Reduction of δ-Aminolevulinic Acid Dehydrase Activity in the Livers of Tumor-bearing Animals

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One of the most striking biochemical findings in both animal and human cancer is the reduction of hepatic catalase activity (4, 6–10, 14). In animals this reduction is progressive with the growth of the tumor and reversible (6, 10). Catalase activity is not reduced in the livers of pregnant animals or animals with subcutaneously implanted whole embryo tissue mashes (7). There are two exceptions to this finding: (*a*) mice bearing slowly growing tumors and (*b*) C57BL mice bearing the rapidly growing Sarcoma 37 (6). A soluble heat- and acid-stable material is produced by tumors which reduces catalase activity *in vivo* (3).

The enzyme involved in porphobilinogen synthesis (δ-aminolevulinic acid dehydrase or ALA dehydrase) from δ-aminolevulinic acid has been studied by Gibson, Neuberger, and Scott (2) and Shemin and co-workers (16). This report describes experiments which demonstrate that ALA dehydrase activity, like catalase activity, is reduced in the livers of tumor-bearing animals.

MATERIALS AND METHODS

The tumor-bearing animals were of the DBA, CDF_1, C57BL, CAF_1, C3H, and C3H/Heston strains. The following transplanted tumors were used: Leukemia L1210, Sarcoma 180, Lymphoma 4, Lymphoma P 388, Lymphoma P 388D1, Glioma 26, Sarcoma 37, Krebs 2 carcinoma, Ehrlich carcinoma, Hepatoma 129, and Hepatoma 134. At the time of study the tumors (except for those which were studied serially) were all large and constituted 10–80 per cent of the carcass weight. The control animals were of the same strain and sex and approximately the same age as the tumor-bearing animals.

ALA dehydrase activity was measured by the method of Gibson, Neuberger, and Scott (2) at 36.5° C. The coarse particulate matter of the homogenate was removed by centrifugation rather than filtration through muslin. The quantity of porphobilinogen in the final solution was calculated from the data of Cookson and Rimington (1).

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RESULTS

All results are expressed in units (1 μmole = 31.7 units) of porphobilinogen synthesized/hour/gm wet weight of tissue.

A-methopterin.—Fifty mg. of A-methopterin/kg was injected intraperitoneally into 35 DBA male mice. Each day for 6 days five treated and two control mice were sacrificed, and the hepatic ALA dehydrase was determined (see Table 1). Although this dose caused a 20 per cent mortality within 6 days, there was no effect on the level of enzyme activity. The effect of daily doses of 1 mg/kg is shown in Table 2; no effect was seen, although the drug was only given for 7 days.

Pregnancy.—Four pregnant CDF_1 mice were found to have normal levels of hepatic ALA dehydrase activity (footnote, Table 5). Another mouse liver (C3H female) examined at the end of gestation was found to have a normal value.

Tumor-bearing animals.—The enzyme activity levels in the livers of animals bearing various tumors are shown in Table 3. The enzyme activity is significantly reduced in all cases below that of controls of the same strain, sex, and age, although to different degrees with different tumors. The exception to this finding is presented in the last column of Table 3. All the tumors are transplanted mouse tumors, with one exception: a 25-month-old Sprague-Dawley female rat with a spontaneous tumor. The control was also a 25 month-old Sprague-Dawley female.

The effect of the progressive growth of leukemia L1210 is shown in Table 4. A distinct reduction in the hepatic enzyme level in the tumor-bearing host is not seen until the 7th day. Some of the reduction in this case may be related to hepatic infiltration, which occurs late in the growth of this tumor.

Comparison of the effect on hepatic ALA dehydrase activity of the rate of tumor growth is

possible through the use of a pair of tumors of common cell origin (P388 and P388dl),[1] but of different growth rates. When transplanted intraperitoneally, each grows as an ascites tumor and simultaneously in the same animal as a solid intramesenteric tumor, but without significant hepatic infiltration. Table 5 shows the progressive decrease in hepatic enzyme activity as tumor growth proceeds. Both the decrease and rate of decrease are greater with the rapidly growing form.

The exception to the usually observed decrease of hepatic ALA dehydrase activity was Glioma 8.4). Table 6 also shows that this tumor (Hepatoma 129) is capable *in vivo* of causing a reduction in hepatic ALA dehydrase activity.

