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THE UPTAKE OF ADRENALINE AND NORADRENALINE BY BLOOD PLATELETS OF THE PIG

BY

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Pig platelets contained 0.2 to 2.6 ng. adrenaline/10^8 platelets (4 experiments). Noradrenaline was not detected in them. When platelet-rich plasma was incubated at 37° with 1 or 10 μg. of added catechol amine/ml., the platelets continued to accumulate adrenaline for at least 120 min.; only about one-third as much noradrenaline as adrenaline was taken up. The concentrations of adrenaline taken up by different platelet samples at the end of incubation for 90 min. were proportional to the concentration of adenosine triphosphate in the platelets.

Mammalian blood platelets contain high concentrations of 5-hydroxytryptamine which they are able to take up from the plasma (Humphrey and Toh, 1954; Hardisty and Stacey, 1955). In some species, notably the rabbit, platelets also contain histamine (Humphrey and Jaques, 1954). The question arises whether the catechol amines which are found in blood (Holzbauer and Vogt, 1954) are likewise concentrated in the platelets. Results obtained with human blood by Weil-Malherbe and Bone (1954) and by Robinson and Stott (1958) suggested that this was so, although Valk and Price (1956) found no catechol amines in human platelets.

This paper reports experiments which show that the platelets of the pig contain adrenaline and that they are able to take up adrenaline from the plasma. We used pig platelets because we expected that the concentration of catechol amines in platelets would be low and because it was easy to obtain pig platelets in comparatively large amounts in blood brought from the slaughter-house: 1 l. of pig blood yielded up to 1 g. (moist weight) of platelets. A preliminary account of this work has appeared (Born and Hornykiewicz, 1957).

METHODS

All glassware with which platelets came into contact was coated with silicone (MS 1107). In the slaughter-house, the pig was stunned electrically and in 1 to 2 min. it was hung up by the hind-legs and bled by cutting the large veins in the neck. Samples of the freely falling blood were caught in a polythene container containing sufficient sodium citrate to give a final concentration of 0.36% (w/v).

The blood was taken to the laboratory and centrifuged at 500 *g.* and 1° for 20 min. to sediment the red and white blood cells. The supernatant plasma which contained the platelets (" platelet-rich plasma ") was removed. Duplicate 0.1 ml. samples were mixed with 1.9 ml. of a solution containing 1% (v/v) formaldehyde in 3.6% (w/v) sodium citrate. The platelets in this diluted suspension were counted in haemocytometer chambers.

In some experiments, samples of platelet-rich plasma were then centrifuged at 6,000 *g.* for 20 min. at 1°. The sedimented platelets were extracted (see below) and the extracts were analysed for catechol amines and for adenosine triphosphate. The total time between the bleeding of the pig and the extraction of the platelets was 1 to 2 hr.

Incubation of Platelets with Added Catechol Amines.—Samples of platelet-rich plasma, about 30 to 40 ml., were incubated in centrifuge tubes in a water-bath at 37°. Catechol amines were added to some samples before incubating; the concentrations are given in the section on results. After incubation, the samples were centrifuged to sediment the platelets. In some experiments the platelets were washed with saline and centrifuged again. They were then extracted.

Adenosine triphosphate was extracted from the platelets with 10% (w/v) trichloracetic acid and determined by the firefly luminescence reaction (Strehler and Totter, 1954).

Catechol amines were extracted from platelets with acid ethanol by the method of Crawford and Outschoorn (1951). This method also extracted 5-hydroxytryptamine. The catechol amines were separated by paper chromatography (Vogt, 1952).

The system did not separate adrenaline from 5-hydroxytryptamine.

