view that the amino acid composition is determined largely by the existing genetic code and the random nature of base changes in evolution. Small but significant deviations from such expectation might be accounted for satisfactorily by assuming selective constraint of amino acid substitutions.

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Iron- and Riboflavin-Dependent Metabolism of a Monoamine in the Rat in vivo

Abstract. n-*Pentylamine enters into intermediary metabolism by the action of monoamine oxidase. [1-^{14}C]Pentylamine injected into rats is rapidly converted to $^{14}CO_2$. The rate of catabolism decreases progressively in the course of nutritional iron deficiency, reaching about 60 percent of control values in 3 weeks. Feeding with iron yields control levels within 6 days. The catabolism of amyl alcohol, which shares a common pathway with* n-*pentylamine by way of valeric aldehyde, is not significantly affected by the deficiency. The results demonstrate that the maintenance of normal monoamine oxidase activity in vivo depends upon an adequate supply of dietary iron.*

Information about possible cofactors of mitochondrial monoamine oxidase (MAO) is necessary for an understanding of the mechanism of action of this widely distributed enzyme. Thus far, no functional metal component has been detected in it, although it is known that MAO activity of rat liver, as measured in vitro, declines significantly during long-term nutritional deficiency of iron, as monitored by body weight changes, hemoglobin levels, and hepatic concentrations of iron (*1*). However, the decreases observed (*1*) with three different substrates were only moderately large and perhaps were insufficient to affect the disposition of monoamines in the intact animal. Hence, it was considered important to assess also the function of MAO in vivo. It has now been observed that the rate of oxidation of a standard substrate for this enzyme is subnormal in the iron-deficient rat.

We used the following compounds labeled in the carbon-1 (Mallinckrodt) position: pentylamine (*n*-amylamine) hydrochloride (specific activity, 1.0 mc/mmole) and *n*-amyl alcohol (specific activity, 1.76 mc/mmole); and unlabeled amylamine (Eastman) which we distilled before use, unlabeled *n*-amyl alcohol (Analyzed Reagent, Baker).

Each compound was injected intraperitoneally into male Sprague-Dawley rats (*2*) in a dose of 100 mg per kilogram of body weight; the injected material consisted of 5 μc of the respective labeled substances per kilogram, suitably diluted with the corresponding unlabeled compound. The animals were immediately placed in individual glass metabolism cages, and the $^{14}CO_2$ in the expired gases was collected in a 1 : 2 mixture of ethanolamine and ethylene glycol monomethyl ether, as described (*3*). About 30 to 40 percent of the administered radioactivity was recovered in the first hour after the labeled amine was injected into normal adult rats (170 g) fed on Purina Chow. A further 30 to 35 percent of the injected ^{14}C was collected over the next 2 hours.

The oxidation of pentylamine is initiated by MAO (*4*), as was demonstrated by our method with rats given the amine 16 hours after an intraperitoneal injection of a MAO inhibitor. With tranylcypromine (5 mg/kg, Smith Kline & French); the oxidation of pentylamine was inhibited by 55 percent during the first hour. With iproniazid phosphate (100 mg/kg; Hoffmann-LaRoche), the amount of injected radioactivity recovered as $^{14}CO_2$ during the first 3 hours was only 3 to 7 percent of that administered.

Control groups of rats, that were fed a semisynthetic diet prepared in this laboratory and containing 312 mg of added ferric citrate hexahydrate per kilogram of feed (Fe-supplemented diet) (*1*), metabolized the injected radioactive pentylamine as readily as those consuming the mixed natural diet (Purina). Other rats that were fed the semisynthetic diet for varying periods of time (Table 1), except for omission of the iron salt (Fe-deficient diet), oxidized the amine at a very much lower rate. Iron deficiency thus caused a reduced rate of metabolism of pentylamine, as judged from the rate of recovery of administered radioactivity, during the first hour. This reduction is apparent as early as 9 days (Table 1) and seems to be fully developed by 3 weeks, at approximately three-fifths of the control rate.

