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Partition Chromatography of Adrenaline and Noradrenaline

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Since *nor*adrenaline has been shown to occur generally in animal organs and tissues together with adrenaline [1-8] it became desirable to work out some chemical methods to identify and separate the substances from each other and from other catechol derivatives which may occur in the tissues. Using the paper partition chromatography method of Consden, Gordon and Martin [9], James [10] demonstrated a separation of adrenaline, *nor*adrenaline and methyladrenaline with water-saturated phenol as a solvent. Euler and Hamberg [4] demonstrated *nor*adrenaline and adrenaline in suprarenal extracts of cattle, using *n*-butanol saturated with *N* HCl as mobile phase. Goldenberg *et al.*[5], Holton [3] and Engel and Euler [8] found the two catechol derivatives in paper chromatograms of adrenal medullary 'tumour extracts.

This report deals with the results of the chromatographic procedure on paper with *n*-butanol/*N* HCl as a solvent and its application on column chromatography with the purpose of separating and identifying various catechol derivatives.

EXPERIMENTAL

Paper partition chromatography

The paper used for the chromatograms was Grycksbo OB and the apparatus the one described by Consden, Gordon and Martin [9] for small paper strips of 9 × 48 cm. A bigger trough for whole filter papers of 48 × 48 cm was made after the same principles. As solvent *n*-butanol saturated with *N* HCl was used. The butanol used was *n*-butanol purum, which, when compared with the *n*-butanol pro analysi gave the same results. The temperature during the runs has been kept at 23—26° C, the papers having been dried before

at the same temperature. The resolving capacity of some other solvents has been tried. *n*-Butanol/H_2O, pyridine, *n*-amyl alcohol/0.5 *N* HCl did not give any separation of the catechols. With *n*-butanol/*N* HCl a good separation of adrenaline and *nor*adrenaline was obtained at 20—24 h in descending runs, allowing in longer runs the separation of other related compounds in addition, such as DOPA and hydroxytyramine. Addition of acetic acid shortened the separation time but tended to spread the spots. About 30 hours ascending run gave small distinct spots with *n*-butanol/*N* HCl at about 25° C, and the necessary amounts of substance could be somewhat smaller than in a descending run.

The adrenaline and *nor*adrenaline used in the experiments was pure synthetic adrenaline (Rhône-Poulenc), *dl-nor*adrenaline and *l-nor*adrenaline hydrochloride (Winthrop). The substances were dissolved in 0.1 *N* HCl and H_2O to give solutions of 5 mg pr ml and were placed on the paper with small capillary tubes to give spots of about 2—4 mm diameter. The amounts used for each spot were 5—10 μg. Hydroxytyramine and dihydroxyphenylalanine (DOPA) were used in some experiments.

When using *n*-butanol/*N* HCl it was not possible to calculate the common *R*-values because the solvent front will disappear at the developing time used. After drying for 20—30 min. at 60—80° C the papers were sprayed with a weak potassium ferricyanide solution of pH 7.7 (James [10]), which indicates the catechols by oxidizing them to coloured quinone compounds. Adrenaline gives a bright red spot and can be clearly distinguished from the bluish red spot given by *nor*adrenaline. The position of adrenaline and *nor*adrenaline are shown in Fig. 1 together with hydroxytyramine and DOPA. The latter two substances also give differently coloured spots as reported by Goldenberg *et al.*[5] with phenol as solvent. By using ninhydrin as an indicator DOPA may be distinguished from the other catechols.

Extracts of animal tissues

The extracts used in the chromatographic analysis have been prepared either by adsorption on alumina[11], or with acidulated alcohol, and suitably concentrated. The amount of catechols present in the extract was determined colorimetrically by the method of Euler and Hamberg [12], or biologically (Euler [1]). By laking the "spots" before developing and biological assay of the extracts it has been possible to verify the identity of the separated compounds. Press juice from the suprarenals may be used directly owing to its high catechol content.

If salts are present the extract may be purified by extraction with butanol in the same way as described later for the column chromatography. Ascorbic