Tumor values.—Table 7 shows that the enzyme level in a number of tumors is in the range of the normal tissues which have the lowest values. The variation among different tumors is less than that of normal tissues.

DISCUSSION

The fact that the enzyme activity involved in porphobilinogen synthesis is reduced in the livers

TABLE 1

THE EFFECT OF A LARGE DOSE OF A-METHOPTERIN (50 MG/KG) ON ALA DEHYDRASE ACTIVITY IN THE LIVERS OF DBA MALE MICE*

	Units (3.15×10^{-2} μmoles) of porphobilinogen synthesized/gm wet weight/hr					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
No. animals	5	5	5	5	3	3
Mean	49.0	48.0	45.9	47.0	47.4	40.3
Range	46.2–50.2	44.0–51.5	40.2–48.8	40.1–50.6	44.0–50.6	37.4–44.0

* The mean value in the livers of 47 normal DBA male mice was 47.4 ± 2.6 (standard deviation).

TABLE 2

THE EFFECT ON ALA DEHYDRASE LEVELS OF DAILY DOSES OF A-METHOPTERIN (1 MG/KG) IN DBA MALE MICE*

		Units (3.15×10^{-2} μmoles) of porphobilinogen synthesized/gm wet weight/hr			
		Day 1	Day 3	Day 5	Day 7
	No. animals	4	4	4	3
Liver*	Mean	48.2	42.7	44.7	45.8
	Range	42.7–55.0	42.3–43.1	41.8–47.5	42.7–48.5
Spleen	Normal		15.9	22.9	
	Treated	18.8	8.2		
Testis	Normal			17.6	
	Treated		15.0	14.4	
Brain	Normal		3.6		
	Treated		3.8		

* The mean value in the livers of 47 normal DBA male mice was 47.4 ± 2.6 (standard deviation).

26 in C57BL mice (Table 3). The level of hepatic ALA dehydrase is normally much lower in C57BL mice than in other strains of mice and was not reduced further by the presence of Glioma 26 after it had reached massive proportions.

Mixture of tumor and normal liver homogenate.—Table 6 shows that *in vitro* mixing of equal amounts of tumor and normal liver homogenate does not inhibit ALA dehydrase activity, since the value for the mixture was approximately equal to the sum of its component parts ($72.2 \cong 61.7 +$

[1] These two forms of a methylcholanthrene-induced mouse lymphoma were kindly supplied by Drs. Dawe and Potter of the National Cancer Institute. The original rapidly growing form (P388) after passage in tissue culture and then serially in mice led to the isolation of a more slowly growing derived form (P388dl).

of tumor-bearing mice appears to be well established by the above data. Whether this generality will hold for spontaneous animal tumors and human tumors remains to be established. The behavior of this enzyme appears, from the evidence thus far available, to parallel catalase in the following respects:

1. Pregnancy does not affect the level of either enzyme in the liver.

2. The level of hepatic activity of both enzymes is much lower (about one-half) in the C57BL strain of mice than in any other strain thus far examined.

3. The rapidly growing Sarcoma 37 does not lower the hepatic catalase level in C57BL mice, and Glioma 26 (a moderately rapidly growing tu-

TABLE 3

LEVELS OF ALA DEHYDRASE ACTIVITY IN THE LIVERS OF TUMOR-BEARING ANIMALS COMPARED WITH NORMALS OF THE SAME STRAIN, SEX, AND AGE*

