Adrenaline and Noradrenaline Concentrations in Rat Tissues

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Noradrenaline and adrenaline concentrations have been little studied in rat tissues; this paper deals with their estimation by an extension of the method of Weil-Malherbe & Bone (1952, 1953, 1954) for blood; a brief report has already been presented (Montagu, 1955). The results do not appear to support the current hypothesis that the concentration of noradrenaline can be correlated with the density of adrenergic-nerve fibres (Euler, 1951*b*, 1954). The proportions of adrenaline in the mixtures of noradrenaline and adrenaline are both higher and more uniform than those usually found (Mirkin, 1953; Euler, 1954; Mirkin & Bonnycastle, 1954).

METHODS

Preparation and chemical analysis of tissue extracts. Male Wistar rats were used, and after overnight starvation were killed by a blow on the head; mean body weights in estimations on different tissues varied from 231 to 250 g. Adrenaline and noradrenaline were estimated in extracts prepared from whole diaphragm (after removal of central tendon and phrenic nerve), heart, spleen, brain and lungs, both kidneys and about 2 g. each of liver, retroperitoneal fat and leg muscles (semitendinosus, semimembranosus, biceps femoris and gastrocnemius). Liver, spleen and brain were dispersed in a Potter–Elvehjem homogenizer in 4 vol. of methanol containing 2·5% (v/v) N-H_2SO_4; the other tissues were ground in a mortar in acid methanol with acid-washed sand. After centrifuging at 3000 rev./min. for 3–10 min. each supernatant was decanted and mixed with 2–4 drops of N-HCl. The residue was re-extracted twice with acid methanol (2 vol.). The three supernatants were combined and shaken with water and *n*-butanol (20 vol. each). Occasionally more water was necessary to effect separation, and more N-HCl to reduce the pH of the aqueous layer below 3. After withdrawing the aqueous layer the *n*-butanol was twice re-extracted with 0·01 N-HCl (10 vol.). An appropriate amount of the aqueous extract was concentrated *in vacuo* at 37–40° to half of its original volume. A solution (5 ml.) containing 1% (w/v) $Na_2S_2O_3,5H_2O$ and 0·5 M-K_2HPO_4 was added and the pH adjusted electrometrically to 8·4–8·6 by addition of Na_2CO_3. [Although the K_2HPO_4 used for this work was satisfactory, some other batches were subsequently found to cause very low recoveries of adrenaline and noradrenaline; in its place 2 ml. of sodium acetate solution (Weil-Malherbe & Bone, 1952) and 5 ml. of a solution containing 2% (w/v) $Na_2S_2O_3,5H_2O$ and 1% sodium ethylenediaminetetraacetate (pH 7·2) were used.] The adrenaline and noradrenaline in the buffered solution were adsorbed on a column of alumina, eluted and condensed with ethylenediamine as described for blood by Weil-Malherbe & Bone (1952), except that only 0·4 ml. of ethylenediamine dihydrochloride solution was used; this was to prevent turbidity developing in the solutions. The solutions were extracted with *iso*butanol. Fluorescence was then determined within 24 hr. with yellow and blue-green filters (Weil-Malherbe & Bone, 1953) and 0·1 or 0·2 μg. adrenaline and noradrenaline standards.

Paper chromatography. Tissue extracts were passed through a column of alumina; the alumina was washed with 5 ml. of acetate solution (Weil-Malherbe & Bone, 1952) and 15 ml. of water, and the amines were eluted with 0·05 N-HCl instead of 0·2 M acetic acid, to reduce the solubility of inorganic salts in acetone. A part of the eluate was removed for estimation of the noradrenaline and adrenaline present at this stage. The rest of the eluate was concentrated *in vacuo* to 0·05 ml., 5–8 ml. of acid acetone (1 ml. of conc. HCl/100 ml. of acetone) was added and the mixture centrifuged; the process was repeated with the supernatant and the second supernatant concentrated before applying to paper. The residues, soluble in water, were kept at 4° for estimation. The chromatograms were run for 10–18 hr. on 8 cm. × 50 cm. paper. When phenol–water was used as solvent the procedure was that described by Weil-Malherbe & Bone (1954). With other solvents the chromatograms were photographed with ultraviolet light (Markham & Smith, 1949, 1951). The guide strip was sprayed with a mixture of 1 part of ethylenediamine and 8 parts of 2 N-NH_3 soln. (Brodie, Axelrod, Shore & Udenfriend, 1954), and the positions of the catecholamines were defined by their fluorescence in ultraviolet light. Appropriate areas of the chromatograms were cut out and extracted for 16–22 hr. at 4° with 10 ml. of 0·01 N-HCl. The noradrenaline and adrenaline in these extracts, in the residues from the acid-acetone precipitations and in the part of the solution removed before paper chromatography were determined after condensation with ethylenediamine.

