

THE RELATIONSHIP BETWEEN 5-HYDROXYTRYPTAMINE AND ADENOSINE TRIPHOSPHATE IN BLOOD PLATELETS

BY

G. V. R. BORN, G. I. C. INGRAM, AND R. S. STACEY

From the Nuffield Institute for Medical Research, University of Oxford, and the Departments of Pathology and Therapeutics, St. Thomas's Hospital Medical School, London

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In normal platelets a proportionality was found between the amount of adenosine triphosphate (ATP) and amount of 5-hydroxytryptamine (5-HT) in platelets both before and after incubating them in plasma to which 5-HT had been added. In patients receiving reserpine and in others with myeloid leukaemia, the amount of 5-HT in the platelets and the uptake of 5-HT by them were depressed, while the amount of ATP was normal. The possibility that ATP is involved in the accumulation of 5-HT by platelets is discussed.

Hardisty and Stacey (1957) showed that, in myeloid leukaemia and some other diseases of the blood, the amount of 5-hydroxytryptamine (5-HT) in the platelets is reduced. In some cases this was associated with an impaired capacity of the platelets to take up 5-HT when they were incubated in plasma to which 5-HT had been added. Further, it has been shown (Born, Ingram, and Stacey, 1956; Born and Gillson, 1957) that the amount of 5-HT in platelets is proportional to the amount of adenosine triphosphate (ATP) which they contain. This paper reports observations on the amounts of 5-HT and ATP in platelets obtained from normal people, from patients suffering from myeloid leukaemia and from others treated with reserpine. Some of the experiments have been briefly reported (Born *et al.*, 1956).

METHODS

The methods described by Hardisty and Stacey (1955) were used for the preparation of platelet-rich plasma and for the determination of the 5-HT content of the platelets and of their ability to take up 5-HT added to the plasma. ATP was extracted from platelets with trichloroacetic acid and estimated by the method of Strehler and Totter (1954), using firefly luminescence.

RESULTS

Platelets obtained from 15 normal persons were analysed for ATP and 5-HT. The results are recorded in Fig. 1, in which the amounts of these

two substances are plotted against one another. Although there is considerable scatter, there is a rough proportionality between the amounts of 5-HT (y) and ATP (x) in the platelets which is expressed by the equation $y = 0.01 + 0.023x$ ($r = 0.67$, $P < 0.01$).

Fig. 2 shows that after platelets from the same people had been incubated with 5-HT the proportionality became closer. The corresponding equation is $y = 0.035 + 0.094x$ ($r = 0.78$, $P < 0.001$). The mean ratios of the number of ATP molecules to the number of 5-HT molecules in the platelets before and after incubation with 5-HT are given in Table I, together with the ranges and standard errors.

Ten patients with myeloid leukaemia were investigated. Of these, five had received no treatment

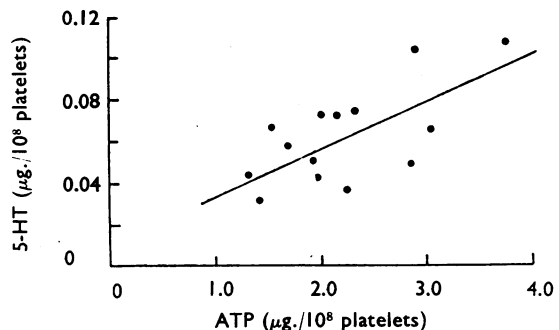


FIG. 1.—The relationship between 5-HT and ATP in normal platelets.

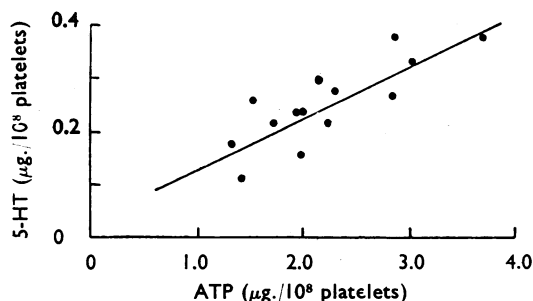


FIG. 2.—The relationship between 5-HT and ATP in normal platelets after incubation at 37° in plasma containing added 5-HT.

and five had started treatment with busulphan. Both groups showed the diminished platelet 5-HT and impaired ability to absorb 5-HT previously described, but the mean concentration of ATP in the platelets of these patients did not differ significantly from the normal, and in 8 of 10 it was within normal range. These changes are reflected in the molecular ratio ATP/5-HT, which is significantly higher than in the normals.

