CONCENTRATION AND HYDROXYLATION OF FREE PHENYLALANINE IN ADRENAL GLANDS*

J. H. FELLMAN AND MARY K. DEVLIN

Division of Neurology, University of Oregon Medical School, Portland, Oreg. (U.S.A.)

INTRODUCTION

The work of GURIN AND DELLUVA[1] and more recently that of UDENFRIEND AND WYNGAARDEN[2] demonstrated that epinephrine is synthesized *in vivo* from phenylalanine. Their experiments as well as those of MCGOODALL AND KIRSHNER[3] and KIRSHNER[4] support the hypothesis advanced by BLASCHKO[5] that epinephrine is biosynthesized *via* a *para*-hydroxylation of phenylalanine to tyrosine; the hydroxylation of tyrosine to DOPA; the decarboxylation of DOPA to hydroxytyramine; the β-hydroxylation of hydroxytyramine to norepinephrine and finally the methylation of norepinephrine to epinephrine.

The enzymic alterations of tyrosine to epinephrine have been shown to take place in adrenal tissue[3,4]. It might be argued that the initial hydroxylation of phenylalanine takes place in liver tissue since phenylalanine oxidase seems to occur only in the liver[6].

In this communication we will describe some of our observations on the presence or large amounts of free phenylalanine in adrenal tissue. The hydroxylation of this amino acid by adrenal tissue was studied and the results of this investigation will also be described.

METHOD

Beef and sheep adrenal glands were obtained from a local abattoir. Rabbit, guinea pig, rat and spider monkey adrenal glands were obtained from stock laboratory colonies. Human adrenal glands were removed at autopsy which was carried out no later than ten hours after death. The whole gland or separately the cortex or medulla were ground with sand and extracted with 10 ml of 0.01 *N* HCl/g of gland. The extract was centrifuged at 25,000 × *g* for ½ h to remove debris. For paper-chromatographic identification of amino-acid constituents of adrenal extracts, a method previously described was used[7]. For chemical determination of phenylalanine, the extracts were treated with 10% trichloroacetic acid to achieve deproteinization. The chemical determination used was a modification of the method of KAPELLER-ADLER[8]**. This proved to be accurate to 5% and more reliable in our hands than the method of UDENFRIEND AND COOPER[9].

For hydroxylation studies, fresh beef-adrenal medulla slices 0.5–1.0 mm thick were used. 200 to 400 mg of the tissue slices were incubated with 1 μC of D,L-phenylalanine-3-^{14}C, and 1 mg of ascorbic acid, 0.03 *M* phosphate pH 7.5 was added to bring the volume of the vessels to 3 ml. All additions were prepared in the phosphate buffer immediately before use. Incubation was

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carried out at 37° C with shaking for 2 h. The gas phase was air. The flask contents were deproteinized by adjusting the pH to 5.3 and boiling for 5 min. The supernatant obtained by centrifugation of this material was chromatogramed on Whatman No. 1 paper. A band of supernatant was permitted to separate in butanol–acetic acid–water (40 : 10 : 50). Next to the extract, a mixture of phenylalanine, tyrosine, and *ortho*-tyrosine was permitted to separate. When the development was complete, this known mixture was cut, developed with ninhydrin and with this as a guide a fraction was obtained by cutting crossways to correspond to the developed strip. This fraction was then eluted with 15 ml of 0.001 *N* HCl and rechromatographed. This method allowed for effective separation of the products of phenylalanine hydroxylation. The chromatogram was permitted to separate with butanol–acetic acid–water, dried and developed with ninhydrin. A series of 0.5 cm strips were cut across the length of the chromatogram and placed on planchets for determination of radioactivity. Radioactivity was determined using a "Micromil" end window gas flow counter. The D,L-phenylalanine-3-^{14}C was obtained from Tracerlab, Incorporated and proved to be chromatographically pure. Radioautographs prepared from these chromatograms indicated the presence of a single radioactive substance which could be superimposed with the phenylalanine on the chromatograph. The ascorbic acid was obtained from Eastman Kodak Co.

RESULTS

The paper-chromatographic identification of phenylalanine in adrenal glands is given in Table I.