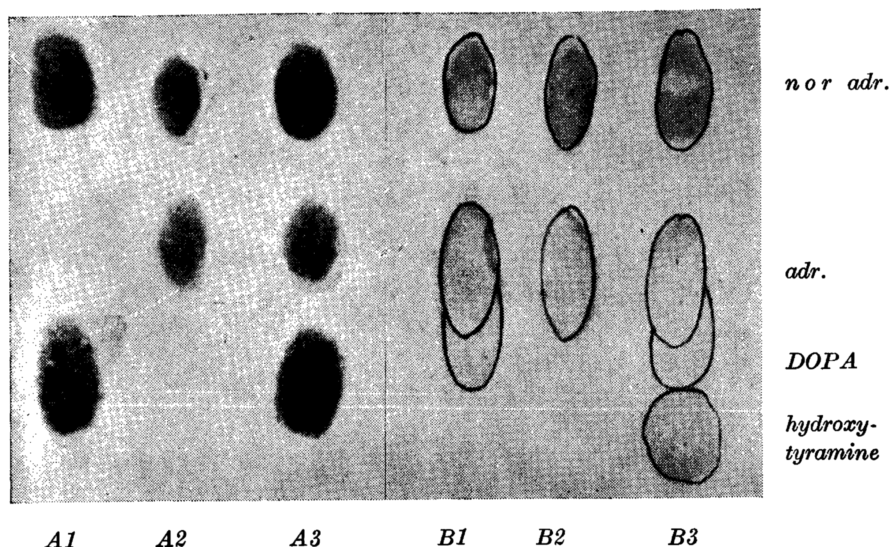

Fig. 1. Paper chromatogram showing A1 separation of n o r adrenaline and hydroxytyramine, A2 n o r adrenaline and adrenaline from suprarenal extract of cattle, A3 n o r adrenaline, adrenaline and hydroxytyramine, B1 n o r adrenaline, adrenaline and DOPA, B2 n o r adrenaline and adrenaline, B3 n o r adrenaline, adrenaline, DOPA and hydroxytyramine, n-butanol/NHCl being used as solvent. Descending run 24 hours at 25° C.

acid present in animal extracts is also adsorbed on alumina and gives spots after developing with potassium ferricyanide. The runs of animal extracts were generally made with three comparative spots: 1) the extract to be analyzed. 2) the comparating substances. 3) 1 + 2. Fig. 1 illustrates also the separation of adrenaline and *nor*adrenaline from cattle suprarenal extract.

Partition chromatography on starch column

Starch (Elsden and Synge [13]) has been used as support for the partition chromatography of adrenaline and *nor*adrenaline in the present series of experiments.

Method

The apparatus used was the one introduced by Edman [14] somewhat modified after the principles given by Moore and Stein [15]. A separatory funnel of 500 ml was connected with a stopper to a column of about 50 cm length and about 2 cm diameter containing a sintered glass filter (Pyrex X). Air pres-

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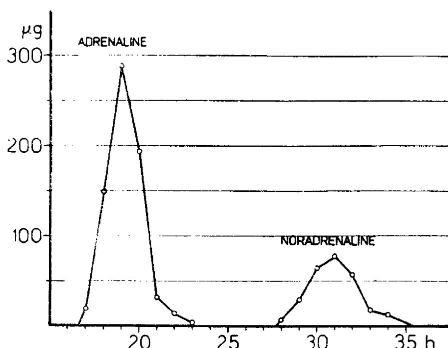

Fig. 2. Partition chromatogram of adrenaline and n o r adrenaline obtained from pure solutions on starch. R adrenaline = 0.544, R n o r adrenaline = 0.350. Amounts added 750 µg adrenaline and 250 µg n o r adrenaline (Exp. IV in Table 1).

sure, controlled by a Hg-manometer, was applied to the separatory funnel. To collect the fractions 24 bottles of 20 ml supplied with funnels (to reduce the evaporation) were placed on the round plate and automatically shifted every hour.

The starch used in the experiments was raw commercial starch which was purified from fats and alcohol-soluble matters by extracting one day in a Soxhlet with methanol. The starch was dried over night at + 60° whereafter it was sieved and kept in air.

The solvent used was *n*-butanol saturated with 0.1 *N* HCl to which one half of the volume 10 *N* HAc was added. Ascorbic acid was added in about 0.1 % to the solvent in order to protect the catechols from oxidation. The strongly acid solvent used in the paper chromatography was found to cause partial racemisation on evaporation of the extracts in vacuo, thereby reducing the biological activity of the catechols. Acetic acid was found useful in shortening the runs and in having a good resolving power.

60 g of starch was suspended in some solvent and pressed together in the column to a length of about 27 cm. Air pressure of 60—160 mm Hg was used to give fractions of about 6—10 ml per hour.

The separation of adrenaline and *nor*adrenaline was usually obtained in about 45 hours' run. The solution or extract to be analyzed, dissolved in 1—2 ml of the solvent, was pressed into the starch with a pressure of 80—160 mm Hg and followed by another 2 ml of the pure solvent. The column above the starch was filled with solvent until about 10 cm from the top. The fractionation was started immediately. The measured fractions were evaporated in a vacuum box at + 35° C. After dissolving the evaporated residue in 1—2 ml H_2O, which gave a pH of about 3, each fraction was tested colorimetrically after oxidation with iodine [4]. The fractions were also tested biologically on the cat's blood pressure and on the hen's rectal cæcum.

