Evidence for the Occurrence of Nor-Epinephrine in the Adrenal Medulla[1]

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NOR-EPINEPHRINE (nor-adrenaline, arterenol) is a primary amine differing from epinephrine only by the absence of an N-methyl group. Its presence in the mammalian body was first demonstrated by pharamacological methods (*9*): extracts of postganglionic adrenergic nerves of cattle showed an activity corresponding to 10–15 μg of L-nor-epinephrine per g of tissue. Its functional importance as sympathin E had previously been suggested by Bacq (*1*) and as a possible precursor of epinephrine by Blaschko (*2*).

Studies on the hemodynamics of epinephrine and nor-epinephrine and its possible relation to the problem of hypertension (*4*) have raised the question as to whether nor-epinephrine is present in the normal adrenal medulla and in tumors of chromaffin tissue (pheochromocytomas).

Since bio-assay of small amounts of nor-epinephrine in mixtures containing large quantities of epinephrine did not seem feasible, chemical methods were used to show the presence of nor-epinephrine in extracts of the adrenal medulla of cattle (U.S.P. epinephrine and U.S.P. epinephrine reference standard). Synthetic epinephrine and nor-epinephrine (Winthrop) were used as standards in these experiments. It was found that the extracts of adrenal medulla contained considerable amounts of nor-epinephrine.

The method employed for the identification and quantitative estimation was chromatography on filter paper, similar to the procedure of James (*6*). Phenol (saturated with water) in an atmosphere of HCl was found to be the most satisfactory solvent and the addition of 8-hydroxyquinoline was found advantageous to avoid tailing. The filter paper used was Schleicher and Schuell No. 597, Whatman No. 1, or Eimer and Amend No. 9–930. All gave satisfactory results. The chromatograms were run for 16–18 hours, in an upward direction (*10*) with the solvent at the bottom of the jar. The substances to be studied were dissolved in acidic alcohol (1 ml conc. HCl per 100 ml 95 percent ethanol) and 0.01 ml applied to the paper. The alcoholic solutions give smaller and better defined spots than aqueous solutions. The spots were developed with 0.44-percent potassium ferricyanide-solution (pH 7.8) according to James (*6*). This leads to the formation of the corresponding adrenochromes (quinones). The colored spots thus produced afforded a better contrast, especially for quantitative estimations, when the paper was subsequently sprayed with a ferric sulfate solution[3] which converted the ferrous salt into Prussian blue. Chromatograms treated in this way are, however, not stable; they gradually turn diffusely blue. In both procedures, the papers were dried and developed at room temperature without heating. Amounts of epinephrine and nor-epinephrine as low as 2 μg could easily be demonstrated.

The colors of the spots obtained by ferricyanide spraying and by spraying with ninhydrin solution (*8*) were useful in distinguishing the spots obtained with nor-epinephrine and epinephrine from those given by Dopa and hydroxytyramine respectively, since the chromatographic demonstration of the latter substances is difficult in the presence of epinephrine and nor-epinephrine. The colors given by these substances are listed in Table 1. Neither Dopa nor hydroxytyramine was found in any of the epinephrine preparations or tumor extracts studied.

TABLE 1

	R_f values*	Color of the spots after spraying with:	
		Ferricyanide	Ninhydrin
Epinephrine	0.50–0.55	pink→yellow	violet
Nor-epinephrine .	0.27–0.33	lavender→brown	mustard
Dopa	0.35–0.38	yellow-pink→blue	dark blue
Hydroxytyramine	0.45–0.50	lavender	mustard

* The R_f values are subject to considerable variations, depending upon conditions.

A quantitative estimation of epinephrine and nor-epinephrine was feasible by planimetry and semilogarithmic calculation (*3*) of the Prussian blue spots. Extracts of the adrenal medulla of cattle (U.S.P. epinephrine) were found to contain 12 to 18 percent

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[3] Ferric sulfate anhydrous 5.0 g; phosphoric acid 85 percent, 75 ml; water to 1000 ml. We are grateful to Dr. Boris Magasanik for suggesting this procedure.

nor-epinephrine; a single sample contained as much as 36 percent, as can be seen in Table 2 (Exp. 2).

The epinephrine fractions from three chromaffin tissue tumors were found to contain 50 to 90 percent nor-epinephrine. Similar findings in adrenal medullary tumors have recently been reported by Holton (*5*).

The hormone of the adrenal medulla thus corresponds in its dual nature to the two known sympathetic mediators: epinephrine and nor-epinephrine. This is of particular interest, since epinephrine and nor-epinephrine differ significantly in their pharmacological actions. Recent hemodynamic studies in man using the method of right heart catherization (*4*) have shown that epinephrine within a physiological range acts as an over-all vasodilator and causes hypertension only by increase of cardiac output. Nor-epinephrine, on the other hand, acts as over-all vasoconstrictor with no change or slight decrease of cardiac output. These two agents also differ in their metabolic effects; e.g., the hyperglycemic action of nor-epinephrine is much less marked than that of epinephrine (ratio 1:8) (*7*). If one assumes that natural epinephrine, as secreted by the adrenal gland, constantly maintains a content of, let us say, 18 percent nor-epinephrine, our present concepts of adrenal secretion are fully valid, since small constant quantities of nor-epinephrine would not significantly alter the functions of epinephrine. The hemodynamic studies quoted above (*4*) were done with extracted epinephrine (U.S.P. epinephrine) and brought out fully the differences between epinephrine and nor-epinephrine and their antagonism. But if one assumes that under varying physiological conditions the nor-epinephrine content of the secreted natural epinephrine varies, current views of the physiology of the adrenal medulla may have to be modified.

TABLE 2

Exp. No.		Epinephrine*	Nor-epinephrine*
		%	%
1	U.S.P. reference standard sample 1	81.5	18.5
2	" " " " 2	64	36
3	" " " " 3	84	16
4	" epinephrine	88	12
5	" "	100	0
6	Pheochromocytoma No. 1	47	53
7	" " 2	12	88
8	" " 3	10	90

* Each value represents at least 5 parallel determinations (concordant within 10%).

There is no doubt that under pathological conditions, e.g., in pheochromocytoma, the nor-epinephrine content of the secreted medullary hormone may increase considerably, since the tumors have been demonstrated to contain up to 90 percent nor-epinephrine. The hemodynamic effects and the influence on carbohydrate metabolism are then profoundly altered.

The tumor extracts were bio-assayed[4]. The proportions of nor-epinephine and epinephrine found were in good agreement with the chemical estimations. The bio-assays were performed by matching the tumor extracts with corresponding mixtures of epinephrine and nor-epinephrine. If the bio-assay is done solely by calculation of the nor-epinephrine content from the responses of nor-epinephrine "insensitive" and "sensitive" test objects, chemical determination and bio-assay do not agree. We think that the error in the bio-assay is due to interference of epinephrine and nor-epinephrine action (competition for equal cell receptors).

In summary, the dual nature of the adrenal medullary hormone has been demonstrated by chromatography on filter paper. Natural epinephrine contains variable amounts of nor-epinephrine.

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