

## Catecholamines in Rat Tissues and Serum determined by Gas Chromatographic Method

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Nanogram order determination of catecholamines in rat tissues and serum was achieved. The recovery of added catecholamines to the acid homogenates was about 50%. The presence of epinephrine in brain was confirmed. The amount of dopamine was unexpectedly low in lung.

The concentration of catecholamines (CA) in biological materials mostly have been measured by fluorometry with THI method. Anton and Sayre<sup>2)</sup> determined CA in tissues of many species by this method.

Gas chromatographic method established by Kawai and Tamura<sup>3)</sup> is selective and sensitive and has been extended to be able to detect a low amount of materials.<sup>4)</sup> We report in this paper on the determination of CA in rat tissues and serum by gas chromatography.

### Experimental<sup>5)</sup>

**Apparatus and Condition**—A Shimadzu Model GC-4AP gas chromatograph equipped with an electron capture detector and with glass tubes sufficient for making columns of 0.1 m (pre) and 2 m (main)<sup>4)</sup> in length and 4 mm in diameter. The column packing was 2% GE-XF 1105 on Gas Chrom P (60—80 mesh). The gas chromatography parameters were as follows: injection temperature, 170°; column temperature, 150°; detector temperature, 170°; nitrogen flow rate, 100 ml/min.

**Isolation of CA**—The tissues were obtained from decapitated male albino rats (Donryu) weighing 250—300 g. Some portions of the tissues were homogenized with 0.4N perchloric acid in a glass homogenizer and centrifugated at 20000 × g at 5° for 30 min. In the case of serum, 0.04 ml of 60% perchloric acid was added to 1 ml of serum. The supernate was extracted two times with an equal volume of ether-benzene (5:2 v/v) to remove lipids.<sup>6)</sup> To the water layer were added 1 ml of 0.2M EDTA and pH of it was adjusted to about 6 with 4N ammonia. Two hundred mg of acid washed alumina<sup>7)</sup> was added to the solution and the pH of it was adjusted to 8.8 with 4N ammonia. The solution was stirred mechanically with a glass rod for 10 min. Then the alumina was taken and adsorbed CA on alumina was eluted with 0.8 ml of 0.2N acetic acid in methanol.

**Preparation of Trifluoroacetyl Derivatives**—The eluate above mentioned was evaporated with a flush evaporator to dryness. For the sake of complete evaporation of the solvent three drops of methanol were added and the solution was evaporated, dried over anhydrous calcium chloride *in vacuo* for 10 min. The residue was trifluoroacetylated with 20 μl of diluted trifluoroacetic anhydride in ethyl acetate (1 to 10, prepared in need). Five minutes later 0.5 ml of *n*-hexane containing 2.3 ng of isodrin (an internal standard) was added to the mixture and 80 μl of the solution was injected with a syringe to a gas chromatograph.

1) Location; *Hongo 7-3-1, Bunkyo-ku, Tokyo.*

2) A.H. Anton and D.F. Sayre, *J. Pharmacol. Exptl. Therap.*, **138**, 360 (1962).

3) S. Kawai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **16**, 699 (1968); *idem, ibid.*, **16**, 1091 (1968).

4) K. Imai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **17**, 1071 (1969).

5) All the reagents were GR and redistilled.

6) J.B. Boylen and J.H. Quastal, *Biochem. J.*, **80**, 644 (1961).

7) Alumina (Woelm Co., Ltd.) was immersed in 2N hydrochloric acid and heated on a gas burner for 1 hr under mechanically stirring with a glass rod. The supernate was decanted and the alumina was washed with distilled water about 20 times, suctioned and dried at 120° overnight.

### Result and discussion

The chromatograms obtained from brain, lung, heart, spleen, adrenal, kidney and serum are shown in Fig. 1a to 1g. Each chromatogram corresponds to the amounts of the samples cited in the parentheses.