For most experiments Whatman no. 1 paper treated with HCl and ethylenediaminetetraacetate (Eggleston & Hems, 1952) was used. For the experiment with diaphragms (Table 1) Grycksbo OB paper (gift from Dr U. Hamberg-Lindgren) was used; this gave a similar blank value to Whatman no. 1 paper with a greater rate of solvent flow (49 cm. in 10 hr.). The Whatman no. 1 paper for the last experiment of Table 1 had been treated with acetic acid, lithium hydroxide and calcium acetate by a shortened modification of the method of Connell, Dixon & Hanes (1955). This removed coloured matter from the paper but appeared to reduce the recovery of the amines.

Bio-assay. For biological estimations elution of the alumina column was carried out with 2–5 ml. of 0·2 M acetic acid only. The method of assay based on regular stimulation by acetylcholine (Jalon, Bayo & Jalon, 1945) was applied to rat uterus and ascending colon as described by Gaddum, Peart & Vogt (1949) in a 3·5 ml. bath. The bath solution was either Gaddum & Lembeck's (1949) fluid, gassed with O_2 but

containing only 0·03 g. of $CaCl_2$/l., or a buffer gassed with $O_2 + CO_2$ (95:5) and containing (g./l.) NaCl, 8·0; KCl, 0·4; $CaCl_2$, 0·03; $NaHCO_3$, 1·4; glucose 1·0 and crude carbonic anhydrase solution prepared from human blood (Roughton & Clark, 1951) equivalent to 1 ml. of washed erythrocytes/l. There was no detectable change of pH when 0·2 ml. of 0·001 N-HCl was added to 2·5 ml. of this buffer; standard solutions and eluates were adjusted to pH 3–4, but to pH 5–7 when the modified Gaddum & Lembeck's fluid was used. Biological estimates were taken as the average of two limiting values varying by a factor of 1 or 2. A part of each test solution was estimated chemically immediately after bio-assay.

Statistics. The function 100 S.E./$\bar{x}$ is termed the 'percentage standard error'. Correlation coefficients (r) were calculated as

$$\frac{\Sigma(xy) - N\bar{x}\bar{y}}{\sqrt{(\Sigma x^2 - \bar{x}\Sigma x)}\sqrt{(\Sigma y^2 - \bar{y}\Sigma y)}}$$

(e.g. Paradine & Rivett, 1953); r was transformed to z, and the significance of z assessed from the formula $t = z\sqrt{(n-3)}$ (Fisher, 1944). Estimates of adrenaline and noradrenaline concentrations have been expressed correct to two significant figures, but three have been used in calculating statistics. The percentage of adrenaline in a mixture of adrenaline and noradrenaline has been referred to in the text as the 'adrenaline percentage'.

RESULTS

Preliminary experiments with rat liver showed that extraction with acid methanol produced considerably larger yields of adrenaline and noradrenaline (140–500 %) than extraction with aqueous 10 % (w/v) solutions of trichloroacetic, phosphotungstic, sulphosalicylic, metaphosphoric, tannic or hydro-

Table 1. *Recovery of noradrenaline and adrenaline by paper chromatography*

The descending method was used for all except the first two experiments. For paper used see Methods. Solvent mixtures: 1, phenol–water (3:1, w/v) as used by Weil-Malherbe & Bone (1954); 2, *n*-butanol satd. with N-HCl (Hamberg & Euler, 1950); 3, *tert.*-butanol–formic acid–water (8:1:2 by vol.) (Ellman, 1955). In the first four experiments, adrenaline (A) and noradrenaline (N) were added to half of the solutions used for chromatography, and each half was treated separately. In subsequent experiments the solution was concentrated as a whole, treated twice with acid acetone, and A and N were added with a micrometer syringe to part of the final supernatant. The amounts of A and N found after addition of these amines to tissue extracts have been corrected by subtracting the amounts of preformed amines.

