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Influence of Specific Nutrients on Catecholamine Synthesis and Metabolism

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Introduction

THE role of cofactors in the formation and metabolism of catecholamines may be studied at various levels. The following is a discussion of results obtained by techniques *in vivo* and *in vitro*, primarily as they are applied at their extremes, *i.e.* by the use of nutritional deficiencies and by the study of the actions of purified enzymes, respectively. Four nutrients will be discussed in the context of ongoing and earlier work in the author's laboratory. These are pyridoxine, riboflavin, copper and iron.

Pyridoxine

Pyridoxine-deficiency has been of great interest in this work because of the role of pyridoxal 5′-phosphate as the coenzyme of dopa decarboxylase (14, 40) and the diminished decarboxylase activity in the tissues of pyridoxine-deficient animals (1–3, 7, 9, 52). In regard to the steady-state concentration of amines, pyridoxine-deficient rats show no change in the catecholamine content of the adrenal gland, liver, brain, spleen or heart which is not attributable to concomitant changes in body weight or organ weight stemming from the deficiency (44, 46, 53). There is no difference in the amounts of dopamine, dopac (dihydroxyphenylacetic acid) and HVA (homovanillic acid, 4-hydroxy-3-methoxyphenylacetic acid) excreted in the urine between control and deficient rats (43). Even when L-dopa is injected in a loading dose the pyridoxine-deficient animals excrete as much of the urinary metabolites as control rats do (43, 44).

In Parkinson's disease, where L-dopa is administered therapeutically, the simultaneous administration of vitamin B_6 interferes with the beneficial response, and this is attributed to increased decarboxylation of the dopa-load peripherally, with less available to the brain centers where the amino acid exerts its effect. The vitamin would have to be converted in the tissues to its coenzyme form, but it is not known whether the coenzyme is attaching to unsaturated apodecarboxylase in various organs or whether it is simply protecting pyridoxal phosphate that is already *in situ*. Studies of decarboxylase activity in crude preparations of rat kidney (3), liver and brain (9) favor the former point of view, but Dr. G. A. Lancaster, who purified hog-kidney dopa decarboxylase in my laboratory, found evidence for the latter possibility as well (25, 26). This evidence comes from the

TABLE 1

*Substrate specificity of purified hog-kidney dopa decarboxylase**

Substrate	Relative Decarboxylase Activity	
	PLP added	No PLP added
3,4-Dihydroxyphenylalanine	100	90
5-Hydroxytryptophan	18	18
p-Tyrosine	†	†
m-Tyrosine	148	59
o-Tyrosine	124	41
threo-3,4-Dihydroxyphenylserine	†	†
erythro-3,4-Dihydroxyphenylserine	2	2

* Data adapted from reference 25.

† No activity detectable. Values are the average of three determinations.

measurement of the rates of decarboxylation of several substrates by an enzyme preparation representing 190-fold purification. In the cases of dopa and 5-hydroxytryptophan decarboxylation was not accelerated by the addition of coenzyme (table 1), and this would indicate the absence of native apodecarboxylase. On the other hand, decarboxylation of *m*- and *o*-tyrosines was greater when the coenzyme was added. These two amino acids, like many others including dopa, are able to form derivatives of the isoquinoline type with pyridoxal (38), and the analogous sequestering of the coenzyme by the substrate would reduce the rate of decarboxylation. Because these two tyrosine isomers were tested with a prolonged incubation (30 min) compared to that used for dopa and 5-hydroxytryptophan (5 or 10 min), reaction with coenzyme could play a role in reducing the amount of active holoenzyme. Thus, the rate with added pyridoxal phosphate should be nearer to the true value.

