A SIMPLE TECHNIQUE INVOLVING SOLVENT EXTRACTION FOR THE ESTIMATION OF NOREPINEPHRINE AND EPINEPHRINE IN TISSUES

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In the course of planning studies on the metabolism of norepinephrine in brain and the changes induced by various drugs, it became apparent that a simple but sensitive technique that could be used routinely for the rapid assay of norepinephrine in brain and other tissues would be of great benefit.

By utilizing the finding that norepinephrine and epinephrine have a surprisingly high n-butanol-aqueous acid partition ratio when the aqueous phase is salt-saturated, such a method was developed (9). Upon extraction with 10 volumes of n-butanol, about 65% of these catecholamines is removed from salt-saturated, acidified tissue homogenates. The amines are quantitatively returned to an aqueous phase by the addition of n-heptane to the butanol and

TABLE 1

Norepinephrine levels (average) in various rabbit tissues

Tissue	Concentration
	μg/g
Brain stem	0.50
Cerebrum	0.19
Cerebellum	Trace
Liver	0.11
Lung	0.08
Kidney	0.17
Spleen	0.69
Intestine (small)	0.30
Gastric mucosa	0.19
Heart	2.0
Aorta	0.85

shaking with dilute HCl. The catecholamines are then estimated fluorimetrically following conversion to highly fluorescent trihydroxyindoles (3). When the fluorescence at 500 mμ resulting from excitation at 400 mμ is measured in an Aminco-Bowman or Farrand spectrofluorometer, the technique is specific for these catecholamines. Norepinephrine can be distinguished from epinephrine by utilization of the differing pH requirements for oxidation (4).

The method of solvent extraction followed by oxidation has been used successfully to measure norepinephrine in brain, heart and other tissues (2, 6, 7, 8). The average levels of norepinephrine found in various rabbit tissues by this technique are shown in Table 1 (10).

By using a special microcuvette and adapter in the Aminco-Bowman spectro-

fluorometer, and only one-tenth the volume of homogenate, solvents and reagents described for the semi-micro method, the sensitivity of the method is increased tenfold. With this adaptation, it has been possible to study the distribution of norepinephrine in various parts of the brain. Thus as little as 50 mg of brain tissue containing as little as 0.03 μg of catecholamine can be analyzed. The distribution studies will be published elsewhere.

It has been found (5) that serotonin, as well as norepinephrine, can be estimated quantitatively by using the norepinephrine-type extraction procedure followed by fluorimetric estimation of the serotonin by the method of Bogdanski *et al.* (1). This has proven of great convenience for the simultaneous assay of these amines.

REFERENCES

1. Bogdanski, D. F., Pletscher, A., Brodie, B. B. and Udenfriend, S.: Identification and assay of serotonin in brain. J. Pharmacol. **117**: 82-88, 1956.
2. Brodie, B. B., Olin, J. S., Kuntzman, R. and Shore, P. A.: Possible interrelationship between release of brain norepinephrine and serotonin by reserpine. Science **125**: 1293-1294, 1957.
3. Lund, A.: Fluorimetric determination of adrenaline in blood. III. A new sensitive and specific method. Acta pharm. tox., Kbh. **5**: 231-247, 1949.
4. Lund, A.: Simultaneous fluorimetric determinations of adrenaline and noradrenaline in blood. Acta pharm. tox., Kbh. **6**: 137-146, 1950.
5. Mead, J. A. R.: Unpublished observation.
6. Pletscher, A.: Wirkung von Isopropylisonikotinsäurehydrazid auf den Stoffwechsel von Catecholaminen und 5-Hydroxytryptamin im Gehirn. Schweiz. med. Wschr. **87**: 1532-1534, 1957.
7. Pletscher, A.: Einfluss von Isopropylisonikotinsäurehydrazid auf den Catecholamingehalt des Myokards. Experientia **14**: 73-74, 1958.
8. Shore, P. A., Cohn, V. H., Highman, B. and Maling, H.: Distribution of norepinephrine in the heart. Nature, Lond. **181**: 848-849, 1958.
9. Shore, P. A. and Olin, J. S.: Identification and chemical assay of norepinephrine in brain and other tissues. J. Pharmacol. **122**: 295-300, 1958.
10. Shore, P. A. and Cohn, V. H.: Unpublished observations.