	After elution from alumina (μmg./g. of tissue)				After paper chromatography (μmg./g. of tissue)		Percentage recovery	
Tissue	N	A	Additions	Solvent mixture	N	A	N	A
Liver	35*	15*	None	1	30	8·0	86	53
			0·5 μg. of A 1·0 μg. of N	1	820	330	82	66
Heart	390	110	None	1	220	70	55	64
			Vitamin C	1	140	70	36	64
			1·0 μg. of A 2·0 μg. of N	1	770	730	39	73
			Vitamin C 1·0 μg. of A 2·0 μg. of N	1	690	850	34	85
Heart	240	87	None	2	98	44	41	50
Kidney	37	12	None	2	19	14	52	120
Diaphragm	49	12	None	2	34	9·9	86	82
			0·5 μg. of A 2·0 μg. of N	2	1470	400	74	81
Brain	150	18	None	3	62	18	42	99
			1·0 μg. of A 4·0 μg. of N	3	1650	890	41	89
Brain	110	34	None	2	68	28	61	83
			0·5 μg. of A 2·0 μg. of N	2	1400	450	70	91
Spleen	200	24	None	3	140	19	70	79
			0·5 μg. of A 2·0 μg. of N	3	1470	340	74	68
Spleen	120	15	None	2	28 39†	8·1 2·7†	— 57	— 72
			0·5 μg. of A 1·5 μg. of N	2	940	370	63	73

* The method of Lund (1949, 1950) gave 42 and 13 respectively.
† Obtained from the residue from acetone treatment.

ferrocyanic acids, or after saturation of aqueous extracts with $(NH_4)_2SO_4$. Concentrations of adrenaline and noradrenaline, and recovery of added amines, were only slightly lower after extraction with aqueous HCl (optimum concentration 0·1 N) than after extraction with acid methanol, but HCl and most of the aqueous acids had the disadvantages that separation from *n*-butanol was slower and the aqueous layers obtained were not usually clear even after concentration, that the filtration rate through alumina was very variable, sometimes very slow, and that the first HCl extract was the most highly coloured; in contrast, successive acid-methanol extracts contained increasing amounts of pigment. With acid methanol the purified tissue extracts were clear and colourless except for those from kidneys (pale yellow), and those from liver and fat which were sometimes turbid. When liver was dispersed in acid methanol and kept at 4° for 2–18 hr., the yields of adrenaline and noradrenaline were increased by less than 5 %.

Specificity of the method

Recovery of added amines. Recovery of a mixture of 0·05 μg. of adrenaline and 0·1 μg. of noradrenaline added to 2 g. of liver homogenate averaged 71 % adrenaline (S.E. 7·5) and 84 % noradrenaline (S.E. 9·8) in fourteen experiments.

Paper chromatography. Table 1 shows the results of nine experiments with from 5 to 33 g. of tissue. Residues from acid-acetone treatment, when dissolved in water, yielded fluorescence after treatment with ethylenediamine. This is partly due to some retention of adrenaline and noradrenaline (Weil-Malherbe & Bone, 1954). Fluorescence appeared to be increased, however, by traces of acetone which were difficult to remove, and the value obtained from the residue was included in percentage recoveries only in the last experiment. Here the residues were large and were taken up in HCl, concentrated, applied to paper, and chromatographed. Pure noradrenaline and adrenaline applied to paper in both acid-acetone and aqueous-acetone solutions showed that acetone does not appear to interfere with the estimation of eluates from paper chromatograms. The percentage recoveries of preformed and of added amines were similar in every experiment. The results justify the provisional conclusion that the substances extracted from tissues and estimated fluorimetrically are noradrenaline and adrenaline.

