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THE ESTIMATION OF ADRENALINE IN NORMAL RABBIT'S BLOOD

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Many attempts have been made in the past to determine the concentration of adrenaline in blood and other fluids, but suitable quantitative methods are few. Using biological test organs, Stewart & Rogoff in 1922 found that the normal adrenaline content of blood from the carotid artery was 1 in 10^9, whilst that in the suprarenal vein was 2 in 10^6. Whitehorn (1935), using a chemical method and silicic acid for adsorption, claimed to estimate adrenaline in blood in a concentration of 0·02μg./ml. Later, Shaw (1938) published a modification of this method which is simpler and more sensitive. He found that rabbit's blood had an apparent adrenaline content of 0·05–0·06μg./g., and most of it probably was adrenaline, as shown by the specific colour increase with alkali. His results with human blood were mostly due to another catechol derivative, as there was little or no colour increase with alkali. Later work (Bloor & Bullen, 1941; Raab, 1943) suggested that adrenaline circulates in the blood of man in very small amounts only.

In recent years, attention has been centred on the fluorescence method of estimating adrenaline in solution (Gaddum & Schild, 1934), and it has been applied to blood (for references see Jørgensen, 1945). The work described below is a modification of Jørgensen's technique, involving the dialysis of haemolysed blood and the subsequent analysis of the dialysate. By using parallel quantitative assays and comparison figures for the activities of adrenaline and noradrenaline (West, 1947*b*), it has been possible to establish which of these two substances is present in normal rabbit's blood.

METHOD

The method of Jørgensen was followed throughout with very slight modifications. The dialysis was carried out in small circular perspex dishes, held together by a metal clasp and each possessing an outlet, closed by a small screw, for introduction or withdrawal of the fluids. The cellophane membrane separating the two halves had a thickness of 25μ. Experience showed that no vaseline was required on the ground edges. Into one side of the dialyser was introduced 5 ml. of blood and 5 ml. of diluent (N/100-HCl containing 0·1% glycine, which caused haemolysis); into the other, 5 ml. of diluent and 2 ml. of Shaw's aluminium hydroxide suspension (to remove traces of other fluorescent substances which pass through the cellophane membrane). The dishes were placed on edge and shaken at the rate of about 120 horizontal movements per min. 5 ml. of the recipient fluid were removed by pipette and centrifuged. 3 ml. of the supernatant solution were transferred to a Monax test-tube, 0·3 ml. of 2N-NaOH added, and the fluorescence compared with a standard eosin solution by two methods as previously described (West, 1947 *a*). Shaw's method was applicable also, but the presence of some soluble aluminium salts inactivated the biological test preparations. This was overcome for the Straub frog heart and frog blood-vessels particularly, by omitting the aluminium suspension from the dialyser and diluting the dialysate before testing.

RESULTS

To determine the efficiency of the method, pure adrenaline solutions were employed in place of the blood. Dialysis was carried out for given times, after

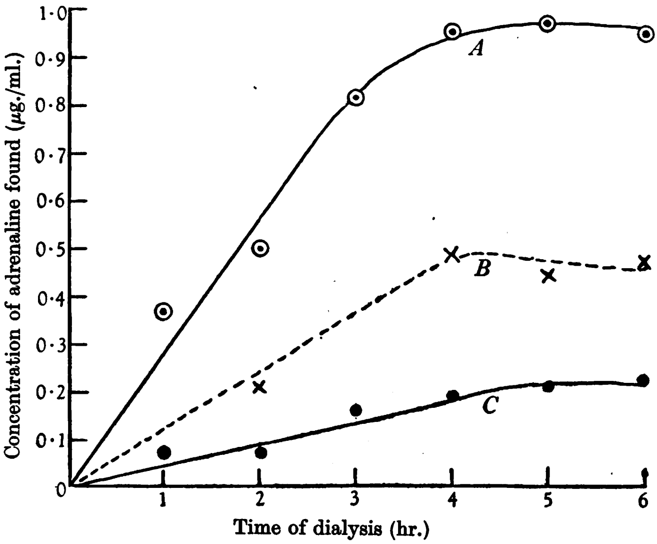

Fig. 1. Recoveries of adrenaline in solution with varying times of dialysis. Concentration of solution $A = 1{\cdot}0\mu$g./ml., $B = 0{\cdot}5\mu$g./ml., and $C = 0{\cdot}2\mu$g./ml. Optimum values were reached after 4 hr.

