J. Physiol. (1971), **217**, *pp.* 679–688
With 2 plates and 1 text-figure
Printed in Great Britain

STORAGE OF ATP AND 5-HYDROXYTRYPTAMINE IN BLOOD PLATELETS OF GUINEA-PIGS

BY M. DA PRADA, A. PLETSCHER AND J. P. TRANZER
From the Research Department of F. Hoffmann-La Roche and Co. Ltd, Basle, Switzerland

(*Received 13 April 1971*)

SUMMARY

1. By density gradient centrifugation of homogenized blood platelets of guinea-pigs, subcellular organelles have been isolated which exhibit much higher concentrations (μmole/μg protein) of ATP than the whole platelets.

2. The organelles also store endogenous 5-HT whose concentration is, however, considerably inferior to that of ATP.

3. Investigated by electron microscopy most of the isolated organelles look empty and only very few show dense osmiophilic cores indicating the presence of 5-HT. Accordingly the occurrence of dense osmiophilic organelles in the whole platelets is also very rare.

4. Exogenous 5-HT taken up by the platelets markedly accumulates in the subcellular fraction containing the organelles storing ATP. Concomitantly, the platelets show a rise in the number of osmiophilic organelles and the frequency of osmiophilic cores in the isolated organelles is increased.

5. From these and previous *in vitro* experiments it is concluded that the so called 5-HT storage organelles of blood platelets primarily store ATP and only subsequently accumulate 5-HT.

INTRODUCTION

Blood platelets of rabbits contain relatively large amounts of 5-hydroxytryptamine (5-HT) and nucleotides, especially adenosine-5′-triphosphate (ATP) (Born, Ingram & Stacey, 1958; Da Prada & Pletscher, 1968; Da Prada & Pletscher, 1970; Goetz, Da Prada & Pletscher, 1971; Paasonen, 1965). These constituents are stored in specific subcellular organelles probably in the main as 5-HT-ATP-aggregates of high molecular weight (Berneis, Da Prada & Pletscher, 1969). The organelles have been isolated in pure form by density gradient centrifugation. They can clearly be distinguished by electron microscopy from the other ultrastructural elements

(e.g. α-granules, mitochondria) mainly because of the high osmiophily of their content, which was shown to be due to the presence of 5-HT (Da Prada, Pletscher, Tranzer & Knuchel, 1967; Tranzer, Da Prada & Pletscher 1966). In contrast, blood platelets of guinea-pigs (like those of man) contain only small amounts of 5-HT (Paasonen, 1965; Tranzer *et al.* 1966) (over 50 times less than platelets of rabbits) and correspondingly the dense osmiophilic organelles storing 5-HT are very scarce (Tranzer *et al.* 1966). The ATP content of platelets of guinea-pigs (like that in man) is however similar to that of rabbits (Goetz *et al.* 1970; Mills & Thomas, 1969). Therefore, the question arises whether in the platelets of guinea-pigs (and possibly of man) the ATP may be stored in the absence of high amounts of 5-HT.

In this paper an attempt has been made to isolate the organelles storing 5-HT in guinea-pig platelets by density gradient centrifugation and to submit them to biochemical as well as to fine structural examinations.

METHODS

Experiments on animals

Guinea-pigs of 500–700 g, of either sex, fasted for 16 hr were bled under light ether anaesthesia through a polyethylene cannula in the carotid artery. Disodium-ethylenediaminetetraacetate (EDTA) (1/10 vol., 5 %) was used as an anticoagulant. Some of the animals received four single I.P. doses each of 230 mg 5-HT-creatinine sulphate/kg (corresponding to 100 mg 5-HT-base/kg) at intervals of 8 hr. The blood was taken 8 hr after the last injection.

Experiments with blood platelets

Blood platelets were isolated as previously described (Bartholini, Pletscher & Gey, 1961) and used for the following experiments.

(1) Platelets (corresponding to 2 ml. of original platelet rich plasma) of 5-HT-treated animals and of controls were washed twice each time with 2 ml. modified Tyrode (no $CaCl_2$ and $MgCl_2$ added) (Da Prada, Bartholini & Pletscher, 1965) and analysed for 5-HT, ATP and proteins and also subjected to electron microscopy.