The iron dependency of this phenomenon was demonstrated by the following parallel experiments. Groups of rats were allowed to consume the iron-deficient diet for 28 days; then they were changed over to the control diet —one supplemented with ferric citrate. In tests carried out during the refeeding phase, these animals showed an increased rate of oxidation of pentylamine within 3 days and attained the same rate of metabolism as the control animals by day 6. There appeared to be a parallel, but slower restoration of the hemoglobin.

The immediate oxidation product of pentylamine is valeric aldehyde (*6*). *n*-Amyl alcohol, which is readily oxidized by the rat in vivo (*7*), also enters metabolism after enzymic conversion to valeric aldehyde. When the alcohol was administered to iron-deficient and iron-supplemented rats there was no significant difference in the respective rates of oxidation, even at 10 weeks (Table 1).

Table 1. Effect of dietary iron deficiency on the metabolism of *n*-[1-^{14}C]pentylamine and *n*-[1-^{14}C]amyl alcohol to $^{14}CO_2$ in the rat in vivo. The result is expressed as the percentage of administered ^{14}C recovered in expired gases as $^{14}CO_2$ in the first 60 minutes.

Diet*	Duration (days)	Rats (No.)	Body weight† (g)	Hemoglobin‡	$^{14}CO_2$ (%)
			n-Pentylamine		
Supplemented with iron	9	3	78 ± 1	12.2 ± 0.4	32.1 ± 1.6
Deficient in iron	9	3	82 ± 2	8.7 ± 0.7	24.2 ± 2.3
Supplemented	15	6	109 ± 2	10.9 ± 0.5	31.1 ± 1.0
Deficient	15	6	94 ± 3	6.3 ± 0.5	20.4 ± 1.4
Supplemented	22	6	145 ± 4	11.9 ± 0.2	34.4 ± 1.2
Deficient	22	6	112 ± 4	5.2 ± 0.4	21.4 ± 1.2
Supplemented	29	6	184 ± 4	12.8 ± 0.2	37.0 ± 0.6
Deficient	29	6	132 ± 7	4.4 ± 0.3	22.0 ± 1.2
Supplemented	31	3	199 ± 7	12.9 ± 0.3	36.2 ± 0.4
Deficient, then supplemented	28 } 3	3	135 ± 12	6.7 ± 0.4	31.9 ± 2.2
Deficient, then supplemented	28 } 6	3	147 ± 13	11.2 ± 0.8	36.2 ± 2.9
Deficient	34	3	163 ± 8	4.0 ± 0.6	21.0 ± 0.6
			n-Amyl alcohol		
Supplemented	30	3	137 ± 8	13.4 ± 0.4	39.1 ± 2.4
Deficient	30	3	134 ± 8	3.3 ± 0.4	42.9 ± 2.4
Supplemented	70	3	262 ± 8		39.2 ± 1.4
Deficient	70	3	207 ± 7		42.6 ± 2.0

* Rats consumed a nutritionally complete, that is, iron-supplemented diet, or the iron-deficient diet for the indicated number of days. In repletion experiments, rats received the deficient diet for 28 days, followed by the supplemented diet for the succeeding 3 or 6 days, respectively. † Mean ± standard error is shown. ‡ Hemoglobin was determined on tail blood by the method of Clegg and King (*5*).

Table 2. Effect of dietary deficiencies of riboflavin and copper on the metabolism of ^{14}C-labeled *n*-pentylamine in the rat in vivo. The result is expressed as the percentage of administered ^{14}C recovered in the expired gases as $^{14}CO_2$ in the first 60 minutes.