Noradrenaline was assayed by its effect on the blood pressure of the pithed rat. At first, adrenaline was assayed in the same way. However, Dr. Marthe Vogt pointed out to us that in such rats the blood pressure might be raised by the 5-hydroxytryptamine present in extracts of platelets. The quantity of 5-hydroxytryptamine in extracts of three samples of platelets, assayed on a strip of isolated rat stomach (Vane, 1957), was found to be 29, 37, and 42 ng./10^8 platelets. The presence of 5-hydroxytryptamine in such amounts would make the determination of adrenaline inaccurate. However, in the experiments in which the uptake of adrenaline was followed we were concerned only with measuring the increase in the quantity of adrenaline in the platelets. For this reason the presence of 5-hydroxytryptamine did not affect the results. In addition, these results were confirmed in other experiments in which adrenaline was assayed on the isolated uterus of the rat by the method of de Jalon, Bayo and de Jalon (1945). Lysergic acid diethylamide (10 ng./ml.) was present in the fluid bathing the uterus in order to abolish any stimulating effect of 5-hydroxytryptamine on the uterus.

Results

Presence of Adrenaline in Freshly Isolated Platelets

When pig platelets were isolated in the way described, they contained adrenaline: the value of 5.8 ± 0.88 ng./10^8 platelets (mean $\pm$ S.E.) was given in the preliminary communication (Born and Hornykiewicz, 1957), but that figure was probably too high because the assays were made with the rat blood pressure. Four later determinations with the rat uterus gave 0.2, 0.5, 1.5, and 2.6 ng./10^8 platelets.

In four samples of platelets which contained adrenaline, noradrenaline was not detectable, and the concentration present was therefore less than 0.3 ng./10^8 platelets. The area in which the concentration of noradrenaline would have been expected was cut from the chromatograms and eluted. When the eluate was assayed on the rat blood pressure there was no effect. This confirmed the conclusion that there was no noradrenaline in the platelet extracts.

Uptake of Catechol Amines by Platelets

When adrenaline was added to platelet-rich plasma it was taken up by the platelets against the concentration gradient. The amount of adrenaline taken up increased for at least 120 min. (Fig. 1).

Table I shows that when platelet-rich plasma was incubated with adrenaline for 90 min., the amount of adrenaline taken up by the platelets increased with increasing concentrations of adrenaline, but the final concentration gradient decreased steadily.

When noradrenaline was added to platelet-rich plasma at concentrations of 1 μg./ml. or 10 μg./ml. the platelets took up only about one-third as much as adrenaline.

Effect of Washing Platelets with Isotonic Saline on their Content of Adrenaline.—In two experiments, adrenaline was added to platelet-rich plasma and, after incubation for 90 min., the platelets were centrifuged down in the usual way. In some tubes the sedimented platelets were extracted at once for adrenaline. In others they were suspended with gentle stirring in 20 to 30 ml. 0.9% (w/v) NaCl, centrifuged again and then extracted.

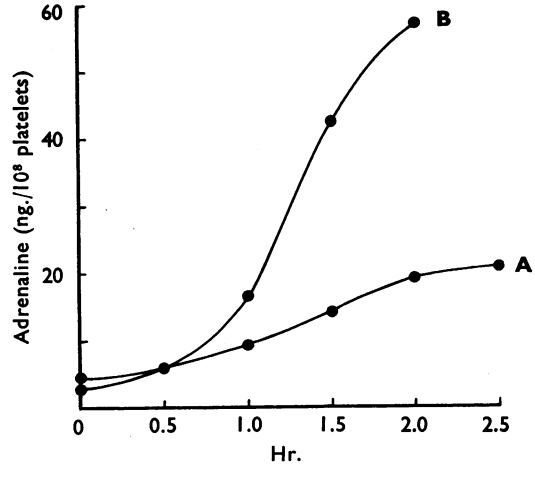

FIG. 1.—Uptake of adrenaline by pig platelets. Observations on platelet-rich plasma of two pigs. A: 1 μg./ml. of adrenaline added to plasma: adrenaline in platelets was assayed on rat blood pressure. B: 10 μg./ml. of adrenaline added to plasma: adrenaline in platelets was assayed on rat uterus.

TABLE I

UPTAKE OF INCREASING CONCENTRATIONS OF ADRENALINE ADDED TO PLATELET-RICH PLASMA OF THE PIG

Incubation: 90 min. at 37°. Average vol. of pig platelet: 12·3 μ^3.

