A., Pletscher, A., Tomich, E. G., Kuntzman, R. & Brodie, B. B. (1956). *J. Pharmacol.*, **117**, 232–236.
Sjoerdsma, A., Weissbach, H., Terry, L. L. & Udenfriend, S. (1957). *Amer. J. Med.*, **23**, 5–15.
Stacey, R. S. (1958). In *5-Hydroxytryptamine*, Editor, Lewis, G. P., pp. 116–122, London: Pergamon Press.
Stacey, R. S. (1961). *Brit. J. Pharmacol.*, **16**, 284–295.
Telkkä, A., Nyholm, M. & Paasonen, M. K. (1964). *Experientia*, **20**, 27–28.
Toh, C. C. (1956). *J. Physiol.*, **133**, 402–411.
Toh, C. C. (1957). *Ibid.*, **138**, 488–494.
Ungar, G., Yamura, T., Isola, J. B. & Kobrin, S. (1961). *J. exp. Med.*, **113**, 359–380.
Uvnäs, B. (1958). *J. Pharm. Pharmacol.*, **10**, 1–13.
Uuspää, V. J. & Airaksinen, M. M. (1963). *Ann. Med. exp. Fenn.*, **41**, 329–339.
Waalkes, T. P. & Coburn, H. (1958). *Proc. Soc. exp. Biol., N.Y.*, **99**, 742–746.
Waalkes, T. P. & Coburn, H. (1959). *Ibid.*, **101**, 122–125.
Waalkes, T. P., Coburn, H. & Terry, L. L. (1959). *J. Allergy*, **30**, 408–414.
Waller, H. D., Löhr, G. W. Grignani, F. & Gross, R. (1959). *Thromb. Diath. Haem.*, **3**, 520–547.
Weissbach, H., Bogdanski, D. F. & Udenfriend, S. (1958). *Arch. Biochem. Biophys.*, **73**, 492–499.
Weissbach, H., Redfield, B. G. & Titus, E. (1960). *Nature, Lond.*, **186**, 99–100.
West, G. B. (1959). *J. Pharm. Pharmacol.*, **11**, 513–534.
Wurzel, M., Marcus, A. J. & Zweifach, B. W. (1965). *Proc. Soc. exp. Biol., N.Y.*, **118**, 468–472.
Zaimis, E. (1964). *Ann. Rev. Pharmacol.*, **4**, 365–400.
Zucker, M. B. & Borrelli, J. (1955a). *J. appl. Physiol.*, **7**, 425–431.
Zucker, M. B. & Borrelli, J. (1955b). *Ibid.*, **7**, 432–442.
Zucker, M. B. & Borrelli, J. (1958). *Ann. N.Y. Acad. Sci.*, **75**, 203–213.