(2) Platelets of normal and 5-HT treated animals were homogenized by ultrasonication and submitted to centrifugation in a continuous Urografin ® gradient in order to isolate the organelles storing 5-HT as previously indicated for rabbit platelets (Da Prada & Pletscher, 1968). This procedure resulted in a bottom layer (fraction 6) attached as a fine film to the tube wall (Da Prada *et al.* 1967). It was analysed for its 5-HT, ATP and protein content or subjected to electron microscopy. The supernatant was divided into five equal parts of about 0·9 ml. each (fractions 1–5) in which the 5-HT and the protein content were measured. These fractions had progressively higher densities with respect to Urografin ®.

(3) Platelets of normal animals were incubated for 1 hr at 37° C with $5{\cdot}7 \times 10^{-4}$ μmole 5-HT-[$^{14}C_3$]creatinine sulphate monohydrate/ml. (Radiochemical Centre Amersham; specific activity 56 μc/μmole) in modified Tyrode, 1 ml. of the medium containing the same amount of platelets as 1 ml. of the original plasma. Thereafter, the platelets were washed twice with Tyrode, and subjected to homogenization and

density gradient centrifugation. In the fractions 1–6 the radioactivity, the total 5-HT and the protein content were determined.

Electron microscopy

The bottom layer (fraction 6) as well as the isolated whole platelets were fixed in a solution of glutaraldehyde 3 % in phosphate buffer, 0·1 M, pH 7·4 at 4° C for 2–4 hr. This procedure was followed by fixation in a solution of OsO_4 2 % in the same phosphate buffer at 4° C for 1 hr, dehydration in alcohol and propylene oxide and embedding into EPON. The ultrathin sections (500 Å) were contrasted with uranyl acetate and lead citrate and examined with a Philips EM 300 (Tranzer *et al.* 1966).

Analytical procedures

5 HT was determined by a spectrophotofluorimetric method (Bogdanski, Pletscher, Brodie & Udenfriend, 1956), ATP with the luciferin-luciferase procedure (Holmsen H., Holmsen J. & Bernhardsen, 1966) and proteins by a colorimetric method (Lowry, Rosebrough, Farr & Randall, 1951). The radioactivity, which, as previously shown (Da Prada & Pletscher, 1969*a*), derived in the main from 5-HT, was measured in a liquid scintillation spectrometer (Da Prada & Pletscher, 1969*a*, *b*).

RESULTS

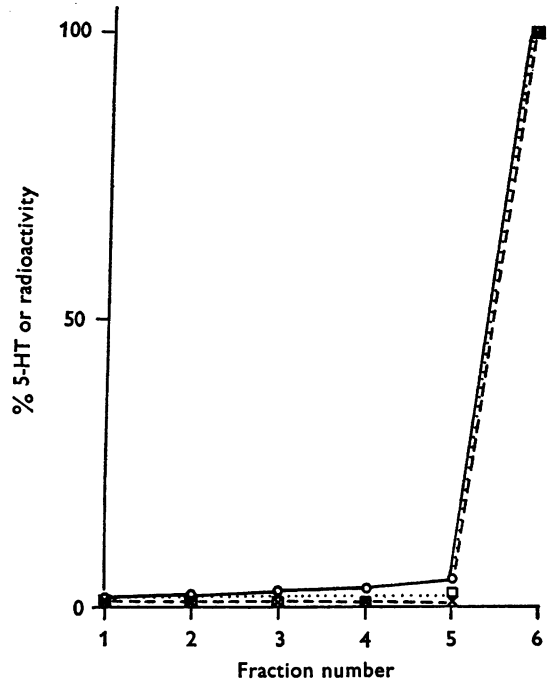
Isolated intact platelets

Isolated platelets of normal guinea-pigs had a very low concentration of 5-HT, i.e. $1{\cdot}0 \pm 0{\cdot}1 \times 10^{-6}$ μmole/μg protein, whereas their ATP content was considerably higher ($2{\cdot}4 \pm 0{\cdot}3 \times 10^{-5}$ μmole/μg protein). The molar ratio between ATP and 5-HT thus amounted to about 24:1. After treatment of the animals with 5-HT I.P. the 5-HT content increased about fifteenfold (to $16{\cdot}1 \pm 3{\cdot}5 \times 10^{-6}$), whereas the ATP levels ($2{\cdot}5 \pm 0{\cdot}3 \times 10^{-5}$) did not markedly change.