Units (3.15×10^{-2} μmoles) of porphobilinogen synthesized/gm wet weight/hr

TUMOR		Sarcoma 37				Sarcoma 180	Hepatoma 129		Hepatoma 134	Krebs 2 carcinoma		Ehrlich carcinoma		Lymphoma 4	Spontaneous rat tumor	Glioma 26
Strain		CAF₁	CAF₁	CAF₁	CAF₁	CDF₁	C3H Heston		C3H Heston	CAF₁		CAF₁		CDF₁	Sprague-Dawley	C57BL
Sex		♂	♀	♀	♀	♀	♀		♀	♂		♀		♀	♀	♂
Tumor-bearing	No. animals	3	3	5	2	5	2	2	6	5	4	5	2	3	1	4
	Mean	39.7	29.0	31.8	32.6	29.1	40.3	34.6	38.3	43.9	35.3	43.0	33.9	29.1	18.1	25.2
	Range†	4.0	3.1	17.6	1.7	6.2	0.4	2.1	14.1	5.3	4.0	12.3	2.6	3.1		8.4
Normal	No. animals	9		3		20	7		7	9		3		20	1	7
	Mean‡	57.8 (2.0)		53.9		52.5 (2.5)	52.9 (2.6)		52.9 (2.6)	57.8 (2.0)		53.9		52.5 (2.5)	28.2	26.1 (2.0)
	Range†	6.1		5.7		9.6	8.1		8.1	6.1		5.7		9.6		6.3

* The tumor-bearing animals represented in a vertical column had tumors which were transplanted on the same day.

† The figures presented as the range represent the difference between the maximum and minimum observed values in a given group.

‡ The figures in parentheses under the mean values for the normals are the standard deviations which have been calculated when there were five or more animals in the group.

mor) does not lower the ALA dehydrase activity level in the liver of this strain.

Further studies are required to determine whether the other factors which are known to affect hepatic catalase activity levels will have an effect on ALA dehydrase.

The possible mechanisms for reduction of enzyme activity in the liver are: (*a*) decreased synthesis of the enzyme, (*b*) increased turnover or degradation rate, and (*c*) inhibition of the enzyme. The mechanisms whereby tumors may accomplish these effects have been discussed by Greenstein (5).

The question of whether the reduction of liver catalase activity in tumor-bearing animals is a result of a catalase inhibitor or whether there is a decrease in its synthesis is unresolved. Evidence exists on both sides. Hargreaves and Deutsch (12) have demonstrated that boiled extracts of tumors and, to a much lesser extent, boiled extracts of certain normal tissues could inhibit the activity of crystalline catalase. The data of Greenfield and Price (13) based on the actual isolation of catalase from the livers of tumor-bearing animals, however, indicate that there is actually less catalase than in normal livers. This would be in keeping with the possibility of a decreased ability to synthesize catalase in the livers of tumor-bearing animals. The fact that cytochrome oxidase is not present in reduced levels of activity in the livers of tumor-bearing animals (11, 15) indicates that, if there is any decrease in the ability of these livers to synthesize porphyrins, it is not of sufficient magnitude to affect the level of this enzyme.

While the simple precursors of the porphyrins are known, the pathway and enzymes involved in the conversion of porphobilinogen to protoporphyrin have not been resolved. From the presently available data it should not and cannot be inferred that reduction in ALA dehydrase activity results in a decreased capacity of the liver to synthesize protoporphyrin and, hence, catalase.

The experiment reported in Table 6 is a preliminary attempt to differentiate between a decrease in amount of enzyme in the liver and diminished activity as a result of an enzyme inhibitor produced by the tumor. An inhibitor could not be demonstrated when equal amounts of homogenates of Hepatoma 129 and normal liver were mixed. The possibility still exists either that an inhibitor might be present, but not in sufficient quantity in 1 ml. of homogenate to produce an effect, or that the conditions for its demonstration were inadequate.

ALA dehydrase activity levels in different tumors (see Table 7) are in the range of the normal

TABLE 4

THE EFFECT ON PROGRESSIVE GROWTH OF LEUKEMIA L 1210 ON ALA DEHYDRASE LEVELS IN VARIOUS TISSUES OF DBA MALE MICE*

Units (3.15×10^{-2} μmoles) of porphobilinogen synthesized/gm wet weight/hr

		Days after transplant							
		1	2	3	4	5	6	7	8
	No. animals	4	4	4	4	4	4	3	6
Liver*	Mean	48.8	49.0	42.1	42.4	46.6	48.9	26.8	26.1
	Range	46.2–50.2	48.4–49.7	41.8–42.3	37–45.7	35.2–53.7	40.1–54.2	22.0–34.3	22.4–31.3
Spleen	Tumor-bearing	25.8	19.4	4.1	19.5	16.3	18.5	9.0	10.8
	Normal		19.4	15.9	27.2				
Testis	Tumor-bearing		15.0	13.1	15.4	13.4	13.9		15.2
	Normal		15.3		17.3	17.6	14.1		20.0
Brain	Tumor-bearing			5.3	3.2		1.9		4.9
	Normal			3.6	1.8		4.6		5.7
Kidney	Tumor-bearing								16.9
	Normal								16.9

* The mean value in the livers of 47 normal DBA male mice was 47.4 ± 2.6 (standard deviation).