TABLE 1. The recovery of adrenaline in aqueous solution, using 5 ml. quantities and dialysis for 4 hr.

Concentration of adrenaline (μg./ml.)	Weight of adrenaline taken (μg.)	Weight of adrenaline found (μg.): Fluorimetric		Physiological		Chemical
		Jørgensen	West	Straub heart	Frog blood vessel	Shaw
1	5	4·7	4·9	4·7	5·4	4·8
0·2	1	0·86	0·94	0·88	—	1·06
0·1	0·5	0·45	0·47	0·46	0·5	0·61
0·03	0·15	0·18	0·20	0·14	0·2	—

which the dialysate was tested fluorimetrically, chemically and physiologically against the adrenaline standard solution. The results, recorded in Fig. 1, confirmed that dialysis for 4 hr. was optimal. Recoveries were good with amounts greater than 0·03μg./ml. (Table 1). The biological tests gave results which agreed closely with those obtained fluorimetrically and chemically, each value in the table being the mean of six readings. A few estimations with solutions of noradrenaline showed correspondingly good agreement, though of course much larger amounts had to be used, since noradrenaline is less active than adrenaline on all the preparations tested.

Blood was then taken from the rabbit's ear vein direct into solid sodium citrate (Jørgensen, 1945) and assayed. Quantitative agreement between the different tests was shown when results were calculated as adrenaline (Table 2), although chemical values were always slightly higher than those found by fluorimetric and physiological methods. Chang & Gaddum (1933) indicated the value of such agreement in the identification of pharmacologically active substances. The average of forty-nine determinations of the adrenaline content of rabbit's blood, made on two samples taken from each of six rabbits was found to be 0·098μg./ml., a value which agrees with that of 0·074μg./ml. found by Jørgensen. The few normal human and cat's blood samples that were assayed indicated a smaller amount of adrenaline, values of between 0·03 and 0·05μg./ml. being detected. The differences in the results with different species may have been due to the emotional response of the rabbits to the bleeding.

TABLE 2. The concentration of adrenaline (μg./ml.) in normal rabbit's blood
Dialysis for 4 hr.

	Method of assay						
	Fluorimetric		Physiological		Chemical		
Rabbit	Jørgensen	West	Straub heart	Frog blood vessel	Shaw	Average value	Shaw's specific test
A	0·110	0·094	0·098	0·090	0·120	0·102	2·1
B	0·119	0·122	0·100	0·120	0·127	0·118	2·3

The addition of known amounts of adrenaline to assayed blood of two rabbits produced slightly variable results (Table 3). Added noradrenaline likewise gave slightly lower values than expected, but generally recoveries were reasonable. Shaw's specific test, indicative of adrenaline or sympatol (but not noradrenaline), was positive when applied to blood dialysates, ratio values of more than 2·0 being recorded (Table 2). Experiments on rabbit's red blood cells and plasma suggested that the majority of the adrenaline is carried in the cells (Bain, Gaunt & Suffolk, 1937), since the amount in rabbit's plasma was unmeasurable.

The results shown in Fig. 2 are from two rabbits which were fed on a fairly constant diet. Samples of blood were taken from these animals about the same time (10 a.m.) each day for 5 days, and then 2 mg. adrenaline was injected subcutaneously in two doses ½ hr. apart. Immediately after the second injection, another sample of blood was taken and yet another at the end of 1 hr. All the samples were assayed for their adrenaline contents by the fluorimetric and biological methods. A rise in blood adrenaline was shown, but the increase had disappeared after 1 hr., thus indicating a fast inactivation process.