On electron microscopic examination the isolated platelets of guinea-pigs showed the usual fine structural elements, i.e. α-granules, mitochondria, glycogen particles and empty vacuoles of various sizes. In addition, very rare vacuole-like organelles occurred, which were surrounded by a single membrane, contained a dense osmiophilic core and had a diameter of about 1500 Å (Pl. 1*a*). They resembled the 5-HT organelles described previously in platelets of other species, such as man, rabbits (Tranzer *et al.* 1966) and cats (Baumgartner, Thoenen & Tranzer, 1969). The average number of these organelles was less than one per platelet section in guinea-pigs. The dense cores varied in size but filled the vacuoles only partially. The isolated platelets of guinea-pigs treated with 5-HT exhibited a similar fine structural appearance with the difference that vacuoles containing dense cores were more frequent and that the latter were larger in size (Pl. 1*b*).

Isolated storage organelles

The various fractions obtained by density gradient centrifugation of the particulate matter of guinea-pig platelets showed a distribution of endogenous 5-HT similar to the fractions obtained from rabbit platelets (Da Prada *et al.* 1967) by the same experimental technique. Thus, the amine was mainly concentrated in the bottom layer (fraction 6)



Text-fig. 1. Content of 5-hydroxytryptamine (5-HT) as well as of radioactivity of various fractions of the particulate matter obtained from platelets of guinea-pigs, subjected to density gradient centrifugation. The endogenous 5-HT and the radioactivity were related to the protein content and expressed in per cent of the values found in the isolated organelles (fraction 6 = 100 %). Representative experiments. Absolute values of isolated organelles: endogenous 5-HT: $8{\cdot}5 \times 10^{-4}$ μmole/μg protein; [^{14}C]5-HT: $1{\cdot}2 \times 10^{-4}$ μmole/μg protein. ○ platelets not incubated *in vitro*: endogenous 5-HT. × platelets incubated *in vitro* with [^{14}C]5-HT: total 5-HT. □ platelets incubated *in vitro* with [^{14}C]5-HT: radioactivity.

which consisted of a fine film attached to the tube wall and represented the isolated storage organelles (see below). The amine content of each of the fractions 1–5 amounted at the most to about 4 % of that of the bottom layer. After incubation of the platelets with [^{14}C]5-HT the

radioactive amine showed the same subcellular distribution as the endogenous 5-HT.

The absolute concentration of ATP in the bottom layer obtained from platelets of guinea-pigs was very high ($108{\cdot}0 \pm 14{\cdot}9 \times 10^{-4}$ μmole/μg protein) and comparable to that previously found in isolated organelles of rabbit platelets (Da Prada & Pletscher, 1968). On the other hand, the 5-HT content was about 35 times lower than that of rabbit organelles. Therefore, the organelles of guinea-pig platelets contained about 0·06 mole 5-HT per mole ATP, whereas in the rabbit 1 mole ATP corresponded to approximately 2·7 mole 5-HT.

The degree of accumulation of endogenous ATP in isolated organelles of guinea-pigs (compared to whole platelets) did not markedly differ from that of 5-HT. Thus, the content of the organelles in ATP ($108{\cdot}0 \pm 14{\cdot}9 \times 10^{-4}$ μmole/μg protein) was approximately 500 times superior to that of the whole platelets ($0{\cdot}2 \pm 0{\cdot}0 \times 10^{-4}$) and the concentration of 5-HT was about 600 times higher in the organelles ($6{\cdot}3 \pm 1{\cdot}9 \times 10^{-4}$) than in the platelets ($1{\cdot}0 \pm 0{\cdot}1 \times 10^{-6}$).

On electron microscopic examination the bottom layer obtained from untreated guinea-pig platelets consisted of a homogeneous population of vesicle-like structures. These organelles were spherical, about 1500 Å in diameter and surrounded by a single membrane. The great majority of the organelles appeared to be empty. Only few were partially filled with a highly osmiophilic content (Pl. 2*a*) and resembled the 5-HT storage organelles of isolated rabbit platelets described earlier (Da Prada *et al.* 1967; Pletscher, Da Prada & Tranzer, 1969).

Exogenous 5-HT

Repeated I.P. injection of 100 mg 5-HT/kg in guinea-pigs raised the concentration of the amine in the isolated organelles as well as their 5-HT/ATP quotient about tenfold (Table 1). The ATP level was only slightly decreased in comparison with untreated controls.