The 5-HT content of platelets and their ability to absorb 5-HT is reduced by the administration of reserpine. This has been shown for human platelets by Haverback, Shore, Tomich, and Brodie (1956) and by Hardisty, Ingram, and Stacey (1956) and is apparent from the results recorded in Table I. One of the subjects of the present investigation was taking 0.25 mg. reserpine by mouth three times a day, and four had received a single dose of 1 mg. intramuscularly 48 hr. before the blood was taken. The mean platelet ATP is not significantly different ($P>0.1$) from the normal, but the ratio ATP/5-HT in the platelets is greatly raised.

DISCUSSION

The results show that, in normal human platelets, the amount of 5-HT is proportional to the amount of ATP, both when the platelets are

freshly isolated from the blood and after they have been allowed to take up 5-HT *in vitro*.

Recent experiments have strongly suggested that, in the intracellular granules of the adrenal medulla, the catechol amines are bound to ATP by ionic bonds (Blaschko, Born, D'Iorio and Eade, 1956). It is interesting to consider whether the proportionality between the amounts of 5-HT and ATP in platelets indicates that a similar mechanism brings about the accumulation and binding of 5-HT in the platelets. Some observations support this idea. One is that, when platelets are present in plasma which is clotting, the ATP in the platelets rapidly breaks down and at the same time 5-HT is released from them (Born, 1956). Another is the remarkably close correspondence between the number of molecules of ATP in platelets and the maximum number of molecules of 5-HT which platelets take up under the best experimental conditions (Born and Gillson, 1957)—namely, one to two molecules of 5-HT for each molecule of ATP.

However, it is possible that the variations in the amounts of 5-HT and ATP might both result from changes in a third factor, such as the platelet volume. In the experiments reported here we found that the concentration of ATP in normal platelets varied 2.8-fold, whereas their volume varied only 1.2-fold (Hardisty and Stacey, 1955). This makes it unlikely that platelet volume determines the amount of ATP which the platelets contained.

Our results show that, in patients treated with reserpine and in others suffering from myeloid leukaemia, the amount of 5-HT that the platelets will hold is reduced while the amount of ATP in them remains the same. Two possible explanations of this must be kept in mind. If 5-HT is bound to ATP in platelets this union might be blocked in these people, or alternatively it may be that there is at least one other factor involved in the accumulation of 5-HT by platelets and that it is through this that reserpine acts and which is possibly defective in myeloid leukaemia.

TABLE I

PLATELET 5-HT AND ATP BEFORE AND AFTER INCUBATION OF PLATELETS IN PLASMA CONTAINING ADDED 5-HT
The numerals in *italics* are significantly different ($P<0.01$) from the corresponding normal figures. The numerals in Roman type are not significantly different ($P>0.1$) from the corresponding normal.

	No. of Subjects	ng. 5-HT/10 ⁸ Platelets		μg. ATP/10 ⁸ Platelets	Molecular Ratio ATP/5-HT	
		Initially	After Incubation with 5-HT		Initially	After Incubation with 5-HT
Normal	15	60.0 ± 5.1	242 ± 21	2.2 ± 0.17	12.8 ± 0.9	3.2 ± 0.24
Myeloid leukaemia (a) untreated ..	5	11.8 ± 3.0	95 ± 12	2.1 ± 0.49	47.4 ± 9.2	8.6 ± 1.7
.. .. . (b) treated ..	5	21.0 ± 5.1	110 ± 30	1.9 ± 0.38		
Reserpine treated	5	5.4 ± 0.6	39 ± 10.5	2.9 ± 0.35	186	27 ± 5.3

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