J. Physiol. (1954) 124, 305–310

THE HISTAMINE AND SEROTONIN CONTENT OF THE PLATELETS AND POLYMORPHONUCLEAR LEUCOCYTES OF VARIOUS SPECIES

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(*Received* 6 *November* 1953)

In a recent review on 'Histamine in blood', Code (1952) discussed the existing literature relating to the distribution of histamine within the leucocytes and platelets of different species, and he drew attention to the relative paucity of information concerning species other than the rabbit. In the course of work on the release of histamine and of 5-hydroxytryptamine from platelets by antigen-antibody reactions *in vitro*, of which a preliminary account has already appeared (Humphrey & Jaques, 1953), we have had occasion to estimate the concentration of these two substances in the platelets and polymorphonuclear leucocytes of various species. We have also obtained further evidence upon the question, which Code regards as not yet conclusively settled, of the relative contributions of platelets and polymorphonuclear leucocytes to the histamine content of whole rabbit blood. Since the matter is one of some general interest, it seemed worth while to present these findings in a separate report.

METHODS

Histamine assay. Histamine was assayed, in the saline extracts or the body fluids, upon the atropinized guinea-pig ileum by comparison with a histamine acid phosphate standard. Results are expressed in terms of the free base. The preparation used, although sensitive to serotonin (5-hydroxytryptamine) is less than 1/10 as sensitive as it is to histamine, and the amounts present in platelet extracts did not usually interfere seriously with histamine assays. In most instances the extracts were re-assayed after inhibition of histamine contractions by mepyramine maleate (which inhibits histamine much more than serotonin) and the difference was taken to represent true histamine activity.

Serotonin (5-hydroxytryptamine) assay. This was performed on rats' atropinized colon preparation (Dalgleish, Toh & Work, 1953), which is insensitive to histamine, or on the rat uterus preparation of Garcia de Jalon, Bayo Bayo & Garcia de Jalon (1945). The standard was serotonin creatinine sulphate, and the results were expressed in terms of the free base. The active material was presumed to be serotonin since it was inhibited by tryptamine (Gaddum, 1953*a*) and by 2-methyl-3-ethyl-5-amino indole (Woolley & Shaw, 1952) to the same degree as was authentic serotonin. In three instances (goat, guinea-pig and rabbit) platelet extracts were examined by paper chromatography by Dr C. E. Dalgleish, and spots were observed, after spraying with

Pauly's diazotized sulphanilic acid reagent, having the same R_F values as serotonin (Dalgleish, 1952).

Extraction of active substances from platelets and leucocytes was effected by freezing the suspension solid, followed by thawing shortly before assay. Solid CO_2 was generally used for freezing, and the frozen material was stored at -5 to $-10°$ C, but the exact temperature was immaterial. While frozen solid, and in the absence of plasma, the active materials were stable for some weeks. The histamine activities found in the supernatant fluids after centrifuging the frozen and thawed materials were compared on several occasions with those found by Code's (1937) extraction procedure and by simply boiling in acid solution (pH 3). The agreement was always within the experimental error of the assays. Since histamine was extracted completely it was assumed that serotonin would behave similarly.

Preparation of platelet suspensions. Blood was collected from heparinized animals without anaesthesia, except in the case of rats and ferrets which were lightly anaesthetized with ether and of dogs and cats which were anaesthetized with sodium pentobarbitone. Care was taken to avoid contact of the blood with cut tissues by the use of silicone-treated needles, and by liberal smearing of surfaces with vaseline. All glass-ware was silicone treated, and all centrifugations were done at 4° C. The blood was usually mixed with sodium citrate (0·5%) or 'Versene' (disodium ethylenediaminetetra-acetic acid) 0·1%, and was centrifuged for ½ hr at 1600 *g*. The buffy layer was collected with a paraffin-coated pipette, and was washed by centrifugation for ½ hr periods at 2000 *g* with 0·9% NaCl, or with Tyrode solution containing 0·1% added gelatin and 0·1% added sodium acetate mixed with one fourth volume of 0·158 M sodium phosphate buffer pH 7·4. After two or three washings (which were shown to be free from histamine) the platelets were suspended evenly and enumerated in a haemocytometer. An approximate count of the red and white cells was made at the same time. Platelet suspensions so prepared contained a few red cells, and the ratio of platelets to white cells ranged between 1 : 200 and 1 : 1000. They were quite stable in buffered gelatin Tyrode solution, losing less than 10% of their contained histamine on overnight storage at 4° C.