TABLE I

	R_F* (B.A.W.)	Ninhydrin	Diazo. Sulfan.**	Radioactive Tracer***
Phenylalanine	0.55	blue violet	fluorescent	coextensive with color
Unknown from adrenal gland	0.55	blue violet	fluorescent	coextensive with color

* R_F values from ascending chromatograms developed with butanol–acetic acid–water.
** Diazotized sulfanilic acid gives fluorescent spot with long-wave ultraviolet source.
*** Phenylalanine-3-^{14}C admixed with unknown.

The results of the quantitative chemical determination of phenylalanine in adrenal tissue are given in Table II. There appears to be a greater amount of phenylalanine in medullary tissue than in cortical tissue. These results, of course, depend on the adequacy of the dissection. While it was quite simple to exclude cortical from medullary tissue, it was not quite as simple to exclude medullary from cortical tissue. This may lead to erroneously high figures of phenylalanine content in the cortex.

When beef adrenal medulla slices were incubated with ^{14}C-labeled phenylalanine, a small amount of radioactive material corresponding to tyrosine and a trace of *ortho*-tyrosine appears. When ascorbic acid was added to the incubation mixture, a much greater amount of radioactive tyrosine and *ortho*-tyrosine could be isolated. These areas of the chromatogram were cut out, extracted as described, admixed with the respective known amino acids and rechromatographed. The isolated radioactivity was co-extensive with the color developed, on spraying the chromatogram with ninhydrin, for tyrosine and for *ortho*-tyrosine. Boiled control slices in flasks containing precisely the same additions as unboiled slices, were almost as effective for hydroxylation of the phenylalanine. See Fig. 1.

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TABLE II

CONCENTRATION OF FREE PHENYLALANINE IN ADRENAL TISSUE IN γ/g WET WEIGHT

Species	*Cause of death or Diagnosis during life*	*Age*	*Concentration of phenylalanine in γ/g wet weight adrenal tissue**
Beef (whole gland)	—	—	553
	—	—	705
	—	—	520
	—	—	769
	—	—	622
Beef (cortex)	—	—	364
	—	—	497
	—	—	539
	—	—	487
	—	—	380
Beef (medulla)	—	—	914
	—	—	904
	—	—	944
	—	—	1053
Rabbit (whole gland)	—	—	319
	—	—	463
	—	—	348
	—	—	273
	—	—	261
Guinea pig (whole gland)	—	—	259
	—	—	331
	—	—	379
	—	—	314
	—	—	327
	—	—	360
Spider monkey (whole gland)	—	—	267
	—	—	267
	—	—	499
	—	—	480
Rat (whole gland)	—	—	154
	—	—	239
	—	—	191
Human (whole gland)	Brain tumor	56	1180
	Hemorrhagic shock	67	1228
	Congestive heart failure	73	964
	Phenylketonuria	14	1015
	Neuroblastoma**	4	683
	Cirrhosis of liver	42	1460
	Cirrhosis of liver***	47	830

* Represents average of duplicate determination. Accuracy to 5%.
** Primary in one adrenal; the other adrenal was used for phenylalanine determination.
*** Died in hepatic coma.

DISCUSSION

The presence of large amounts of free phenylalanine in adrenal tissue suggests that this amino acid plays a role in epinephrine biosynthesis. A further implication of this

