Finally, it is unlikely that the discordant results in the literature on chlorpromazine activation of the adrenal cortex can be due to differences in the absolute quantity of drug administered. Over the wide range of body weights employed in this study there appeared to be no correlation between absolute quantity of administered drug and any of these indices of adrenocortical activation.

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Catecholamine biosynthesis *in vivo*: An application of thin-layer chromatography*

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ALTHOUGH thin-layer chromatography has been used previously in the separation of epinephrine and norepinephrine[1, 2] and also of various epinephrine metabolites[3, 4] the application of this technique to the separation of norepinephrine and its biosynthetic precursors, dopa and dopamine, has

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not previously been reported. This paper describes a method for the rapid, complete separation of dopa, dopamine, and norepinephrine, by means of thin-layer chromatography, and also the application of the method in a study of norepinephrine biosynthesis after the injection of titrium-labeled dopa.

Chromatoplates were prepared by coating glass plates (20 cm × 20 cm; by means of a Desaga/Brinkmann model S11 adjustable spreader) to a depth of 0·4–0·5 mm with a suspension of 15 g MN-cellulose powder 300 G (containing approximately 1·5 g $CaSO_4.1/2H_2O$) in 88 ml water. Routinely 1 μg dopa-^{3}H,† 1 μg dopamine-^{3}H,† and 0·01 μg norepinephrine-^{3}H† (the latter with 1 μg norepinephrine), each in a volume of 0·5–1·0 μl, were applied to a plate. During the application of the materials, the solvent was evaporated under a gentle stream of nitrogen, and the spot diameter was maintained at 2–3 mm unless tissue extracts were applied, when the diameter was 5–7 mm. Of the several solvent systems[5] tested initially, the following gave the best bidimensional separation of dopa, dopamine, and norepinephrine and were used subsequently; (1) methanol: *n*-butanol: benzene: water (4:3:2:1); (2) acetone:*t*-butanol:formic acid:water (180:180:1:39). Both solvent systems contained 0·01% of the sodium salt of ethylenediamine tetraacetic acid (NaEDTA), and were prepared shortly before each run. Bidimensional development was carried out with solvent (1), followed by solvent (2). The total time necessary to develop both solvents 17 to 18 cm was between 3 and 3·5 hr. The indicator spray used to detect the catecholamine and dopa spots after development consisted of a mixture of potassium ferricyanide (0·1 g), ethylenediamine (5 ml), absolute ethanol (45 ml), and water (50 ml).

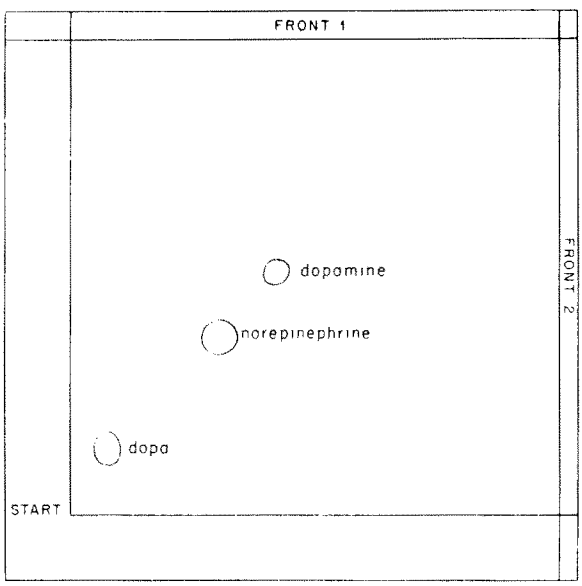

FIG. 1. Location of dopa, dopamine, and norepinephrine on thin-layer plate after bidimensional development (see text).

After spraying, the chromatoplate was placed either in an oven at 50°–60° for 3·5 min, or under a stream of warm air (55°) for the same length of time. After this treatment, the areas where each substance was concentrated appeared as highly fluorescent spots when observed under a portable Mineralight with a long-wave u.v. filter. The location of the spots after a typical run is shown in Fig. 1. After development and location of the spots, the chromatoplate was lightly sprayed with water

† Dopa-2,3,-^{3}H (dihydroxyphenylalanine, 50·2 mc/mmole) and dopamine-β-^{3}H (dihydroxyphenylethylamine, 25·4 mc/mmole) obtained from Tracerlab; DL-norepinephrine-7-^{3}H as the hydrochloride (7·6 c/mmole) purchased from the New England Nuclear Corp. were used.

‡ Woelm alumina, neutral grade, was employed.

and the spots removed with a spatula and added directly to 10 ml phosphor.[6] This mixture was shaken at room temperature for 6–8 hr and its radioactivity determined in a Packard Tri-Carb liquid scintillation spectrometer having an efficiency for tritium of 25%. Quenching (tritiated water internal standards) was found to be between 1% and 3%.

Table 1 presents the R_F values of the materials investigated, the sensitivity of the detection reagent for each compound, and the recoveries of the materials when developed bidimentionally under the conditions described above. Although the mobility of each substance was found to be influenced by the normal variables (for example, the thickness of the absorbent, the amount of material applied, and the degree of saturation of the developing chamber[7]), the R_F values nevertheless were quite reproducible. That binding of the amines to cellulose could account for the low recovery figures (Table 1) is supported by the following observations. (1) Extraction of labeled material from the

TABLE 1. R_F VALUES OF SUBSTANCES INVESTIGATED

	R_F Values × 100		Sensitivity* to detection reagent (μg)	Recovery after bidimentional development† (%)
	Solvent (1)	Solvent (2)		
Dopa	11–14	6–7	0·05	20, 23 (n=2)
Dopamine	46–51	42–49	0·05	21–26 (n=4)
Norepinephrine	32–37	30–37	0·02	18–20 (n=4)

* Minimum amount easily detectable (see text) after bidimensional development.