Bio-assay. Table 2 shows the result of thirteen simultaneous chemical and biological determinations of noradrenaline and adrenaline in tissue extracts. Although the concentrations of noradrenaline and adrenaline cover a wide range, which has enhanced the significance of the correlation coefficients *r* and *z*, the mean values show excellent agreement. The fact that the mean noradrenaline concentration determined biologically was greater than that from chemical estimation is evidence against erroneously high chemical estimates due, for example, to hydroxytyramine or 3:4-dihydroxyphenylacetic acid. The 'adrenaline percentages' in these experiments were lower and more variable than usual (see Table 3); this was probably due to the extra time

Table 2. *Comparison of biological with chemical estimation of noradrenaline and adrenaline*

Tissue extracts were eluted from alumina columns with acetic acid. Amounts of noradrenaline (N) and of adrenaline (A) are expressed as μmg./g. of tissue. %A is the 'adrenaline percentage'.

	Biological method			Chemical method		
Tissue	N	A	%A	N	A	%A
Heart	330	81	20	310	43	12
	390	49	11	280	71	20
	350	26	6·9	340	50	13
	500	73	13	350	73	17
	290	40	12	180	20	10
	210	160	42	360	130	26
Kidneys	120	25	18	99	9·3	8·6
	210	14	6·4	95	22	19
	100	3·6	3·5	82	14	14
	77	22	22	89	11	11
Liver	24	0·8	3·1	56	1·8	3·1
Diaphragm	380	0·9	0·2	260	4·2	1·6
Skeletal muscle	20	0·7	3·2	30	1·4	4·6
Mean values	**230**	**38**	**12**	**190**	**35**	**12**

	Noradrenaline	Adrenaline	Adrenaline percentage
r and *P*	0·8491 <0·001	0·9134 <0·001	0·6226 <0·02
z and *P*	1·253 <0·001	1·548 <0·001	0·7287 <0·01
t and *P*	3·962 <0·01	4·895 <0·001	2·304 <0·05

needed to extract the necessarily larger amounts of tissue and to assay the extracts biologically. In particular, in the experiment on skeletal muscle, the entire musculature except for diaphragm and head muscles was put through a Latapie mill before being mixed with acid methanol; the estimations on liver and diaphragm suffered a corresponding delay. The experiments were not intended to indicate the true concentrations of noradrenaline and adrenaline in tissues, but to compare two different methods of assay.

Enzymic destruction of catecholamines in liver. Two 2 g. samples of liver were dispersed in 2 ml. of water and incubated for 1 hr. at 37°; afterwards they were diluted with 12 ml. of acid methanol and treated as usual. No fluorescence was detected.

Catalytic oxidation of tissue catecholamines. Twelve eluates obtained from liver, and one from diaphragm, were adjusted with NH_3 soln. to pH 12, and $CuSO_4$ was added to a concentration of 10^{-4}M. Half were kept at 4° for 2 hr. and the others boiled for 15 min. After condensation with ethylenediamine no fluorescence was detected.

Test for interference by coloured tissue components. Unpurified acid-methanol extracts of leg muscles, fat and brain are colourless in contrast to those of the other organs investigated. Acid-methanol extracts of leg muscles, after treatment with *n*-butanol and extraction with HCl, were divided into three parts. One was shaken with the combined highly coloured *n*-butanol residues from spleen and lung after these had been extracted with HCl, and the aqueous layers were concentrated before filtration through alumina. Another was concentrated after addition of a similarly treated *n*-butanol extract from a diaphragm; the remainder was treated as usual and gave slightly higher values for both noradrenaline and adrenaline (43 and 8·0 μmg./g. respectively) than either of the other two (39 and 41 μmg. of noradrenaline/g., 7·3 and 6·4 μmg. of adrenaline/g.). If impurities soluble in *n*-butanol contribute to the relatively high fluorimetric readings of diaphragm and spleen, some of these impurities would be expected to be present also in subsequent extractions of the *n*-butanol with HCl.