The administration of D-dopa results in the excretion of excess urinary dopamine (43); the pathway of formation in this case is presumably by deamination of the amino acid to 3,4-dihydroxyphenylpyruvic acid, a reaction that is mediated by the yellow enzyme D-amino acid oxidase, and by asymmetric transamination of the keto acid to L-dopa (5). The L-dopa is the immediate precursor of the urinary dopamine. Although there is no significant effect of pyridoxine deficiency on the conversion of L-dopa, administered *in vivo*, to urinary dopamine there is a pronounced effect when D-dopa is given in its place (43, 46). This interesting effect, detected *in vivo*, parallels the known effects of pyridoxine-deficiency on enzyme activities: transaminase is more sensitive than amino acid decarboxylase to the deficiency (7). Transamination *in vivo* has been demonstrated also in another way. Injection of the keto acids 3-hydroxy- and 3,4-dihydroxyphenylpyruvic acids in suitably prepared animals elicits a pressor response (5, 6, 37). The effect seems to depend upon the re-amination of the keto acids and their subsequent decarboxylation to *m*-tyramine and dopamine, respectively. Pyridoxine-deficient animals show less of a pressor response.

Riboflavin

Deficiency of riboflavin in the rat brings about decreases in the concentrations of epinephrine and norepinephrine in the liver (67% and 62% decrease below controls, respectively) and norepinephrine concentration in the brain (11% decrease). These changes are partly, but not entirely, accounted for by the altered organ weight resulting from the deficiency. No changes occur in the catecholamine content of the adrenal glands, spleen (46), or heart (27) as a result of riboflavin deficiency (table 2). The pronounced effect of the deficiency on hepatic catecholamines probably points to some defect in the mechanism of receiving catecholamines from the blood as preparation for their metabolic conversion by catechol O-methyltransferase, monoamine oxidase (MAO) and aldehyde dehydrogenase to vanilmandelic acid. This may represent a defect in the uptake$_2$ mechanism described by Iversen (19).

Recently we have introduced a simple method of assessing *in vivo* the activity of one enzyme in this series, MAO. *n*-Pentylamine-$^{14}C_1$ is injected into rats that are housed for the course of the experiment in individual all-glass metabolic cages from which the expired gases can be collected and sampled periodically (28). The pathway of catabolism of pentylamine, a classical substrate of MAO, is shown in figure 1. Animals convert about one-third of the injected radioactivity to $^{14}CO_2$ in 1 hr, but very much less than this if they are pretreated with a MAO-inhibitor such as iproniazid or tranylcypromine. Rats that have been made deficient in riboflavin through dietary means metaboilse the pentylamine at a much slower rate than under the control conditions, but the slowed rate can be

TABLE 2

*Organ weights and catecholamine content of organs in riboflavin-deficient rats**

Organ	Item	Mean Values		
		+B_2	No B_2	P
—	Final body weight, g	252	129	<.01
Adrenals	Weight, mg/100 g body weight	13.1	25.4	<.01
	Epinephrine, μg/pair	18.6	15.7	>.05
	Norepinephrine, μg/pair	6.2	5.7	>.05
Liver	Weight, g/100 g body weight	3.9	4.0	>.05
	Epinephrine, μg	2.7	0.9	<.01†
	Norepinephrine, μg	6.3	2.4	<.01†
Brain	Weight, g/100 g body weight	0.42	0.68	<.01
	Norepinephrine, μg	1.0	0.9	<.01†
Spleen	Weight, g/100 g body weight	0.20	0.26	=.05
	Norepinephrine, μg	1.6	1.6	>.05
Heart	Norepinephrine, μg	0.52	0.60	>.05

* Data for heart (28 days of deficiency), reference 27. Other data (19–54 days of deficiency), reference 46, to which the final body weight shown above corresponds.

† After adjusting for regression of organ weight on amine content of the organ, P was <.05, but >.01.