Diet*	Duration (days)	Rats (No.)	Body Weight (g)	$^{14}CO_2$ (%)
	Riboflavin deficiency			
Supplemented with riboflavin	7	3	70 ± 2	36.1 ± 2.0†
Deficient in riboflavin	7	3	59 ± 3	33.9 ± 0.7
Supplemented	15	6	107 ± 4	34.7 ± 1.0
Deficient	15	6	67 ± 2	28.3 ± 0.9
Supplemented	21	6	146 ± 4	36.1 ± 1.4
Deficient	21	5	73 ± 3	29.8 ± 1.7
Supplemented	27	3	177 ± 3	38.0 ± 1.4
Deficient	27	3	74 ± 7	25.4 ± 1.7
Deficient, then supplemented	34 } 7	3	126 ± 5	30.5 ± 2.7
Deficient	41	3	75 ± 9	21.2 ± 1.6
Deficient, then supplemented	34 } 14	3	137 ± 15	33.6 ± 2.4
Deficient	48	3	76 ± 10	22.8 ± 2.6
	Copper deficiency‡			
Supplemented with copper	14	3	90 ± 2	29.9 ± 1.3
Deficient in copper	14	3	88 ± 4	27.2 ± 2.6
Supplemented	21	3	102 ± 2	29.8 ± 1.0
Deficient	21	3	103 ± 3	27.4 ± 2.0
Supplemented	28	3	119 ± 1	30.7 ± 1.1
Deficient	28	3	111 ± 6	28.5 ± 1.3
Supplemented	41	5	158 ± 6	28.7 ± 1.2
Deficient	41	4	156 ± 13	29.9 ± 1.2

* Rats consumed nutritionally complete diets, supplemented with the respective nutrient, or a diet deficient in that nutrient, for the indicated number of days. † The mean ± standard error is shown. ‡ The serum oxidase activity of these rats was determined by the method of Rice (*17*). Control rats (10 animals, 16 determinations) had 0.079 ± 0.002 optical density units (540 nm) per 15 minutes per 0.1 ml of serum. Values for the animals consuming the copper-deficient diet were very low even at 14 days and remained low throughout the experiment; their mean ± standard error (10 rats, 14 determinations) was 0.007 ± 0.002.

Our results indicate that the deficient oxidation of the amine caused by iron deficiency is related to some action at the level of MAO. However, they do not shed light on the specific role of iron in the function of this enzyme, that is, whether the metal is necessary for MAO biosynthesis, as a prosthetic group, for the proper action of the enzyme in the mitochondrial membrane, or for some other purpose.

In regard to the possibility of a role of iron in the orientation (*8*) of MAO in its mitochondrial location (*1*), Dallman and Goodman (*9, 10*) have demonstrated that a dietary deficiency of iron brings about morphological changes in the mitochondria. If the structural changes lead directly to malfunction of MAO, then the same disturbance of MAO activity ought to be observed no matter how these ultrastructural abnormalities are produced. In fact, similar changes occur in the course of deficiencies of riboflavin (*11*) and of copper (*9*), yet the former is associated with a great reduction of MAO activity (*12, 13*), whereas copper deficiency has no such effect (*1*). These results are, of course, understandable on the basis of the presence of riboflavin in the prosthetic group of rat liver MAO (*14*) but lack of significant amounts of copper in the purified enzyme (*13, 15*). In order to assess the relative effects of these nutrients by our method in vivo, we fed rats a diet deficient in riboflavin (*16*) and copper (*1*), respectively, and we then tested the rate of metabolism of pentylamine. Riboflavin deficiency, like that of iron, causes a diminution in MAO activity in vivo, whereas copper deficiency, even of 6 weeks duration, is without effect (Table 2). Hence, it is unlikely that iron and riboflavin deficiencies act in this instance through ultrastructural changes in the mitochondria (*18*). It may be pertinent that purified MAO obtained from rat liver contained a significant amount of iron (*15*) and that the enzyme is inhibited by iron-chelating agents (*1, 19*). These facts suggest that the metal may have some role as a prosthetic group of this enzyme. It is conceivable that the iron is a functional part of an enzyme catalyzing the incorporation of riboflavin into the apoprotein of MAO, in a covalent linkage (*14, 20*).

In our work MAO activity in vivo has been shown to depend upon an adequate supply of dietary iron. The possibility must now be considered that

the metabolism of endogenous monoamines, such as catecholamines, serotonin, and related compounds, is disturbed in states of iron deficiency.