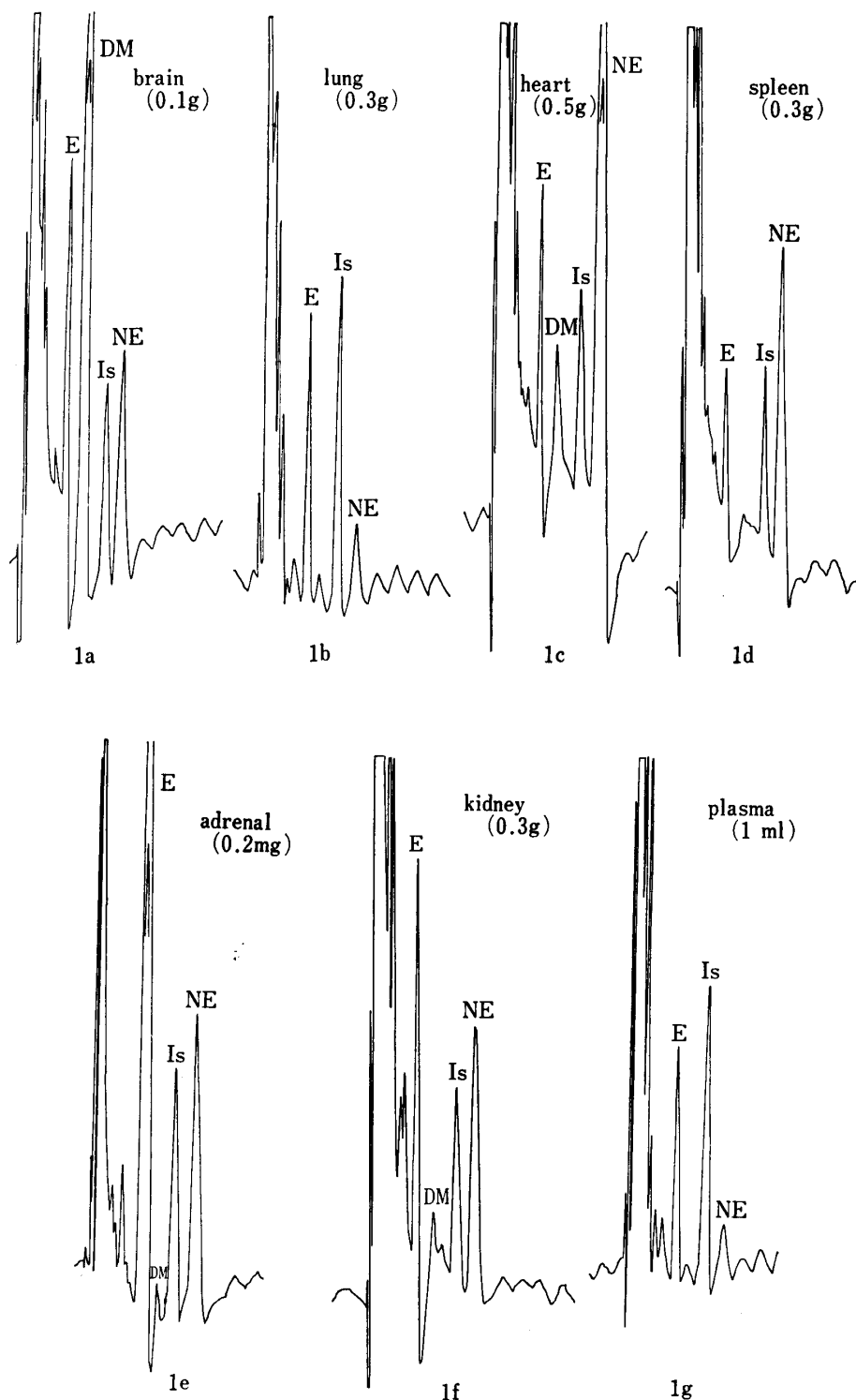


Fig. 1. Gas Chromatograms of Catecholamines in Several Tissues and Serum of Rat

E: epinephrine, DM: dopamine, NE: norepinephrine, Is: internal standard (isodrin)

The recovery tests were performed with the addition of an appropriate volume of 0.1N acetic acid solution of authentic samples to the perchloric acid homogenates before centrifugation. The overall recovery was about 50% (St. Dev. 6.5%) in any tissue or serum. However, in the case of thymus and liver, interfering substances could not be removed and the good results were not obtained.

The corrected values are shown in Table I. The concentrations are almost identical with those reported by Anton and Sayre<sup>2)</sup> who used THI method, except those of brain and lung.

TABLE I. Corrected Values of Catecholamines in Rat Tissues and Serum

Serum or tissues	Brain (5) <sup>a)</sup>	Lung (4) <sup>a)</sup>	Heart (3) <sup>a)</sup>	Spleen (5) <sup>a)</sup>	Kidney (2) <sup>a)</sup>	Adrenal (7) <sup>a)</sup>	Serum (7) <sup>a)</sup>
Epinephrine ng/g	150	48	40	37	9.6	520 $\mu$ g/g	8.8 ng/ml
Norepinephrine ng/g	810	44	250	440	100	190 $\mu$ g/g	6.3 ng/ml
Dopamine ng/g	570	2.7	9.7	7.8	3.2	4.6 $\mu$ g/g	2.2 ng/ml

a) Each value is the average of several experiments which numbers are cited in the parentheses.

Since the suggestion by Euler<sup>8)</sup> was made that Sympathin exists in brain, many investigations have been made on the problem. The ratio of the quantity of epinephrine to the sum of the amounts of epinephrine and norepinephrine in rat brain was reported 4.5% by Gunne<sup>9)</sup> and 34% by Montagu<sup>10)</sup> using fluorometric method. On the other hand, Sano and Taniguchi<sup>11)</sup> found no epinephrine in brain, using the column of Amberlite IRC 50 for the separation of the amines and THI method. Carlsson<sup>12)</sup> and McGeer and McGeer<sup>13)</sup> also did not detect by fluorometry. Probably the large amounts of dopamine in brain interfere with the measurement of epinephrine.<sup>2)</sup> Chromatogram (Fig. 1a) showed the presence of epinephrine in brain. The per cent of epinephrine to the sum of epinephrine and norepinephrine is about fifteen per cent. Presence of epinephrine was further confirmed by comparison of the retention times of the peak on columns of 2% GE-XF 1105 and 2% QF-1 with those of authentic epinephrine.

It is interesting that the chromatogram (Fig. 1b) showed a low existence of dopamine in lung although the dominant catecholamine in it was said to be dopamine.<sup>14)</sup>

Further study on catecholamines in rat is under continuation.

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9) L.M. Gunne, *Ann. N.Y. Acad. Sci.*, **96**, 205 (1962); *idem*, *Acta Physiol. Scand.*, **56**, 324 (1962).

10) K. Montagu, *Biochem. J.*, **63**, 559 (1956).

11) I. Sano and K. Taniguchi, *Klin. Waschr.*, **38**, 57 (1960).

12) A. Carlsson, *Pharmacol. Rev.*, **11**, 490 (1959).

13) E.G. McGeer and P.L. McGeer, *Can. J. Biochem.*, **40**, 1141 (1962).

14) H.J. Schümann, *Arch. Exptl. Path. Pharmac.*, **234**, 282 (1958).