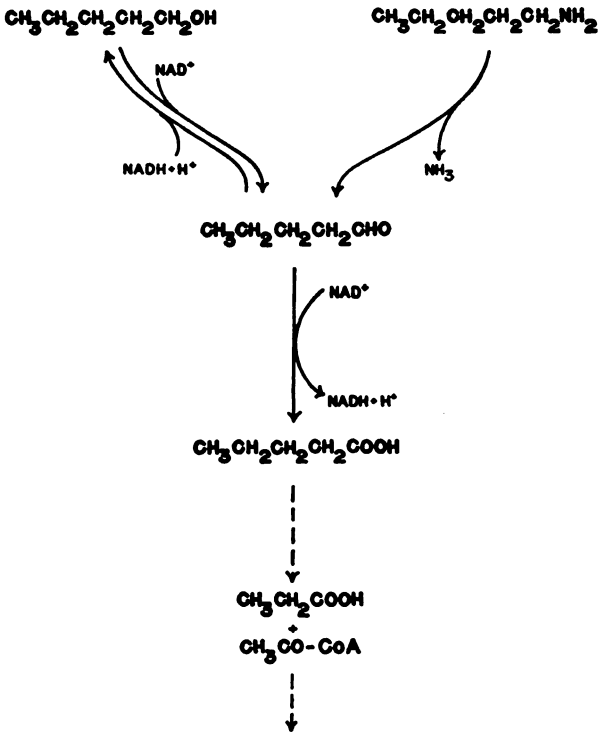

FIG. 1. Catabolism of *n*-pentylamine and *n*-pentanol, showing their common pathway through valeraldehyde.

restored to normal in approximately 6 days after the introduction of the vitamin into the deficient diet (48). These results (figure 2) correspond to (a) the decreased hepatic levels of MAO in riboflavin-deficient rats, discovered by Hawkins (17) and studied extensively in my laboratory (8, 39, 41, 56); and (b) the slow restoration of these levels to control concentrations on repleting the animals with the vitamin (8).

All attempts to reconstitute the activity of MAO extracted from the livers of deficient rats by addition of flavin coenzymes *in vitro* have so far failed, for a reason that has now become clear: the enzyme contains covalently bound flavin adenine dinucleotide (FAD). In beef-liver mitochondrial MAO the bond is between the 8α-methyl group of the isoalloxazine nucleus, known for some years to be a chemically reactive site (18), and a cysteine residue of the polypeptide chain (20, 51). Other MAOs (10, 36, 58, 59) also have FAD tightly bound and, in the case of rat-liver MAO, this bond is associated with ninhydrin-positive material (58, 59). After riboflavin deficiency in rats has been terminated by addition of the vitamin to the diet, the gradual increase of MAO activity entails the biosynthesis of the enzyme *de novo*, or at least involves a time-consuming resynthesis of the FAD-apoenzyme linkage.[1]

[1] Covalent binding of FAD to the peptide chain has also been described in the case of succinic dehydrogenase; linkage is through a histidyl residue (29).

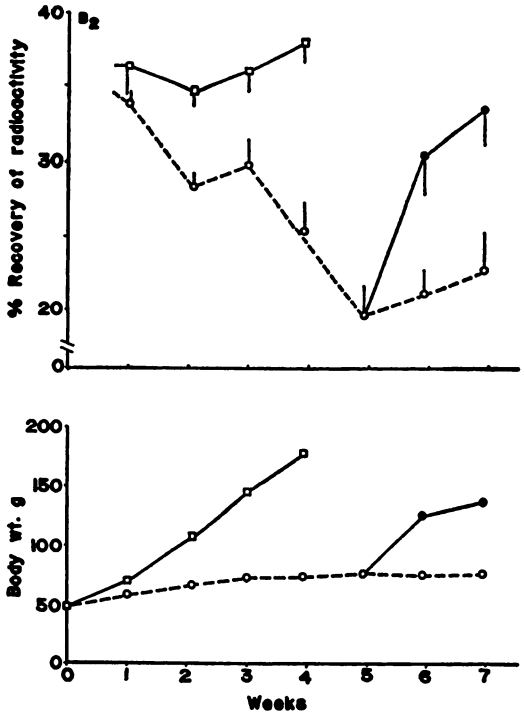

FIG. 2. Effect of riboflavin deficiency on initial rate of oxidation of *n*-pentylamine *in vivo* (upper section) and body weight (lower section). □ Control (riboflavin-supplemented) rats; ○ deficient rats; ● deficient for 34 days, then re-alimented with riboflavin. (Based on data of table 2, reference 48.)