Experimental error

Variation of results was investigated by analysing four samples of liver from the same rat, and one sample from each of four different rats on each of 4 days. Analyses of variance showed that variations between different rats were significantly greater when the analyses were carried out on different days than when undertaken simultaneously; when carried out simultaneously, variations between different rats, with the probable exception of noradrenaline concentration, were not greater than variations in different samples from the same rat. Since day-to-day variations were known to occur in the power supply to the fluorimeter, the effect of different lighting conditions was investigated with a voltage regulator (Variac; Claude Lyons Ltd., London) to vary the power supply to the mercury lamp by stages over a range of 125–250v. A photomultiplier tube in conjunction with a galvanometer ('Scalamp'; W. G. Pye and Co. Ltd., Cambridge) replaced the photocell attachment to the Unicam spectrophotometer previously used.

Readings of blank, pure noradrenaline and adrenaline solutions increased as the voltage was increased, and vice versa; the changes were rapid above 200–215v. The readings of the noradrenaline standard varied from 36 to 42 % of those of adrenaline with the yellow filter; with the blue-green filter they varied from 125 to 95 % when the voltage was increased from 125 to 215v; with 240–250v the percentage readings decreased to half or one-third of their previous values, that is, differentiation between adrenaline and noradrenaline was reduced; this was only partly reversed when the voltage was subsequently reduced to 125. With voltages increasing from 125 to 215, the maximum deviation of calculated from theoretical composition of a mixture of 0·1 μg. of adrenaline and 0·2 μg. of noradrenaline was 12 %; but with 240 and 255v there appeared to be no noradrenaline in the mixture; adrenaline estimates were increased. However, the very high readings of all solutions and the reduced differentiation between noradrenaline have never been observed in normal practice; readings usually correspond with those obtained between 200 and 215v in this experiment. Varying light intensity as a cause of day-to-day variation remains an unproven possibility; more recent investigations suggest that the power supply to the units measuring the fluorescence, which was not intentionally varied in the above experiments, may be a source of error. Experimental errors, whatever their cause, are incorporated in the statistical evaluation of results.

Noradrenaline and adrenaline concentrations in rat tissues

Mean concentrations of noradrenaline and adrenaline and mean 'adrenaline percentages' found in nine rat tissues are shown in Table 3. The concentration of noradrenaline found in spleen has been compared with those in the other tissues and the significance of the differences is shown in the table.

Since experiments still in progress have indicated that there may be considerable seasonal variation of the catecholamine contents of heart, kidneys and liver, it should be stated that the values in Table 3 were obtained from analyses carried out mostly in October, but also in September and November. Several tissues were always analysed together, and

if five sets of analyses carried out simultaneously on the different tissues during one of these months only are compared, alterations of the relative values shown in Table 3 are not large (maximum 14%).

DISCUSSION

The method provides good average recoveries of adrenaline and noradrenaline; bio-assay and paper chromatography have provided satisfactory evidence of its specificity under the experimental conditions used; the estimated substances disappeared when means were used which are known to destroy adrenaline and noradrenaline. Although absolute specificity can never be guaranteed, no evidence was obtained that these were not the substances estimated.

Table 4 shows results of other workers for noradrenaline and adrenaline concentrations in rat tissues other than suprarenals; those of Leduc, Dubreuil & D'Iorio (1955), which also were obtained by an extension of the method of Weil-Malherbe & Bone, are in good agreement with those found here. The other noradrenaline concentrations show fair agreement.

Optimum extraction of amines was obtained with concentrations of both sulphuric acid in methanol and of aqueous hydrochloric acid which resulted in homogenates of about pH 5. The poor results obtained with, for example, 10% aqueous trichloroacetic acid, which is in common use for extraction of 'sympathins', may therefore have been due to its lower pH; a 10% solution of this acid in *n*-butanol, in which ionization is

Table 3. *Concentrations of noradrenaline and adrenaline in rat tissues*

Results are given as means ± S.E. with percentage standard errors in parentheses. The last two columns give means of limiting values found in sheep tissues by Euler (1954).