On electron microscopy the bottom layer obtained from platelets of 5-HT treated animals had an appearance similar to that from controls with the difference that the majority of the organelles now contained strongly osmiophilic dense cores of about 400–1000 Å (Pl. 2*b*).

DISCUSSION

Storage sites of ATP and 5-HT

The subcellular organelles isolated from platelets of guinea-pigs by density gradient centrifugation are probably specific storage sites for ATP. In fact, the nucleotide shows a considerable concentration (108×10^{-4} μmole/μg protein) in the bottom layer (fraction 6) corresponding to the

organelles, which is about 500 times higher than that in intact platelets. In contrast, the content of 5-HT in the organelles is markedly inferior ($6{\cdot}3 \times 10^{-4}$ μmole/μg protein) to that of ATP. Nevertheless, the amine shows a much higher concentration in the organelles than in the whole platelets (600 times) and the subcellular distribution experiments demonstrate a rather selective 5-HT accumulation in the bottom layer containing the organelles. The storage organelles of guinea-pig platelets thus

TABLE 1. Content of 5-HT and ATP in isolated storage organelles of blood platelets of guinea-pigs and rabbits

	Rabbit* controls	Guinea-pig		
		Controls (C)	5-HT I. P. (T)†	T/C
5-HT	$210 \pm 17 \times 10^{-4}$	$6{\cdot}3 \pm 1{\cdot}9 \times 10^{-4}$	$62{\cdot}1 \pm 3{\cdot}9 \times 10^{-4}$	$12{\cdot}5 \pm 3{\cdot}3$
ATP	$85 \pm 9 \times 10^{-4}$	$108{\cdot}0 \pm 14{\cdot}9 \times 10^{-4}$	$81{\cdot}0 \pm 15{\cdot}0 \times 10^{-4}$	$0{\cdot}77 \pm 0{\cdot}13$
5-HT/ATP	$2{\cdot}7 \pm 0{\cdot}2$	$0{\cdot}06 \pm 0{\cdot}01$	$0{\cdot}83 \pm 0{\cdot}14$	—

Values of 5-hydroxytryptamine (5-HT) and adenosine-5′-triphosphate (ATP) are expressed in μmole/μg protein and represent means ± S.E. of three to four experiments.

* Values of rabbit organelles were taken from a previous publication (Da Prada & Pletscher, 1968).

† Pre-treatment with four doses of 5-HT I.P. (see Methods).

resemble those of rabbits (Born *et al.* 1958; Da Prada & Pletscher, 1968; Da Prada *et al.* 1967) with the exception that the organelles of guinea-pigs exhibit a much lower 5-HT concentration. It can therefore be concluded that the subcellular organelles of guinea-pig platelets mainly store ATP, but that the small amount of endogenous 5-HT present in these platelets is also accumulated in the organelles.

Electron microscopy of organelles

The scarce dense osmiophilic cores observed in the isolated organelles on electron microscopy are probably due to the presence of 5-HT. In previous experiments only 5-HT but neither ATP nor histamine has been demonstrated to reduce OsO_4 under the present experimental condition (Tranzer *et al.* 1966). Catecholamines, which also react with OsO_4, are probably not present in the organelles in sufficient amounts to induce osmiophilia, since the concentration of these amines in platelets of guinea-pigs is more than 50 times lower than that of 5-HT (M. Da Prada *et al.*, unpublished results). Furthermore, exposure of guinea-pig platelets to exogenous 5-HT (by repeated I.P. injection of the amine) increases the frequency of dense osmiophilic cores in parallel to the 5-HT content of the organelles.

The absence of osmiophilic cores in the majority of the normal isolated

organelles may be due to various reasons. On the one hand, as mentioned before, ATP does not reduce OsO_4 with the present experimental technique. On the other hand the osmiophilic content (which represents 5-HT) might have been lost during the isolation and fixation procedure. Furthermore, if the organelles, whose diameter is about 1500 Å, are incompletely filled with the osmiophilic material, the ultrathin sections which have a thickness of about 500 Å might derive from the 'empty' part of the organelles. These artifacts alone, however, can hardly explain the scarcity of the osmiophilic material, since the organelles of rabbit platelets, which have been isolated and prepared for electron microscopy by the same technique as those of guinea-pigs, are almost uniformly filled with osmiophilic cores (Da Prada *et al.* 1967; Pletscher *et al.* 1969). In addition, isolated organelles of guinea-pigs treated with 5-HT show a relatively high frequency of osmiophilic cores which would not be the case if the osmiophilic material was lost during the isolation and fixation procedure. These findings indicate that the scarcity of osmiophilic cores in the isolated organelles of normal guinea-pigs is at least partly related to their low 5-HT content. The electronmicroscopic findings are thus in agreement with the biochemical results.