MAO from several sources contains 2 moles of FAD per mole of enzyme (10, 35, 50, 56–58), or 1 mole of FAD per minimum molecular weight of 150,000 in the case of rat liver (57).

Copper

Copper is an essential part of dopamine-β-hydroxylase (12), and the administration of copper-chelating agents to animals effects a reduction in the activity of this enzyme, prevents conversion of dopamine and tyramine to the corresponding β-hydroxy derivatives (31), lowers the norepinephrine content of the heart and brain of rats (4, 13, 31, 32) and decreases the chronotropic action of tyramine on the rat heart (54). As these chelators may engender auxiliary effects beside immobilising prosthetic copper, it is desirable to produce copper deficiency not only by binding copper already in the tissues but by withholding the metal from the diet of the animals. In the latter case, the tissue levels of this metal would decrease as it is gradually lost from the body during normal turnover processes. In our experiments with copper-deficient rats a number of years ago, we observed that when they are injected with labeled dopamine they convert a smaller proportion of this to cardiac norepinephrine than their controls do (table 3). This process, which involves the action of dopamine-β-hydroxylase,

is restored to normal even in chronically deficient animals by the sole addition of 50 μg of copper as the sulfate to their daily diet (30).

As norepinephrine exerts an inhibitory action on the enzyme tyrosine hydroxylase, the first enzyme involved in its biosynthesis (47), a decreased activity of dopamine-β-hydroxylase should be reflected in increased rates of conversion of tyrosine to catecholamines. This has been demonstrated by several investigators.

Falardeau in my laboratory is interested in determining whether the enzymic activity changes also. He has treated male rats with sodium diethyldithiocarbamate in divided doses totaling 2 g/kg body weight, given intraperitoneally, over a period of 10 hr, and has assayed the activity of tyrosine hydroxylase of the adrenal glands and brain 24 hr after the first injection (11). In rats given this copper chelator and inhibitor of dopamine-β-hydroxylase, adrenal activity is increased to 240% of control levels (table 3). Activity of the enzyme in the neostriatum is not affected by this treatment. The increased activity of the adrenals as tested *in vitro* is not likely to stem from changes in the concentration of norepinephrine in the extracts.

In addition to our interest in the metabolic consequences of copper-deficiency in the rat we have been concerned with various biochemical alterations in the

TABLE 3

*Effect of copper-deficiency on catecholamine metabolism in the rat**

Diet	Item Measured	Mean Values		
		Cu added (controls)	No Cu added to diet	P
Cu-deficient	Percent of ^{14}C-catecholamines in heart as ^{14}C-norepinephrine†	52	26	<.001
	Cardiac copper, μg/g	8.1	4.0	<.001
	Hepatic copper, μg/g	4.4	2.3	<.001
	Heart weight, g	0.56	0.88	<.001
Cu-deficient 13 wk	Percent of ^{14}C-catecholamines in heart as ^{14}C-norepinephrine	50	14	<.001
	Cardiac copper, μg/g	7.4	5.2	<.001
	Hepatic copper, μg/g	5.2	1.8	<.001
Purina chow, copper chelator injected‡	Tyrosine hydroxylase			
	Adrenal glands, nmoles/hr per pair	10.6 ± 1.4	25.1 ± 1.4	<.001
	Corpus striatum, nmoles/hr	3.6 ± 0.2	3.6 ± 0.3	>.05

* Data for copper-deficiency, piebald rats, reference 26. Data for tyrosine hydroxylase, reference 11 in which the method of Nagatsu *et al.* (33) was used.

† Determined 1 hr after intramuscular administration of ^{14}C-dopamine by published procedures (30).