Platelets free from leucocytes were obtained from the blood of rabbits which had received doses of nitrogen mustard (1·75 mg/kg) intravenously 4 days previously. This dose is sufficient to reduce the polymorphonuclear leucocyte count 100-fold, while diminishing the platelet and lymphocyte counts by less than half. Suspensions prepared from such animals contained not more than one leucocyte per 10,000 platelets.

Polymorphonuclear leucocyte suspensions were prepared by intraperitoneal injections of warmed horse meat extract broth (Mackie & McCartney, 1946). 16–18 hr later the peritoneal cavity was irrigated with 1·5% sodium citrate in 0·7% sodium chloride solution. The exudate cells were collected and washed twice on the centrifuge with the buffered gelatin Tyrode solution described above. Differential and total counts were made, and the proportion of polymorphonuclear cells was usually found to be 85–90%, except in the rats where it was only 50%. Cells prepared in this way, and stored at 2° C, became actively motile when warmed to 37° C after a week and were presumably little, if at all, damaged. The cells were lysed by freezing and thawing, as described for platelets. On two occasions the histamine values obtained in this way were checked by the full Code (1937) extraction method, and on several occasions the washings were shown to contain negligible amounts of histamine.

RESULTS AND DISCUSSION

Histamine and serotonin

The results are summarized in Tables 1 and 2. While they confirm the conclusion that platelets are often rich sources of histamine and serotonin, there appears to be a remarkable species variation not only in the absolute but also in the relative amounts of the two substances contained. The values for histamine in platelets are in general agreement with those quoted by Code

(1952), who also mentions low values for platelets of the horse. Our direct estimations of histamine in polymorphonuclear leucocytes were limited to relatively few species; only those of the rat contained large amounts. Indirect calculations, by comparison of the histamine contents of leucocyte-rich and leucocyte-poor portions of the buffy layer gave estimates of 15 $\mu g/10^9$ leucocytes in man and $<0{\cdot}3$ $\mu g/10^9$ leucocytes in dog. Owing to the relatively

TABLE 1. Histamine content (μg) of 10^9 platelets or leucocytes

Species	No. of samples tested	Range	Mean	Comment
Rabbit platelet	23	2–6·7	3·5	Samples from nitrogen mustard treated rabbits averaged 3·0 μg. A third substance was also present
Rabbit polymorph	7	0·5–1·5	1·0	—
Guinea-pig platelet	6	0·1–0·28	0·18	Four out of six samples contained an unidentified third substance
Guinea-pig polymorph	3	0·1	0·1	—
Guinea-pig eosinophil	1	—	1·5	Eosinophil exudate obtained after paraffin injection
Dog platelet	8	0–<0·05	0	Quoted from Humphrey & Toh (1954)
Man platelet	3	0·01–0·12	0·06	The samples contained an unidentified third substance
Rat platelet	3	—	0	—
Rat leucocyte	4	10–18	16	—
Cat platelet	2	0·06–0·18	0·12	The samples contained an unidentified third substance
Ferret platelet	2	0·025–0·05	0·04	The samples contained an unidentified third substance
Ferret leucocyte	2	0·03–0·07	0·05	—
Goat platelet	3	0·16–0·3	0·25	Maximum values—activity may be partly due to serotonin

TABLE 2. Serotonin content (μg) of 10^9 platelets

Species	No. of samples tested	Range	Mean
Rabbit platelet	8	4·5–12	7·5
Guinea-pig platelet	3 (pools)	0·12–0·3	0·21
Dog platelet*	8	0·6–2·7	1·7
Man platelet	2	0·2–0·3	0·25
Rat platelet	1 (pool)	—	0·4
Goat platelet	2	3·5–5	4·3
Cat platelet	3	0·7–1·1	0·9

* Quoted from Humphrey & Toh (1954).

much greater numbers of platelets than of leucocytes (usually around 100 : 1), the contribution of platelets to whole blood histamine must be the predominating factor. From our experiments with rabbits deprived of polymorphonuclear leucocytes by treatment with nitrogen mustard we obtained unequivocal evidence that the platelets in this species contribute practically all the histamine of the blood.