† $\frac{\text{Radio-activity extracted from spot}}{\text{Total radioactivity applied to plate}} \times 100.$

cellulose was more complete in 0·2 M hydrochloric acid than in either phosphor or water. (2) Recovery of each substance from a single spot (with no solvent development) decreased as the time between application and extraction increased. (3) Recovery of the labeled material increased as the extraction time increased (i.e. the spot from the chromatoplate shaken for longer periods in 10 ml phosphor); thus, during the first 4 hr of shaking, the amount extracted increased by 16% for norepinephrine, 24% for dopa, and 11% for dopamine. After 8 hr of shaking, the *further* increase in extracted material was 4% for norepinephrine, 1% for dopa, and 1% for dopamine. (4) The recovery of labeled material consistently was less after bidimentional than after unidimensional development, although the time of contact between catecholamine and cellulose was maintained constant in both cases at 3 to 3·5 hr; when the substances were applied to the plates and left in contact for the same time *without* solvent development, recovery was highest of all (50–70%).

The following results are presented to illustrate the application of the above method to the investigation of catecholamine biosynthesis. A male albino Sprague-Dawley rat (220 g), treated 16 hr earlier with iproniazid (100 mg/kg, i.p.), was given 0·71 mg dopa-^{3}H (3·23 mg/kg) via the tail vein. Ten minutes later the animal was killed by decapitation; the heart, brain, and a portion of the spleen were removed, washed thoroughly in ice-cold isotonic saline, blotted, and weighed. A sample of blood was also taken and mixed with 0·1 volume of 4 M perchloric acid and 0·01 volume of 1% NaEDTA, and stored in ice for 30–40 min. The tissue samples were cut into small pieces and homogenized in 4 volumes of 0·4 M perchloric acid containing 0·01% NaEDTA, by means of a glass homogenizing tube with a ground-glass pestle. The resulting homogenates and the acidified blood sample were centrifuged for 15 min in an International clinical centrifuge. The supernatant solutions were separated and adjusted to pH 8·4 by the careful addition of 4 N sodium hydroxide (to pH 2) followed by 0·5 M sodium carbonate and finally by 0·1 M sodium carbonate. Aliquots (1·6–2·0 ml) of the tissue extract at pH 8·4 were passed over alumina‡ columns (0·5 cm × 25 cm; 0·6 g). The columns were washed with 0·5 ml 0·2 M sodium acetate buffer, pH 8·4, followed by 0·5 ml water. Dopa, dopamine, and norepinephrine were eluted from the columns with 2·0 ml of 0·2 M hydrochloric acid. The recovery of radioactivity from the alumina columns was 95%; 68% of the total radioactivity was present in the hydrochloric acid eluate. After adding 0·02 ml of 1% NaEDTA to the hydrochloric acid eluates, aliquots of 50 μl were applied to the chromatoplates along with 1μg each of dopa,

dopamine, and norepinephrine and developed as described above. Although significant radioactivity was obtained by extraction of each of the resulting spots after development, it was felt that the application to the plates of a more concentrated hydrochloric acid eluate from the alumina column would yield higher count values for each spot and therefore would give more reliable data. Accordingly, the hydrochloric acid eluates described above were evaporated to dryness in the frozen state and then brought to a final volume of 0·2 ml with 0·2 M hydrochloric acid. Aliquots (50 μl) of these solutions, chromatographed in the same manner, gave counts (cpm-background) ranging from 87 to 2,200 for the resulting spots. The relative distribution of radioactivity among the dopa, dopamine, and norepinephrine spots was very similar whether the original or concentrated acid eluate was applied to the plate, indicating that no measurable destruction of the compounds had occurred during the freeze-drying process. The recovery of radioactivity from the spots after development of the chromatoplate was 21%, which is in accord with the recoveries of the pure materials (see Table 1). About 5% of the total radioactivity applied ot the plates was found in the solvent front after development in solvent (1) and about 2% remained at the origin. No significant amount of radioactivity was found in spots taken from other areas on the plate.

The results of this experiment are presented in Table 2 and demonstrate the conversion of dopa-^{3}H to dopamine-^{3}H and norepinephrine-^{3}H in the various tissues and in the blood. These data are in

TABLE 2. THE BIOSYNTHESIS OF DOPAMINE AND NOREPINEPHRINE FROM INTRAVENOUSLY INJECTED DOPA

Tissue	Dopa	Dopamine	Norepinephrine
	(counts per minute/g tissue)*		
Heart	48,923	30,799	13,448
Spleen	105,348	4,196	14,122
Brain	9,025	8,325	8,633
Blood	77,077	29,801	36,415

* Values corrected for the average recovery of each material from the plate (see Table 1).

agreement with the postulated biosynthetic pathway leading from dopa to norepinephrine[8] and confirm the findings of Burack and Draskoczy[9] who reported a rapid turnover of catecholamines *in vivo*.

The data presented in this paper indicate that thin layer chromatography has definite application as a convenient and reliable investigational tool in biochemical or pharmacological research involving catecholamines.

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