Transport of Epinephrine and Norepinephrine in Human Plasma.* (23632)

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(Introduced by G. W. Thorn)
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Plasma proteins may interact with other proteins, with ions, and with small molecules. The present communication concerns the transport of epinephrine (E) and norepinephrine (NE) in human plasma and their binding with plasma proteins.

Materials and methods. The blood employed was collected either in ACD† solution or through cationic exchange resins (Dowex-50) in the ADL-Cohn Blood Fractionator(1). The cold ethanol Method 6, described by Cohn and his collaborators, was employed for the fractionation of the plasma pools(2). The separated fractions (Table I) were dissolved or suspended in the smallest possible volume of 0.15 M sodium chloride and were dialyzed for a limited period of time (2 to 3 hours) against 0.15 M sodium chloride at a pH approximately 6.0, at 2°C. Chemical determinations for E and NE were performed with the method of Weil-Malherbe and Bone(3) as modified by Aronow(4). Bioassays for E and NE were performed by a modification of the method described by Gaddum and Lenbeck(5). The bioassay is based on the quantitative inhibition by the catechol amines of the contraction induced with acetylcholine in an *in vitro* preparation of rat colon and rat uterus. The concentration of the fractions, tested with the bioassay, over the original plasma volume was 8 to 10 fold.

Results. Two ACD and 2 resin-collected plasma pools were fractionated within 24 hours from the collection of the blood. The separated fractions, after a limited dialysis, were stored in the frozen state at –5°C.

Table I shows distribution of E and NE among the plasma fractions. It is suggested, from the results, that the E is almost completely bound to human plasma proteins and only a trace is unbound (supernatant fluid V). The protein responsible for the major binding and the transport of E is the albumin, which is present in Fraction V. In one case Fraction IV-4, determined by the chemical method, also was found active. The NE is also bound to albumin of Fraction V; however, an appreciable amount of NE seems to be unbound (supernatant fluid V).

Fraction V was subjected to acid hydrolysis by boiling for 15 minutes at pH 1. The amount of E and especially NE after the acid hydrolysis was found significantly increased, as determined with the chemical assay. Duplicate determinations were used in these experiments. The amount of E before the acid hydrolysis was found to be .8 μg per liter of original plasma; after the acid hydrolysis it was 1.0 μg per liter of plasma. The amount of NE before the acid hydrolysis was 2.0 μg per liter of plasma and 8.0 μg after the acid hydrolysis. It is of interest to notice that the amount of NE of Fraction V determined after the acid hydrolysis by the chemical method is very close to the amount in the untreated Fraction V as determined by the bioassay method (9.4 μg/liter of plasma).

Summary. Plasma pools collected in ACD solution or through cationic exchange resins were fractionated with the cold ethanol Method 6 of plasma fractionation, and the separated fractions were analyzed for E and NE, chemically and biologically. Although the E is almost completely bound to plasma proteins, an appreciable amount of NE seems to be unbound. The plasma protein responsible for the binding and the transport of both E and NE is in the albumin. A part of E possibly binds also with Fraction IV-4. The increase of E and NE activity following acid

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† ACD solution was prepared according to NIH Formula A: 8 g citric acid • 1 H_2O; 22 g dextrose (anhydrous); 26 g sodium citrate • 2 H_2O; made up to 1 liter with distilled water. Five hundred ml of blood is collected into 75 ml of the above solution.

TABLE I. Distribution of Epinephrine and Norepinephrine in Human Plasma Fractions.

Plasma fractions	Epinephrine (μg/l of plasma) Chemical method Exp. 1*	Exp. 2†	Bioassay Exp. 3‡	Norepinephrine (μg/l of plasma) Chemical method Exp. 1§	Bioassay Exp. 2‡
Plasma control	2.1		Incr. contr.	5.4	Incr. contr.
Precipitate I	.2	.1	Inactive	.1	Inactive
II + III	.2	.2	"	.2	"
IV-1	.2	.2	"	.2	"
IV-4	.2	.6	"	.16	"
V	.9	1.0	.62 .8	.85	9.4 2.0‖
Supernatant fluid V	.16		Trace	1.2	2.38

* Resin-collected plasma (6 donors). ‖ Determined by chemical method.
† ACD- " " (10 ").
‡ Resin- " " (9 ").
§ ACD- " " (5 ") one wk old.

hydrolysis suggests the presence of a conjugated form, binding to albumin, which is released by acid hydrolysis.

1. Tullis, J. L., Surgenor, D. M., Tinch, R. J., D'Hont, M., Gilchrist, F. L., Driscoll, S., Batchelor, W. H., *Science*, 1956, v124, 792.

2. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., Taylor, H. C., *J. Am. Chem. Soc.*, 1946, v68, 459.

3. Weil-Malherbe, H., Bone, A. D., *Biochem. J.*, 1952, v51, 311.

4. Aronow, L., Howard, F. A., *Fed. Proc.*, 1955, v14, 315.

5. Gaddum, J. H., Lembeck, F., *J. Pharmacol.*, 1949, v4, 401.

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Device for Simultaneous Microscopic Observation of Peripheral and Visceral Circulation.* (23633)

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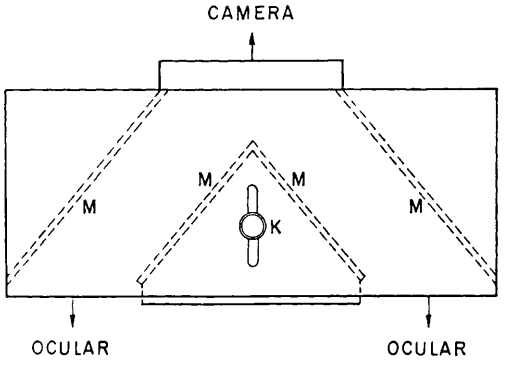
A method has been developed for simultaneous comparison and photographic recording of peripheral and visceral vascular areas in the same experimental animal.

Methods. The apparatus consists of a field-splitting device† (Fig. 1) which is placed over the oculars of 2 microscopes so adapted that the interocular distance is equal to the average distance between the cheek pouch and mesocecum of the hamster. The cheek pouch is pinned over a small transparent lucite block and prepared as a single membrane(1). The method for exposing and spanning the meso-



M = FRONT SURFACE MIRROR.
K = SET KNOB. CENTER PAIR OF MIRRORS MAY BE ADJUSTED UP OR DOWN.

FIG. 1. Field-splitting device.

* Aided by contract from Air Force Office of Scientific Research.

† Made to author's specifications by Mr. John B. Sanroma, Mass. Inst. Tech.