Adrenaline Conc.			Platelet Conc./ Plasma Conc.
In Plasma	In Platelets		
(μg./ml.)	(ng./10^8 Platelets)	(μg./ml. Platelet Volume)	
1	14·2	11·5	11·5
10	60	49	4·9
50	216	176	3·5
250	678	552	2·2
500	918	746	1·5

Table II shows that washing with saline did not affect the amount of adrenaline in the original platelets nor in those incubated with 1 μg. adrenaline/ml. plasma. However, when the plasma had contained 10 μg. adrenaline/ml. the amount of adrenaline in the platelet sediment was reduced by about 12%.

TABLE II
EFFECT OF WASHING WITH ISOTONIC SALINE ON ADRENALINE CONTENT OF SEDIMENTED PLATELETS

Adrenaline Conc.		
Added to Platelet-rich Plasma (μg./ml.)	In Unwashed Platelets (ng./10^8 Platelets)	In Platelets Washed Once with Saline (ng./10^8 Platelets)
Nil	1·6	1·6
1	8·0	8·0
10	83·3	73·1

In two other experiments estimations were made of the amount of plasma trapped with platelets which had been centrifuged. This was done by determining the "inulin space" (Creese, 1954) in the pellet of platelets. From this the amount of adrenaline present in this volume of plasma was determined. It was calculated that, when the plasma contained 10 μg. adrenaline/ml., about 14% of the "platelet adrenaline" was accounted for by adrenaline outside the platelets. This suggested, therefore, that washing the platelets with saline merely removed plasma containing adrenaline and did not remove any adrenaline from the platelets themselves.

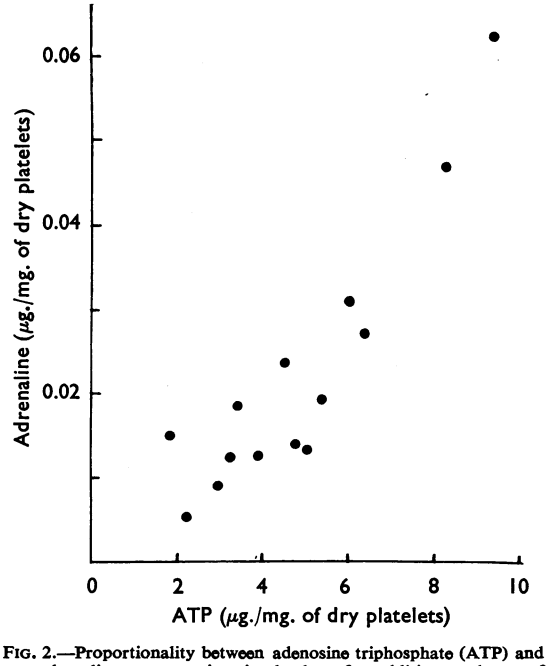

FIG. 2.—Proportionality between adenosine triphosphate (ATP) and adrenaline concentrations in platelets after addition to plasma of 1 μg. adrenaline/ml. and incubation at 37° for 90 min. $r=0.905$; $P<0.01$.

Proportionality Between the Amount of Adrenaline Taken Up by the Platelets and their Content of Adenosine Triphosphate.—Platelets were incubated for 90 min. in plasma to which 1 μg. adrenaline/ml. had been added, and adenosine triphosphate and adrenaline were determined in the platelets. It was found that there was a proportionality between the concentrations of adrenaline and of adenosine triphosphate in the platelets. This is shown in Fig. 2 in which the results are expressed on the basis of dry weight of platelets. The proportionality was also seen, but not so well, when the results were expressed in terms of the platelet count.

DISCUSSION

In these experiments pig platelets were found to contain adrenaline. On the basis of the amount of adrenaline in the platelets, pig plasma contained up to about 15 μg./l. This is much more than the concentrations of adrenaline found by Holzbauer and Vogt (1954) in the plasma of undisturbed, conscious dogs (between 0.04 and 0.25 μg./l.) and in one healthy man (below 0.06 μg./l.).