Preliminary experiments indicate that isolated storage organelles of human platelets resemble those of guinea-pigs with regard to ultrastructure as well as 5-HT and ATP content.

Prevailing storage of ATP

The chemical and ultrastructural analysis of the isolated storage organelles of guinea-pigs indicate that ATP can be stored without major amounts of 5-HT being present. Thus, the ATP content of these organelles is about equally high as that of rabbit organelles, but the 5-HT/ATP quotient amounts only to 0·06 compared with 2·7 in rabbits. The storage of ATP in the organelles may be explained by the property of the nucleotide to form high molecular weight aggregates by vertical stacking in the presence of bivalent cations as previously shown *in vitro* (Berneis, Da Prada & Pletscher, 1970*a*; Pletscher, Berneis & Da Prada, 1970). In fact, preliminary results indicate that the content of bivalent cations (Mg + Ca) is over 50 times higher in the organelles than in the intact platelets.

Exogenous 5-HT

Like the endogenous 5-HT the exogenous 5-HT accumulates mainly in the organelles storing ATP. This is indicated by the studies on the subcellular distribution of [^{14}C]5-HT (Text-fig. 1) and by the mentioned findings that higher doses of exogenous 5-HT increase the number of osmiophilic cores as well as of the 5-HT content of the isolated organelles.

Furthermore, in the intact platelets of 5-HT-treated animals the dense osmiophilic organelles become more numerous than in controls. They probably originate from preexisting ATP- storing organelles, which look empty on electron microscopy and which can hardly be distinguished from other empty-looking platelet vesicles unless they have accumulated considerable amounts of exogenous 5-HT. Accumulation of this amine in ATP-storing organelles may be explained by previous *in vitro* findings with artificial solutions (Berneis *et al.* 1970*b*; Berneis, Pletscher & Da Prada, 1970). The amine seems to be reversibly incorporated into the ATP-metal aggregates to produce 5-HT-ATP-metal aggregates. Their apparent average molecular weight depends on the concentration and the temperature and reaches its maximum if the molar ratio between 5-HT and ATP amounts to 2:1 and more.

Concluding remarks

These findings indicate that the presence of nucleotides, e.g. ATP in the organelles is essential for the storage of biogenic monoamines. Organelles storing 5-HT, but devoid of nucleotides have not been found up to now, nor do monoamines form aggregates in the presence of bivalent cations. The process of amine storage might therefore be explained in the following way: The storage organelles probably accumulate primarily nucleotide-metal aggregates, which due to their large size cannot leave the organelles, since they are unable to penetrate the surrounding membrane. This accumulation may occur already in an early phase of the platelet development, e.g. in the megakaryocytes. The 5-HT probably enters the platelets from the plasma in a later stage by an active transport at the level of the cytoplasmic membrane (Pletscher, 1968). Thereby platelets of rabbits seem to take up more 5-HT during their life span than those of guinea-pigs. Part of the amine, penetrated into the platelets, enters the organelles, where it is incorporated into the nucleotide-metal aggregates. As a result of this incorporation the concentration gradient of the free amine between the external medium (cytoplasm) and the interior of the organelles would be maintained, enabling more of the amine to enter the organelles and to be incorporated into nucleotide-metal aggregates. This might lead to an accumulation of 5-HT in the form of amine–nucleotide–metal aggregates. Since 5-HT seems to be reversibly bound to the nucleotides (Berneis *et al.* 1970*b*; Berneis, Pletscher & Da Prada, 1969) a state of equilibrium between aggregated and non-aggregated molecules may exist within the organelles, whereby the majority of the amine is probably present in the aggregated form and only very little in monomolecular solution. This might be the physico-chemical explanation for the finding that the 5-HT stored in platelets is freely exchangeable with exogenous 5-HT (Born & Gillson, 1959).

REFERENCES

BARTHOLINI, G., PLETSCHER, A. & GEY, K. F. (1961). Diminution of 5-hydroxytryptamine in thrombocytes *in vitro* by chloropromazine and related compounds. *Experientia* **17**, 541–542.