Tissue	No. of estimations	Noradrenaline (μmg./g. of tissue)	Adrenaline (μmg./g. of tissue)	'Adrenaline percentage'	Significance of differences between splenic and other noradrenaline concentrations *P*	Sheep tissues (μmg./g. of tissue) Noradrenaline	Sheep tissues (μmg./g. of tissue) Adrenaline
Heart	25	530±44 (8·2%)	160±17 (10%)	25±1·8 (7·2%)	<0·001	850	150
Brain	11	440±41 (9·5%)	210±7·8 (3·7%)	34±2·7 (7·8%)	<0·001	80	—
Spleen	13	260±33 (13%)	64±8·4 (13%)	22±2·6 (12%)	—	3150	55
Diaphragm	20	220±12 (5·5%)	54±6·5 (12%)	20±2·1 (11%)	<0·3	—	—
Kidney	20	160±17 (11%)	43±5·0 (12%)	22±1·5 (6·8%)	<0·01	500	60
Liver	25	80±6·7 (8·3%)	22±3·1 (14%)	21±1·8 (8·5%)	<0·001	175	9
Retroperitoneal fat	13	69±17 (24%)	19±3·9 (20%)	23±3·3 (14%)	<0·001	—	—
Lung	13	62±6·5 (10%)	23±2·1 (9·2%)	28±2·2 (7·8%)	<0·001	90	6
Leg muscles	20	52±3·9 (7·4%)	15±1·5 (10%)	22±1·9 (8·4%)	<0·001	25	1·3

Table 4. *Concentrations of noradrenaline and adrenaline in rat heart, spleen and liver*

The results of Leduc *et al.* (1955) were given as μmg./g. of nitrogen. Their values have been converted into μmg./g. of tissue by assuming that there were 32 mg. of nitrogen/g. of tissue.

Tissue	Noradrenaline (μmg./g. of tissue)	Adrenaline (μmg./g. of tissue)	Authors
Heart	670 to 720	20 to 44	Goodall, 1951; Hökfelt, 1951
	500	97	Leduc *et al.* 1955
Spleen	410	79	Euler & Hökfelt, 1951
	300	67	Leduc *et al.* 1955
Liver	40 to 59	2	Goodall, 1951; Hökfelt, 1951
	45	21	Leduc *et al.* 1955

reduced, produced much better yields of catecholamines.

Whole organs were usually extracted to avoid possible irregularity due to unequal distribution of adrenaline and noradrenaline within an organ. Unequal distribution has been found in heart (Goodall, 1951) and brain (Vogt, 1954). Since the percentage standard error for liver, only a part of which was taken, was not greater than those of organs taken *in toto*, no evidence was obtained for unequal distribution in this organ. On the other hand, this might be the cause of the larger 'percentage standard errors' of estimations on retroperitoneal fat.

The absolute concentrations of adrenaline and the 'adrenaline percentages' found here are higher than those usually reported (Euler & Hökfelt, 1951; Goodall, 1951; Hökfelt, 1951; Euler, 1951*b*, 1954; Mirkin & Bonnycastle, 1954), but accord fairly well with those found by Leduc *et al.* (1955), and with that found in human blood by Weil-Malherbe & Bone (1953, 1954). The amounts of adrenaline found here have been verified as stated above, and 'adrenaline percentages' were similar with a modification of the method which produced higher absolute values of both amines. 'Adrenaline percentages' were more uniform in different organs than those found by Euler (1951*a*, *b*; 1954) in cattle and sheep. Since adrenaline concentrations are lower than those of noradrenaline, failure to extract adrenaline adequately would result in a ratio which varied unduly; the greater uniformity found here could be taken as further support for the method. Brain had the highest adrenaline percentage of the nine tissues examined; this might be related to the greater efficacy of adrenaline compared with noradrenaline in inducing electrical activity of the hypothalamus (Porter, 1952), an area known to be rich in catecholamines (Vogt, 1954).

The noradrenaline and adrenaline concentrations found here in rats have been compared in Table 3 with the most recent findings of Euler (1954) in sheep; species differences are very obvious in spleen and brain, and in rats the very much higher concentrations in diaphragm than in leg muscles are notable. The noradrenaline contents of organs have been attributed to their contents of adrenergic-nerve fibres (Euler, 1950, 1951*b*, 1954; Euler & Uddén, 1951), partly on account of a correlation between sympathetic innervations and such noradrenaline concentrations as were found by Euler; a similar noradrenaline concentration in all adrenergic-nerve endings of any species is implied. But in rats, injections of insulin (Montagu, in preparation), of ACTH or propylthiouracil (Hökfelt, 1951), injection of adrenaline or feeding of iodinated casein (Leduc *et al.* 1955), which cannot increase the numbers of adrenergic-nerve fibres, each significantly increases the noradrenaline content of some but not of other organs; seasonal changes in noradrenaline distribution may also occur. Either, therefore, noradrenaline is not always confined to adrenergic-nerve fibres or its concentration can be different in different fibres.