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Murine Leukemia Virus: High-Frequency Activation in vitro by 5-Iododeoxyuridine and 5-Bromodeoxyuridine

Abstract. *Cells of embryos of the high leukemic mouse strain AKR can be grown in culture as virus-negative cell lines. However, these lines and clonal sublines uniformly have the capacity to initiate synthesis of murine leukemia virus. Exposure of the cells to 5-iododeoxyuridine or 5-bromodeoxyuridine induced synthesis of virus in as high as 0.1 to 0.5 percent of the cells; many of the cells were producing virus as soon as 3 days after initiation of treatment. Induction of virus by these drugs is several orders of magnitude greater than that obtained with any other treatment tested. These studies indicate that the full genome of murine leukemia virus is present in an unexpressed form in all AKR cells and provide a potentially powerful technique for activating leukemia virus genomes in other cell systems.*

For many years it has been recognized that murine leukemia virus (MLV) can be "activated" in mice by certain carcinogenic stimuli or aging [see (*1*)]. Also, Aaronson *et al.* (*2*) have presented evidence that spontaneous activation of virus can occur in mouse embryo cells propagated in vitro. However, because of the complexity of in vivo systems, and the rarity of emergence of virus in vitro, it has not been possible to study the activation process or to rigorously eliminate the possibility that emergence of virus represents dissemination of a low-grade carrier state.

We have studied activation of MLV in cell lines from embryos of the high leukemic AKR mouse. Although newborn AKR mice are uniformly positive for infectious MLV, 15- to 17-day embryos contain little or no virus (*3*). By initiating tissue culture cell lines with small numbers (250 or 2000) of AKR embryo cells, we have been able to establish two cell lines (Nos. 26 and 32) that have been carried as virus-negative lines for more than 70 passages, over a 17-month period (*4*). No evidence of MLV expression was observed by extensive testing for infectivity, viral antigens, morphologic particles, or reverse transcriptase activity. Clonal sublines, derived by growing single cells in microcups, as well as subclones thereof, have also been carried as virus-negative lines. The tissue culture passage history of each line and clone includes at least 60 tests for virus by the various techniques mentioned above with completely negative results.

On rare occasions, infectious MLV has appeared spontaneously in sublines of cell lines 26 and 32, as well as in several of the clonal lines of each. The majority of the clonal lines has not shown spontaneous induction of virus. When virus emergence occurred, the infection spread throughout the cultures and was readily detected by the above-mentioned tests. We estimate the frequency of spontaneous activation to be no more than 10^{-8} to 10^{-9}. More frequent emergence of virus occurred in cultures that were exposed to x-ray (1000 r) or transformed by SV40 virus (*4*), or which were treated with 50 μg of 3-methylcholanthrene per milliliter or 10 μg of 7,12-dimethylbenzanthracene per milliliter for 48 hours (*5*). With none of these treatments did the rate of activation appear to exceed 10^{-5}. Comparable low-efficiency induction of avian leukosis virus after similar treatment of primary chick embryo cell cultures has been reported (*6*).

Other compounds that we have tested at a number of dose levels for ability to activate the AKR cell lines include 5-fluorodeoxyuridine, cytosine arabinoside, 8-azaguanine, cyclic dibutyryl adenosine monophosphate, cyclophosphamide, uracil mustard, 6-mercaptopurine, 6-azathymine, 6-azauridine, and hydroxylamine. With the exception of rare activation by 5-fluorodeoxyuridine, all of these drugs gave negative results.

In contrast, exposure of the cells to 5-iododeoxyuridine (IUDR) or 5-bromodeoxyuridine (BUDR) has consistently and rapidly induced MLV synthesis by a relatively high proportion of the cells. Exposure of growing cultures to 20 or 100 μg of IUDR or BUDR per milliliter for 24 to 48 hours has induced synthesis of MLV by as many as 0.5 percent of the cells within a week after addition of the drug, an increase of the activation rate by about 10^6 times, compared to the spontaneous rate. Activation of virus by IUDR or BUDR or both has been observed with lines 26 and 32, all 10 clones from them, and all 6 subclones tested. The efficiency of induction differs significantly between clonal lines. Induction of MLV synthesis by IUDR and BUDR also occurs in primary AKR embryo cells treated on the day after explantation. Table 1