BAUMGARTNER, H. R., THOENEN, H. & TRANZER, J. P. (1969). Accumulation of 5-hydroxydopamine in 5-HT storage organelles of cat platelets and concomitant changes in platelets aggregation. *Experientia* **25**, 857–859.

BERNEIS, K. H., DA PRADA, M. & PLETSCHER, A. (1969). Micelle formation between 5-hydroxytryptamine and adenosine triphosphate in platelet storage organelles. *Science, N.Y.* **165**, 913–914.

BERNEIS, K. H., DA PRADA, M. & PLETSCHER, A. (1970*a*). Metal-dependent aggregation of nucleotides with formation of biphasic liquid systems. *Biochim. biophys. Acta* **215**, 547–549.

BERNEIS, K. H., DA PRADA, M. & PLETSCHER, A. (1970*b*). A possible mechanism for uptake of biogenic amines by storage organelles: incorporation into nucleotide-metal aggregates. *Experientia* (in the Press).

BERNEIS, K. H., PLETSCHER, A. & DA PRADA, M. (1969). Metal-dependent aggregation of biogenic amines: a hypothesis for their storage and release. *Nature, Lond.* **224**, 281–283.

BERNEIS, K. H., PLETSCHER, A. & DA PRADA, M. (1970). Phase separation in solutions of noradrenaline and adenosine triphosphate: influence of bivalent cations and drugs. *Br. J. Pharmac.* **39**, 382–389.

BOGDANSKI, D. F., PLETSCHER, A., BRODIE, B. B. & UDENFRIEND, S. (1956). Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.* **117**, 82–88.

BORN, G. V. R. & GILLSON, R. E. (1959). Studies on the uptake of 5-hydroxytryptamine by blood platelets. *J. Physiol.* **146**, 472–491.

BORN, G. V. R., INGRAM, G. I. C. & STACEY, R. S. (1958). The relationship between 5-hydroxytryptamine and adenosine triphosphate in blood platelets. *Br. J. Pharmac.* **13**, 62–64.

DA PRADA, M., BARTHOLINI, G. & PLETSCHER, A. (1965). Effect of monoamine liberators on the metabolism of 5-hydroxytryptamine in blood platelets. *Biochem. Pharmac.* **14**, 1721–1726.

DA PRADA, M. & PLETSCHER, A. (1968). Isolated 5-hydroxytryptamine organelles of rabbit blood platelets: physiological properties and drug-induced changes. *Br. J. Pharmac.* **34**, 591–597.

DA PRADA, M. & PLETSCHER, A. (1969*a*). Storage of exogenous monoamines and reserpine in 5-hydroxytryptamine organelles of blood platelets. *Eur. J. Pharmac.* **7**, 45–48.

DA PRADA, M. & PLETSCHER, A. (1969*b*). Differential uptake of biogenic amines by isolated 5-hydroxytryptamine organelles of blood platelets. *Life Sci. Oxford* **8**, 65–72.

DA PRADA, M. & PLETSCHER, A. (1970). Identification of guanosine-5′-triphosphate and uridine-5′-triphosphate in subcellular monoamine-storage organelles. *Biochem. J.* **119**, 117–119.

DA PRADA, M., PLETSCHER, A., TRANZER, J. P. & KNUCHEL, H. (1967). Subcellular localization of 5-hydroxytryptamine and histamine in blood platelets. *Nature, Lond.* **216**, 1315–1317.

GOETZ, U., DA PRADA, M. & PLETSCHER, A. (1971). Adenine, guanine and uridine-5′-phosphonucleotides in blood platelets and storage organelles of various species. *J. Pharmac. exp. Ther.* (in the Press).

HOLMSEN, H., HOLMSEN, I. & BERNHARDSEN, A. (1966). Microdetermination of adenosine diphosphate and adenosine triphosphate in plasma with the firefly luciferase system. *Analyt. Biochem.* **17**, 456–473.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

MILLS, D. C. B. & THOMAS, D. P. (1969). Blood platelet nucleotides in man and other species. *Nature, Lond.* **222**, 991–992.

PAASONEN, M. K. (1965). Release of 5-hydroxytryptamine from blood platelets. *J. Pharmac. Pharm.* **17**, 681–697.

PLETSCHER, A. (1968). Metabolism transfer, and storage of 5-hydroxytryptamine in blood platelets. *Br. J. Pharmac.* **32**, 1–16.