Tissue concentrations of noradrenaline vary under many conditions such as thyroid activity, which in most species affect the metabolism of other cells besides neurons (e.g. Euler, 1951*a*, *b*; Euler & Hökfelt, 1951; Euler & Uddén, 1951; Hökfelt, 1951; Leduc *et al.* 1955); noradrenaline unassociated with adrenergic nerves has already been shown in several tissues of different species (brain, gliomas, chromaffin tissues; Blaschko, 1950; Bülbring, Philpot & Bosanquet, 1953; Euler, 1951*a*; Vogt, 1954). The fact that section of the sympathetic innervation of certain organs reduces or abolishes the sympathetic activity of extracts of those organs (kidney, spleen, heart and salivary glands; Cannon & Lissák, 1939; Euler, 1951*b*; Euler & Purkhold, 1951; Goodall, 1951) can be taken as evidence for the fact that adrenergic nerves contain noradrenaline, but could be due in part to removal of an effect of sympathetic innervation on synthesis, uptake or storage of 'sympathin' by surrounding cells.

SUMMARY

1. The method of Weil-Malherbe & Bone (1952, 1953) for estimating adrenaline and noradrenaline in blood has been extended to rat tissues; satisfactory evidence for its specificity has been obtained in a number of ways, including paper chromatography and comparison with bio-assay.

2. Adrenaline and noradrenaline have been estimated in nine rat tissues; mean values, in μmg./g. of tissue, were noradrenaline (adrenaline), heart 530 (160); brain 440 (210); spleen 260 (64); diaphragm 220 (54); kidney 160 (43); liver 80 (22); retroperitoneal fat 69 (19); lung 62 (23); leg muscles 52 (15).

3. The proportion of adrenaline in mixtures of noradrenaline and adrenaline varied from 20 to 34%; it was highest in brain and lung.

4. It is concluded that noradrenaline may not be confined to adrenergic-nerve endings.

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Fractionation of Basic Proteins and Polypeptides

CLUPEINE AND SALMINE

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Interest in the biological action of polypeptides and low-molecular-weight proteins with a high arginine content led to a study of the purity of such compounds. Classical methods of purification have already been used on clupeine, e.g. fractional precipitation or extraction, or both, of the methyl ester hydrochlorides (Felix & Dirr, 1929; Felix & Mager, 1937), of the picrates (Ando, Ishii, Hashimoto, Yamasaki & Iwai, 1952) and of the sulphates (Waldschmidt-Leitz, Kuhn & Zinnert, 1951). Until recently the application to these compounds of methods of purification such as chromatography and countercurrent distribution between solvents had not been described. The present paper describes the application of these techniques to a commercial sample of clupeine leading to the partial separation and identification of the components of clupeine and, to a lesser extent, salmine.

The method of assay used in the present work was based on the modified Sakaguchi–Weber procedure (Weber, 1930) for determination of arginine, but was applied to the whole protein. This method readily lends itself to the routine analysis of the large number of protamine samples obtained by chromatographic and countercurrent fractionation procedures, and has been used in the fractionation studies (to be published) of another polypeptide containing high arginine, namely, licheniformin.

A number of two-phase systems was examined for suitability in countercurrent distribution but only one system, containing *n*-propanol and a concentrated salt solution, was found to give satisfactory fractionations. A similar system for the fractionation of clupeine had been described by Felix (1953), but at the time when our investigations were made we were not familiar with this work. In view of the complexity of the results obtained a brief outline of our main methods and results of fractionation is given here.

A preliminary countercurrent distribution showed the presence of at least two major and several minor components in our crude clupeine sulphate. By the use of a 50 tube machine and a greater number of transfers a distribution curve (Fig. 1) was obtained