TABLE 5

THE EFFECT OF THE PROGRESSIVE GROWTH OF LYMPHOMAS P388 AND P388D1 ON ALA DEHYDRASE ACTIVITY IN THE LIVER OF CDF_1 FEMALES*

Units (3.15×10^{-2} μmoles) of porphobilinogen synthesized/gm wet weight/hr

P388

	Days after transplant			
	3	6	9	10
No. animals	5	5	5	5
Mean	49.5	41.9	20.1	19.1
Range	46.7–56.4	34.8–48.8	19.4–21.2	16.0–20.8

P388D1

	Days after transplant		
	5	12	15
No. animals	5	4	3
Mean	56.1	36.7	24.5
Range	53.7–60.7	33.0–39.2	23.8–26.4

* The mean values in the livers of 20 CDF_1 females was 52.5 ± 2.5 (standard deviation).

The mean value in the livers of two normal pregnant CDF_1 females was 55.9, and in two pregnant CDF_1 females 5 days after transplantation of P388D1 it was 57.2.

TABLE 6

THE EFFECT ON ALA DEHYDRASE ACTIVITY OF MIXING TUMOR (HEPATOMA 129) HOMOGENATE WITH NORMAL LIVER HOMOGENATE

	Animal no.	Units of porphobilinogen/gm wet weight/hr
Normal liver (C3H ♀)	1	61.7
Liver from C3H ♀ bearing Hepatoma 129	2a	43.0
Hepatoma 129	2b	8.4
Mixture of equal parts (1 ml. each) of normal liver homogenate and tumor homogenate	1+2b	72.2*

* This value represents the total units of porphobilinogen synthesized by 1 gm. wet weight of normal liver (1) and 1 gm. wet weight of hepatoma 129(2b) when mixed in equal proportions and is approximately equal to the sum of the component parts.

TABLE 7

ALA DEHYDRASE LEVELS IN TRANSPLANTED MOUSE TUMORS

		Units (3.15×10^{-2} μmoles) of porphobilinogen synthesized/gm wet weight/hr			
Sarcoma 37		4.9	4.0	3.5*	3.7*
Sarcoma 180			11.4		
Hepatoma 129			7.9	8.4*	
Glioma 26			3.2		
Harding-Passey melanoma			2.5		
Ehrlich carcinoma			4.2		
Krebs-2 carcinoma			6.0	5.4*	
Leukemia 1210			3.1		
Lymphoma 4			13.8		
P388 lymphoma	Solid form		8.6		
	Ascites form		9.5		
P388D1	Solid form		2.8	3.3*	

* These values are from separate tumors and do not represent duplicates done on the same tumor mass.

tissues which have the lowest values. There is less variation among different tumors than among normal tissues. These observations have been made on many other enzymes. The only tumor type whose normal tissue of origin was studied carefully was the heptaoma. It is seen that in the malignant transformation considerable loss of enzyme activity has occurred.

A-methopterin was studied for its effect on the enzyme, because it has been shown that folic acid deficiency in animals causes a marked drop in free erythrocyte protoporphyrin, thereby suggesting that folic acid is involved in porphyrin synthesis (17). The data in Table 1 indicate that a dose of A-methopterin sufficient to cause a profound interference with folic acid utilization had no effect on ALA dehydrase activity.

SUMMARY

1. The enzyme involved in the synthesis of porphobilinogen from δ-aminolevulinic acid (ALA dehydrase) has been shown to be present in reduced levels of activity in the livers of mice bearing a number of transplanted tumors.

2. From the data thus far available, the variations in ALA dehydrase activity levels resemble those previously found for catalase in that (*a*) pregnancy does not lower the hepatic activity of either enzyme and (*b*) the levels of both enzymes are much lower normally in the C57BL strain of mice than in others thus far examined and do not appear to be reduced further by tumors.

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