PLETSCHER, A., BERNEIS, K. H. & DA PRADA, M. (1970). A biophysical model for the storage and release of biogenic monoamines at the level of the storage organelles. In *Advances in Biochemical Psychopharmacology*, vol. 2, pp. 205–211, ed. COSTA, E. & GIACOBINI, E. Raven Press.

PLETSCHER, A., DA PRADA, M. & TRANZER, J. P. (1969). Transfer and storage of biogenic monoamines in subcellular organelles of blood platelets. In *Progress in Brain Research*, vol. 31, pp. 47–52, ed. AKERT, K. & WASER, G. Amsterdam: Elsevier Publishing Co.

TRANZER, J. P., DA PRADA, M. & PLETSCHER, A. (1966). Ultrastructural localization of 5-hydroxytryptamine in blood platelets. *Nature, Lond.* **212**, 1574–1575.

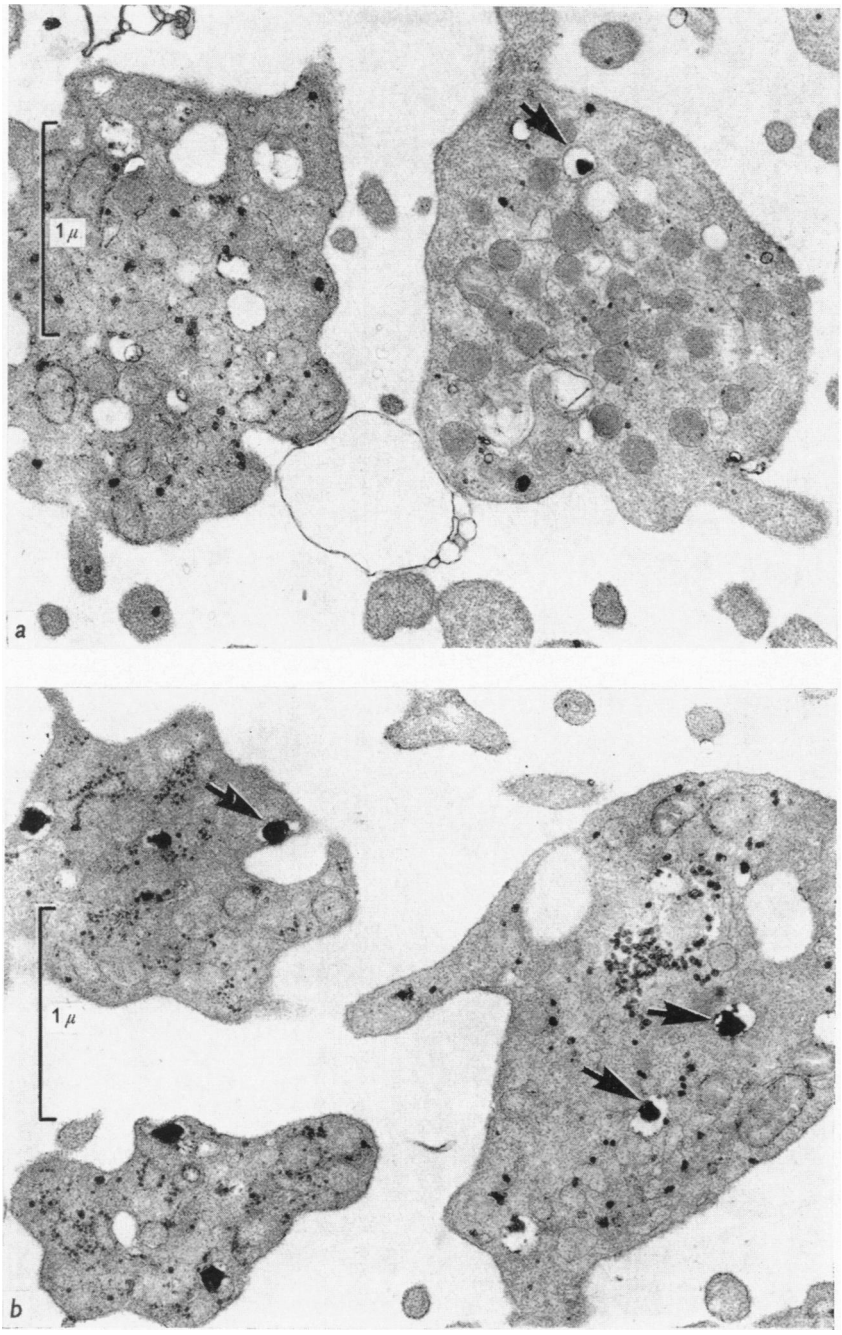
EXPLANATION OF PLATES

PLATE 1

Isolated blood platelets of guinea-pigs (× 28,000): *a* from normal animals and *b* from animals pre-treated with 5-HT I.P. Arrows indicate 5-HT organelles.