TABLE 3. The recovery of adrenaline added to 5 ml. quantities of normal citrated rabbit's blood. Dialysis for 4 hr.

Blood	No. of samples	No. of assays	Mean adrenaline content (μg./ml.)	Adrenaline added to vessel (μg.)	Theoretical concentration (μg./ml.)	Concentration found (μg./ml.)	No. of samples	No. of assays
A	3	12	0·122	1·0	0·322	0·361	3	9
				0·5	0·222	0·257	3	10
B	3	10	0·072	1·0	0·272	0·269	3	10
				0·5	0·172	0·168	3	10

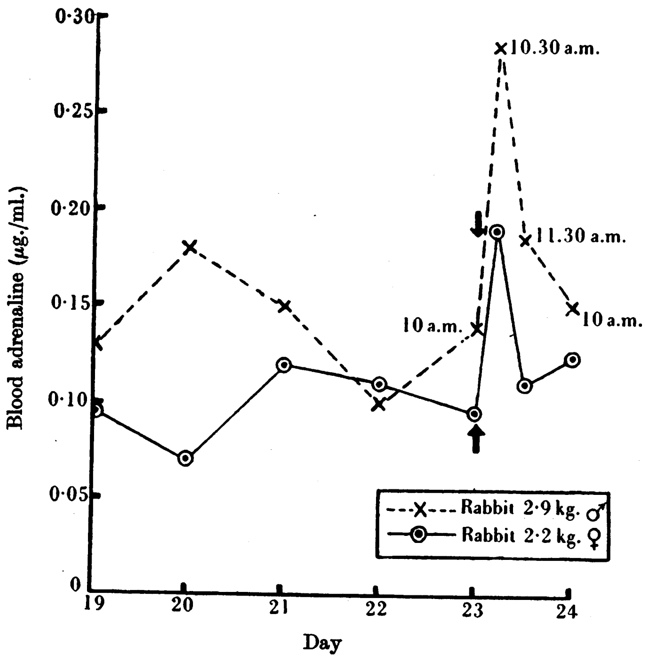

Fig. 2. The effect of 2 mg. adrenaline injected subcutaneously (at the arrows) on the blood adrenaline of two rabbits. After 1 hr. the rise had disappeared. Each point represents the mean of values obtained by the fluorimetric, Straub heart and perfused frog blood-vessel methods.

DISCUSSION

By means of parallel quantitative assays and comparison figures for the activities of adrenaline and noradrenaline, normal rabbit's blood has been found to contain adrenaline at a concentration of 0·098μg./ml. On no occasion have the assays indicated the presence of noradrenaline, since quantitative agreement between the different tests was shown when the results were calculated as adrenaline. The proof that rabbit's blood contains adrenaline and not noradrenaline is important, since the work of Euler (1946*a*, *b*, *c*) showed that extracts of mammalian spleen, heart and sympathetic nerves contain an active substance similar in action to noradrenaline. It adds weight to the suggestion that noradrenaline might be the precursor of adrenaline (Blaschko, 1942), and that methylation might occur on stimulation, the methyl group being derived from the choline constituent of lecithin. Examples of biological transmethylation are quite numerous, methionine and choline usually acting as donors of the methyl group.

SUMMARY

1. By means of chemical, fluorimetric and physiological methods, it has been possible to demonstrate the presence of adrenaline (and not noradrenaline) in normal rabbit's blood.

2. The average concentration in duplicate blood samples from six rabbits was 0·098μg./ml., and this value was nearly doubled after 2 mg. of adrenaline were injected subcutaneously.

3. The possible relation of noradrenaline and adrenaline to the mediator liberated by adrenergic nerves is discussed, and transmethylation of noradrenaline suggested.

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