‡ Solution of sodium diethyldithiocarbamate injected intraperitoneally at zero time and at 2, 4, 6, 8 and 10 hr into 6 male albino rats weighing about 130 g. Total dose, 2 g/kg. Animals were killed 24 hr after the first injection. Controls (n = 6) received 0.9% NaCl.

copper-loaded rat (15, 22, 23). Such investigations may throw some light on the mechanism of the changes in the retention of copper in the tissues or on the somewhat erratic disturbances in monoamine metabolism in Wilson's disease (42, 45). Other drug-induced models can be employed for the study of copper metabolism in the rat in conditions resembling Laennec's cirrhosis or biliary cirrhosis (21). In the course of this work we have examined the effects of copper-loading in the rat on the activity of dopa decarboxylase and MAO in some organs. The results are set out in table 4. The dosage with copper sulfate has resulted in significant increases in the concentration of copper in liver, kidneys and brain, although the absolute increase in the last organ is small. The two enzymes that were measured underwent decreases only in the liver. These decreases were not seen after 2 weeks of copper-loading, but were becoming evident at the 4th week. The dopa decarboxylase activity of the adrenal glands did not change. Whether the liver is unique in undergoing change because of the greater accumulation of copper or for some other reason is not known.

There is one more point to make about the role of copper in monoamine metabolism. When we obtained purified MAO in our laboratory in 1966 (57) we looked for copper in our preparations, for Nara *et al.* (34) had claimed that beef-liver mitochondrial MAO is a cuproprotein. The concentration of copper in the rat-liver MAO was well below that reported for authentic cuproproteins. Subsequent reports from other laboratories confirmed our finding (10, 55). At

TABLE 4

*Effect of copper-loading of the rat on dopa decarboxylase and monoamine oxidase activities**

Organ	Copper Injection	Concentration of Cu	Enzymic Activities	
			Dopa decarboxylase	Monoamine oxidase
		μg/g		
Liver	−	4.2 ± 0.1	4.92 ± 0.27	102 ± 2
	+	376 ± 21	2.77 ± 0.13	76 ± 5
	P	<.001	<.001	<.001
Kidneys	−	4.7 ± 0.2	4.80 ± 0.33	9.2 ± 0.2
	+	65 ± 6	4.73 ± 0.28	9.5 ± 0.4
	P	<.001	>.05	>.05
Brain	−	1.6 ± 0.1	0.025 ± 0.002	28 ± 2
	+	2.2 ± 0.1	0.029 ± 0.002	29 ± 1
	P	<.001	>.05	>.05
Adrenal glands	−		0.28 ± 0.05	
	+		0.29 ± 0.006	
	P		>.05	

* Rats received 3.75 mg of copper (as solution of copper sulfate) per kg of body weight by intraperitoneal injection, daily for 6 weeks. Controls received an equal volume of 0.9% NaCl under the same conditions. Dopa decarboxylase activity is expressed as μmoles of serotonin formed from 5-hydroxytryptophan/g of tissue per hr; monoamine oxidase activity is expressed as nmoles of 4-hydroxyquinoline formed from kynuramine/mg protein per hr. In the table the means of each enzyme activity (± S.E.) are based on determinations in 6 to 10 rats (24).

the time that the issue about copper in MAO had been raised, we examined the livers of copper-deficient rats for MAO activity. Prolonged feeding of a diet very low in copper content was without effect on the enzyme, whose activity toward three substrates was tested (49). There was no question about copper depletion of these rats for the concentration of ceruloplasmin in the serum and of copper in the liver had clearly declined significantly. Despite these findings, obtained by different methods and in different laboratories, as well as a withdrawal of the original claim about the presence of copper in MAO (55), there are still papers appearing with the statement that this enzyme is copper-linked.