PLATE 2

Bottom layer (fraction 6) obtained by density gradient centrifugation (× 28,000): *a* from platelets of control animals and *b* after treatment with 5-HT I.P.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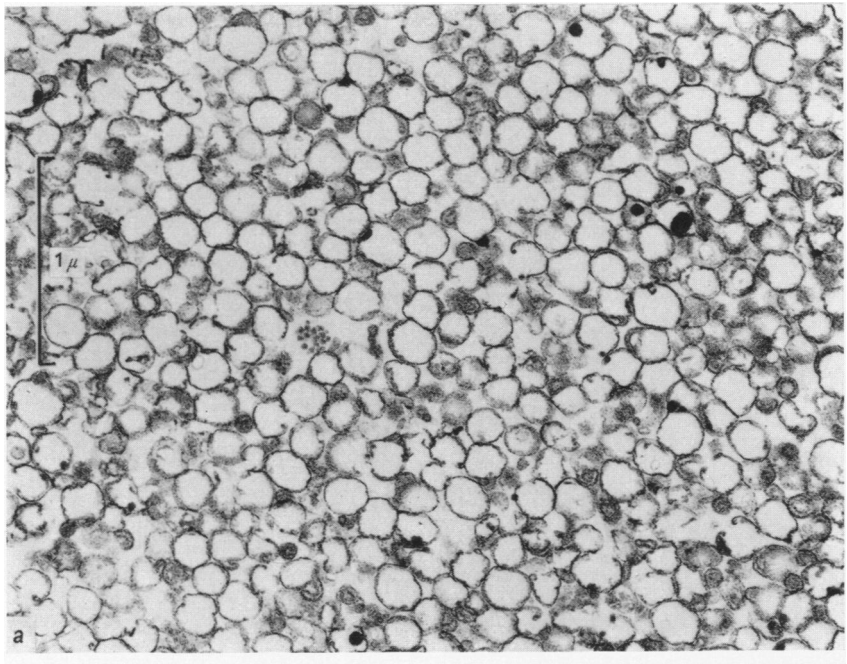

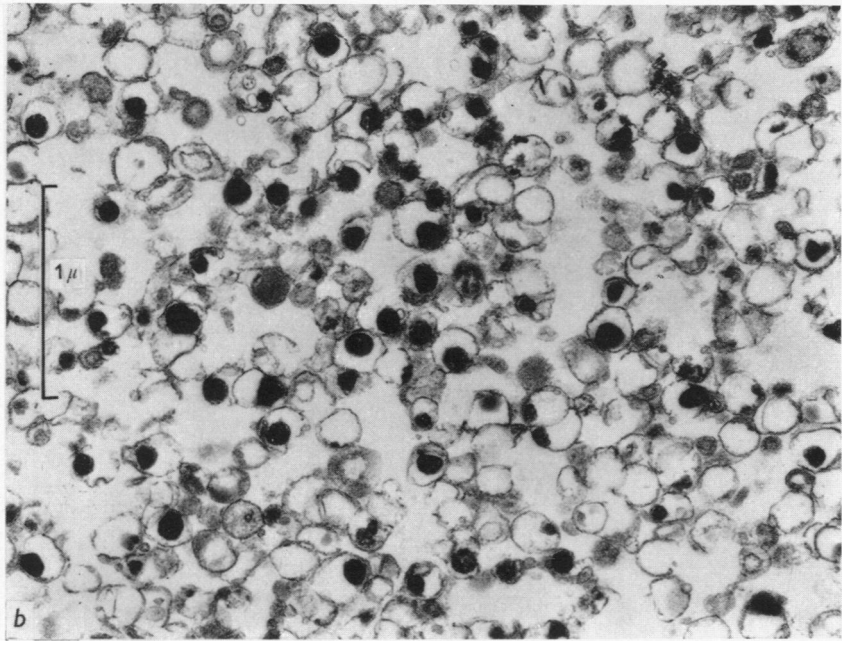

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