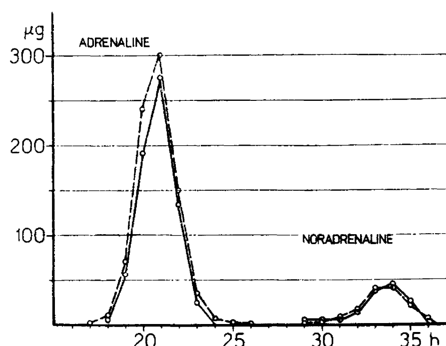

Fig. 3. Partition chromatogram of adrenaline and n o r adrenaline obtained from cattle suprarenal extract on starch. R adrenaline = 0.545, R n o r adrenaline = 0.350. Full line: colorimetrical estimation, dotted line biological assay on cat's blood pressure.

Separation of adrenaline and n o r adrenaline from pure solutions on starch.

The results of four experiments with varying amounts of adrenaline and *nor*adrenaline directly dissolved in the solvent are given in Table 1.

Table 1. Chromatographic separation of adrenaline and n o r adrenaline from pure solutions on starch column.

Expt.	μg added Adr. + *Nor*adr.	Recovered after separation μg		Percentage recovery	
		Adr.	*Nor*adr.	Adr.	*Nor*adr.
I.	400 + 500	287	389	72	80
II.	400 + 500	348	508	87	102
III.	750 + 250	634	196	84	72
IV.	750 + 250	698	265	93	106

Figures refer to the hydrochlorides. *l-nor*adrenaline was used in expts. I and II, *dl*-noradrenaline in expts. III and IV.

Fig. 2 shows a diagram of the separation.

The *R*-values calculated from expt. IV are for the synthetic hydrochlorides of adrenaline: 0.545 and *nor*adrenaline: 0.350. The results of the colorimetric assays were checked biologically and showed good agreement.

Extracts of cattle suprarenals on starch

The extracts used were prepared from fresh cattle suprarenals with acid alcohol to 5 g/ml. After colorimetric determination of the adrenaline and *nor*-adrenaline present, a suitable amount containing about 10 mg of catechols (20—30 % *nor*adrenaline) was mixed twice with *n*-butanol containing 0.1 m

N-HCl in 25 ml n-butanol and evaporated *in vacuo*, in order to remove water and precipitate the salts. The butanol extract thus obtained was shaken three times with 2 volumes of water which quantitatively transferred the catechols into the water phase. The butanol treatment generally entailed a loss of some 10 p. c.

The treatment with butanol did not induce any gross chemical changes of adrenaline and *nor*adrenaline as shown on the paper chromatogram. The butanol extract was filled up to 10 ml with butanol containing some drops of 0.1 N HCl. Of this extract 1 ml was used to the column. The extracts were run on the column in exactly the same way as the pure substances described above. The first few fractions contained some coloured matter but no catechols. The partition chromatogram obtained from cattle suprarenal extracts (expt. IV) is shown in Fig. 3. It was confirmed by colorimetrical and biological tests that the first curve represents the adrenaline and the second one the *nor*-adrenaline as indicated by the R-values. Moreover, the quantitative agreement between the colorimetrical estimation and the biological assay was quite satisfactory as illustrated by the Fig. 3. The total yield of adrenaline from the partition chromatograms of cattle suprarenal extracts was 83—90 p. c. of the amount added and of *nor*adrenaline 57—61 p. c.

Comment

Certain catechol derivatives such as DOPA do not easily separate from adrenaline with n-butanol/N HCl as solvent. The colour of DOPA after indicating the spots with potassium ferricyanide is not much different from adrenaline but is easy to separate from hydroxytyramine. After the drying procedure DOPA changes and gives a similar quinone colour as hydroxytyramine. In phenol the separation of the catechols gives another picture according to James [10], DOPA appearing between *nor*adrenaline and adrenaline. This difference is therefore of great use for the identification of DOPA if present in extracts. This has recently been achieved by Goodall [16] who found the characteristic spots of DOPA and hydroxytyramine with extracts of sheep suprarenals.

SUMMARY

1. With n-butanol/N hydrochloric acid as solvent adrenaline and *nor*-adrenaline and related compounds can be separated by paper chromatography.

2. By partition chromatography on starch column adrenaline and *nor*-adrenaline have been separated in cattle suprarenal extracts, n-butanol/0.1 N hydrochloric acid/acetic acid being used as solvent.

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