The identification of the vasoconstrictor substance in ox platelets with serotonin was made by Rand & Reid (1951), and it is widely recognized that

platelets are an important source of this substance. Erspamer & Faustini (1953) quote values for the serotonin content of serum from many species. Since clotting is accompanied by complete release of histamine and serotonin from platelets, the serum values are probably derived from the platelets. In Table 3 our estimates of platelet serotonin have been multiplied by mean blood platelet counts, and the values obtained are in general agreement with Erspamer & Faustini's estimates of serum serotonin, with the striking exception of those for the cat. It appears that serotonin occurs more uniformly, and in greater quantities than histamine in platelets. We examined only two specimens of polymorphonuclear exudate, one from rabbit and one from guinea-pig, and found the serotonin content to be less than 0·01 μg/10^9 cells.

TABLE 3. Comparison of mean serum serotonin values from Erspamer & Faustini (1953) with mean whole blood values calculated from our findings (Humphrey & Jaques) on the basis of platelet content alone

	Serotonin (μg/ml.) in	
Species	Serum (E. & F.)	Blood (H. & J.)
Rabbit	3·53	5·2
Goat	2·18	3·0
Cat	3·8	0·68
Rat	0·57	0·34
Dog	0·21	0·49
Guinea-pig	0·21	0·18
Man	0·12	0·19

Occurrence of other active substances in platelets

After treatment of the atropinized guinea-pig ileum with doses of mepyramine and tryptamine sufficient greatly to depress the responses to histamine and serotonin, it was observed that the response to platelet extracts was diminished to a smaller extent than was the response to amounts of histamine and serotonin which had been equivalent to the platelet extracts when tested on the untreated gut. It appeared that some 5–10% of the pharmacological activity of platelet extracts was not abolished by these antagonists. Mr W. E. Brocklehurst, using lysergic acid diethylamide (Gaddum, 1953*b*) instead of tryptamine to inhibit serotonin, has since confirmed some of these observations (private communication). Residual activity of this kind was most noticeable in testing guinea-pig and ferret platelets, but was found also to a lesser extent in human, cat and rabbit platelet extracts. The nature of the substance or substances responsible was not further investigated. In most instances this third type of activity showed a delayed onset of contraction and thus resembled that of the so-called 'slow reacting substances' described by Feldberg & Kellaway (1938); but in the case of rabbit platelets the extracts caused contraction of the desensitized guinea-pig ileum without any apparent latency. Compared with the histamine and serotonin activity, the residual activity was always small, but it may to some

extent have exaggerated our estimated values—particularly in assays of serotonin on the rat colon, in which we could not be certain of specifically inhibiting the serotonin contractions.

The importance of platelets as packets of very active pharmacological substances, which circulate in the blood stream and are capable of releasing their contents locally when slightly damaged, is obvious, and has already been pointed out by Rand & Reid (1952). A point of purely technical importance, whose seriousness has not always been sufficiently recognized, is that unless great care is taken to avoid platelet damage when blood is drawn, incorrect estimates will be obtained for free histamine or serotonin in plasma. Our own findings in rabbit, dog and human plasmas have been values of the order of 0·002 μg/ml. for both substances. Even these small amounts might be due to traces derived from damaged platelets.

SUMMARY

1. The average amounts of histamine found in the platelets of various species were as follows (per 10^9 platelets): rabbit, 3·5 μg; cat, goat and guinea-pig, 0·1–0·3 μg; man, dog, rat and ferret, less than 0·1 μg.

2. The average amounts of serotonin were: rabbit, 7·5 μg; goat 4·3 μg; dog, 1·7 μg; cat, 0·9 μg; man, guinea-pig and rat, 0·2–0·4 μg.

3. Platelets from man, rabbit, cat, guinea-pig and ferret contained a further unidentified substance when tested on the isolated guinea-pig ileum.

4. Histamine was found in the polymorphonuclear leucocytes obtained from peritoneal exudates of rabbits, guinea-pigs, ferrets and rats, but only in rats was the amount large.

5. It is concluded that histamine and serotonin found in the blood of most species is almost all contributed by the platelets. When damage to platelets is minimized, the amounts found in the plasma are very small indeed.

We wish to thank Dr R. K. Richards of Abbott Laboratories for providing a sample of serotonin creatinine sulphate, and Dr D. W. Woolley of the Rockefeller Institute for Medical Research for a gift of 2-methyl-3-ethyl-5-hydroxyindole. These were made available to us through the kindness of Dr W. Feldberg, F.R.S. We are also indebted to Mr W. E. Brocklehurst for doing the estimations of histamine and serotonin in cat platelets.

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