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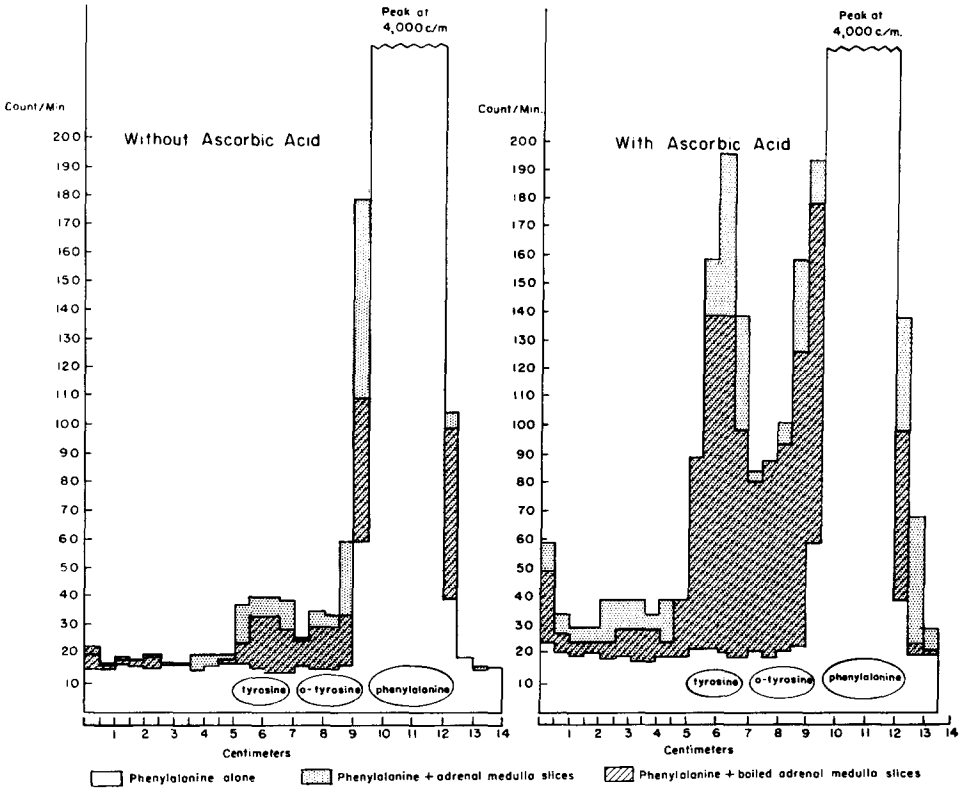

HYDROXYLATION OF PHENYLALANINE BY ADRENAL MEDULLA SLICES

Fig. 1.

observation is that there exists within this gland some mechanism for phenylalanine hydroxylation, or the hydroxylation of some metabolic product of this amino acid such as phenylethylamine. That the latter possibility is unlikely has been demonstrated by UDENFRIEND who reported that ^{14}C-labeled phenylethylamine was not incorporated into epinephrine when injected into rats. On the other hand, he was able to demonstrate that ^{14}C-labeled phenylalanine, tyrosine and DOPA did serve as precursors of adrenal epinephrine and norepinephrine[2].

The only tissue thus far demonstrated to contain phenylalanine oxidase has been the liver[6]. In phenylpyruvic oligophrenia, this enzyme is absent and this deficiency accounts for the accumulation of phenylalanine in the tissue fluids of these afflicted individuals. Nevertheless, UDENFRIEND AND BESSMAN, using ^{14}C-labeled phenylalanine have found that tyrosine is formed from phenylalanine to a small extent by these patients[10]. Further evidence that phenylalanine is hydroxylated by these patients is supported by the fact that epinephrine biosynthesis takes place[7] and also by the appearance in the urine of these individuals of abnormal hydroxylation products of phenylalanine[11].

The hydroxylation of phenylalanine by adrenal tissue bears a striking analogy to the chemical hydroxylating system of UDENFRIEND, *et al.*[12] which utilizes ascorbic

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acid, ferrous ion, and ethylenediaminetetracetic acid. The adrenal system depends on ascorbic acid, undoubtedly contains iron and very effective chelating materials in the form of proteins or polypeptides. This system would be expected to carry out non-specific hydroxylation and lead to products such as *ortho*-tyrosine as well as tyrosine. The *ortho*-tyrosine formed, being essentially a by-product in the metabolic sequence, would be expected to undergo metabolic degradation to *ortho*-hydroxyphenylacetic acid. Thus the adrenal system might be responsible for the appearance of *ortho*-hydroxyphenylacetic acid in normal urine[13] and also account for the appearance of *ortho*-hydroxylated aromatic acids in the urine of phenylpyruvic oligophrenics[14]. DALGLIESH[15] has considered, "That two routes exist for the hydroxylations in aromatic amino-acid metabolism, the one specific, the other non-specific and presumably much slower". In the absence of the specific liver phenylalanine oxidase, the nonspecific adrenal system carries on this essential metabolic function, albeit inadequately.

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SUMMARY

The adrenal glands of man, beef, spider monkey, rabbit, guinea pig, and sheep contain large amounts of free phenylalanine.

Beef-adrenal medulla is capable of hydroxylating phenylalanine by a mechanism suggestive of the chemical system of UDENFRIEND.

The significance of these findings to aromatic hydroxylation products found in normal urine and those isolated from the urine of individuals afflicted with phenylpyruvic oligophrenia is discussed.

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