It is possible that the difference may be explained as follows. Whereas the samples analysed by Holzbauer and Vogt (1954) were obtained under resting conditions, platelets for use in the experiments described here were obtained from the blood of pigs which had been driven with some violence into the slaughtering pen, had then been stunned electrically and had finally been bled out from the veins in the neck. All these procedures are known to increase greatly the secretion of adrenaline from the suprarenal medulla into the blood.

It is not known whether platelets contain adrenaline when they are in the blood of the living pig. It took 1 to 2 hr. to isolate the platelets from the blood of the pig after it had been killed. The results on the rate at which adrenaline is taken up by platelets *in vitro* are consistent with the possibility that all the adrenaline which was found in the platelets was taken up by them during the time which elapsed between the death of the pig and the isolation of the platelets. The amount of free adrenaline, if any, left in the plasma was not determined.

It is interesting that the platelets contained adrenaline but no detectable noradrenaline. Moreover, when they were exposed to the same concentration of adrenaline and noradrenaline *in vitro*, they took up more adrenaline than noradrenaline.

The uptake of adrenaline by platelets was proportional to the amount of adenosine triphosphate in the platelets. In this the uptake of adrenaline by platelets resembles the uptake of 5-hydroxytryptamine (Born, Ingram and Stacey, 1958), an observation which led to the suggestion that in platelets amines are bound to adenosine triphosphate through their ionized groups. Work with granules which can be isolated from the cells of the adrenal medulla has made it likely that such compounds exist, because the granules contain both catechol amines and adenosine triphosphate in exceptionally high concentrations; moreover, the number of molecules of amine is probably just sufficient to satisfy all the acidic groups of the adenosine triphosphate (Hillarp, Högberg and Nilson, 1955; Blaschko, Born, D'Iorio and Eade, 1956). In the platelets this may be so in the case of 5-hydroxytryptamine (Born and Gillson, 1957) but not in the case of adrenaline. When platelets have taken up as much adrenaline *in vitro* as they can, they still contain far less adrenaline than adenosine triphosphate. If, therefore, an ionic bond is formed between the amine and adenosine triphosphate it is only one feature of a more complicated process. In pig platelets the preferential uptake of adrenaline over noradrenaline shows that the uptake is more specific than can be explained by ionic binding alone.

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REFERENCES

Blaschko, H., Born, G. V. R., D'Iorio, A., and Eade, N. R. (1956). *J. Physiol.*, **133**, 548.

Born, G. V. R., and Gillson, R. E. (1957). *J. Physiol.*, **137**, 82P.

—— and Hornykiewicz, O. (1957). Ibid., **136**, 30P.

——, Ingram, G. I. C., and Stacey, R. S. (1958). *Brit. J. Pharmacol.*, **13**, 62.

Crawford, T. B. B., and Outschoorn, A. S. (1951). Ibid., **6**, 8.

Creese, R. (1954). *Proc. roy. Soc., B.*, **142**, 497.

Hardisty, R. M., and Stacey, R. S. (1955). *J. Physiol.*, **130**, 711.

Hillarp, N.-A., Högberg, B., and Nilson, B. (1955). *Nature, Lond.*, **176**, 1032.

Holzbauer, M., and Vogt, M. (1954). *Brit. J. Pharmacol.*, **9**, 249.

Humphrey, J. H., and Jaques, R. (1954). *J. Physiol.*, **124**, 305.

—— and Toh, C. C. (1954). Ibid., **124**, 300.

de Jalon, P. G., Bayo, J. B., and de Jalon, M. G. (1945). *Farmacoter. act.*, **2**, 313.

Robinson, R., and Stott, F. D. (1958). *Biochem. J.*, **68**, 28P.

Strehler, B. C., and Totter, J. R. (1954). In *Methods of Biochemical Analysis*. Ed. Glick, D., **1**, p. 341. London: Interscience.

Valk, A. T., and Price, H. L. (1956). *J. clin. Invest.*, **35**, 837.

Vane, J. R. (1957). *Brit. J. Pharmacol.*, **12**, 344.

Vogt, M. (1952). Ibid., **7**, 325.

Weil-Malherbe, H., and Bone, A. D. (1954). *Nature, Lond.*, **174**, 557.