Iron

Although our purified rat-liver mitochondrial MAO had very little copper present, there was sufficient iron (57) to warrant further investigation. Recently, Oreland (36) has reported the presence of iron in MAO of pig liver. As a first step in our investigation of this metal we measured MAO activity in the livers of iron-deficient rats and found that this led to a moderate, but significant, decrease

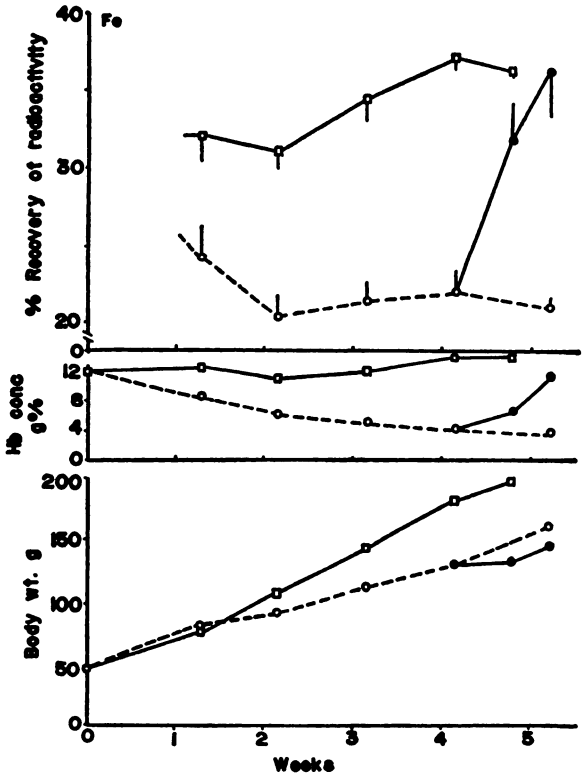

FIG. 3. Effect of iron deficiency on initial rate of oxidation of *n*-pentylamine *in vivo*, hemoglobin concentration, and body weight of rats. Symbols, as in figure 2, except that realimentation of some deficient rats with iron began on day 28. (Based on data of table 1, reference 48.)

in the enzyme activity (49). This encouraging result led us to test the effects of iron-deficiency in the intact animal. For this purpose we made use of the ^{14}C-pentylamine assay (48). The results demonstrated conclusively that nutritional deficiency of iron in the rat, with concomitant anemia and decreased hepatic iron stores, leads to a marked decrease in the rate at which the animals metabolize pentylamine to CO_2 (figure 3). The validity of this result has been checked by two associated experiments. In the first, chronically deficient animals were repleted with iron, the addition of the iron salt to their diet being the only dietary alteration. The ability of these animals to metabolize pentylamine rose steadily toward normal over a period of 3 to 6 days. In the second experiment, similarly labeled *n*-pentanol was administered to the deficient rats. This compound is also readily metabolized to CO_2 and water, and its initial oxidation product is the same as for pentylamine, *viz.* valeraldehyde. In other words, any effect upon the oxidation of valeraldehyde or one of its products would be detected if either the alcohol or the amine were used, but if the effect were upon MAO alone then one should expect to find a change in the rate of metabolism of pentylamine, but not of pentanol. Our experiments showed that the iron-deficient rat metabolizes the alcohol at precisely the same rate as the iron-supplemented controls. These experiments, then, localize the effect of iron-deficiency at the level of MAO. The specific function of iron in the activity of this enzyme still remains to be explained, and we are carrying on further work to detect this role (60). Iron might be present as a metal prosthetic group of the enzyme, necessary for the actual oxidative reaction. Several iron-flavoproteins are known and their mechanisms of action have been studied. They seem to be characterized by an asymmetric electron paramagnetic resonance (EPR) signal at 1.94 gauss, attributable to non-heme iron bound to the protein in some special way (16).

Iron could conceivably act in other ways: as a bonding agent between the enzyme and other portions of the external membrane of the mitochondrion, where MAO resides; or as the cofactor of another enzyme that is needed for the biosynthesis of MAO, *e.g.*, in the step whereby FAD is covalently linked to the peptide chain. The answer will require further novel experiments.

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