Changes in the Succinoxidase Activity of Livers from Rats during the Development of Hepatic Tumors on Feeding *p*-Dimethylaminoazobenzene

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In a series of papers, Salter and his collaborators (3, 11, 16) reported the results of their investigation of the respiratory activity of tumors of various origins in the presence of succinate or *p*-phenylenediamine as substrate. They took the oxygen uptake of homogenate or slices in a Ringer-glucose medium as basic oxygen consumption and found in tumor tissues a very small response to the addition of these substrates. The response in homologous normal tissue was much higher. These authors consider that the lowered capacity to oxidize the above substrates in tumor tissue "might be a useful adjunct to routine morphological investigation" (3). Rosenthal and Drabkin (15) point out that different normal tissues differ widely in their capacity to oxidize succinate or *p*-phenylenediamine and one might expect a difference between neoplastic and normal parent tissue only in cases when tumors arise in a tissue with normally high succinoxidase content. Detailed studies of the components of the succinoxidase system of tumors and normal tissues were carried out by Potter and his associates. DuBois and Potter (4) found that the cytochrome *c* is diminished in the tumor but unchanged in precancerous tissues. Using homogenized tissues Schneider and Potter (18) found a fairly constant succinic dehydrogenase and cytochrome oxidase content in 10 kinds of experimental tumors. These values were lower than those of normal tissue of high succinic dehydrogenase and cytochrome oxidase content but some normal tissues such as lung or spleen had activities even lower than those of tumor tissues. Liver tumors had only about one-fourth of the succinic dehydrogenase activity and about one-third of the cytochrome oxidase activity of normal liver.

Since Warburg's (19) discovery of the high aerobic glycolysis of tumor slices, theories have repeatedly been brought forward to explain carcinogenesis in terms of changes of enzymatic processes. A new impetus to these theories came from the work of Kensler and his group (10) who demonstrated that substances which were isolated from urine of rats fed *p*-dimethylaminoazobenzene (17) depress the activity of the diphosphopyridine enzyme system. Potter (13) found an inhibition of the succinic dehydrogenase system by similar substances. Potter (14) advanced a theory that cancer may be the result of a competition between a hypothetical enzyme (X) and a derivative thereof arising from it by the action of carcinogenic agents. While confirming the inhibition of the succinoxidase activity in normal liver tissue by aromatic diamines of the same type as the metabolites of *p*-dimethylaminoazobenzene, Elson and Hoch-Ligeti (6) established the fact that no depression by these aromatic diamines occurred in the succinoxidase activity of tumor tissue, or normal tissue of low metabolic activity. On the contrary, a prolonged increase of the O_2 uptake was observed in such tissues, due to slow oxidation of the diamines. It seemed possible that, as a consequence of treatment with *p*-dimethylaminoazobenzene, circulating metabolites might at first depress the high succinoxidase activity of normal liver. On further treatment with *p*-dimethylaminoazobenzene they might not be dealt with further as in normal tissue and so by cumulative action might introduce changes leading to neoplasm. It seemed, therefore, of interest to follow up the changes of the succinoxidase activity of livers from rats from the beginning of the treatment with *p*-dimethylaminoazobenzene until the development of tumors, and of the tumor tissue itself. Since the effect of the diet on the production of liver tumors with *p*-dimethylaminoazobenzene is well established the effects of the different diets on the succinoxidase of the liver were also studied.

EXPERIMENTAL

Albino rats of both sexes were used weighing about 100 gm. at the beginning of the experiment. They

were housed in the same room, in cages containing not more than 10 rats of the same sex. The diets were as shown in Table I. *p*-Dimethylaminoazobenzene in amount of 0.06 gm. was added in 2 ml. arachis oil to 100 gm. of the diet. The rats were allowed to consume the food and water *ad libitum*. The food consumption was higher in males and varied with the different diets, even in the absence of *p*-dimethylaminoazobenzene. It was highest, 12 to 16 gm. per day, in the groups of males receiving milk with diets not containing *p*-dimethylaminoazobenzene. On addition of *p*-dimethylaminoazobenzene to this diet the consumption dropped to about 6 to 8 gm. per day and was similar to that of animals on the basic rice diet. On addition of *p*-dimethylaminoazobenzene no further reduction of the food intake occurred on these diets without milk. Details of food intake and body weight are given elsewhere by Hock-Ligeti (7). The animals on the high-fat diet consumed about 10 gm. per day without, and 7 gm. per day with, addition of *p*-dimethylaminoazobenzene. Animals were sacrificed from the third day onwards at different intervals; the oldest rat was one killed on the 460th day of the experiment. Rats suffering from any disease other than tumors were discarded. The livers were removed as quickly as possible, weighed, and the succinoxidase content determined simultaneously in slices and in homogenates. Warburg-type manometers were used. The experiments were carried out at 38° C. Readings were taken every 5 minutes. Homogenate was prepared in M/30 phosphate buffer according to Potter and Elvehjem (12). The reaction mixture consisted of 1 ml. homogenate containing 40 mgm. of tissue; 0.5 ml. of 6 per cent succinate in M/10 phosphate buffer (pH.-7.35), 0.2 ml. of 1 per cent cytochrome *c* prepared according to Keilin and Hartree (9), 1 ml. M/10 phosphate buffer pH.7.35 and H_2O to make 3 ml. The thickness of the slices was 0.2 to 0.3 mm., the area about 50 sq. mm. and the final dry weight varied from 2 to 8 mgm. In experiments with slices 0.2 ml. of 20 per cent KOH solution was placed in the central cup and the volume of the homogenate was replaced by an equal volume of M/30 buffer. The gas phase was air, or in experiments with slices, oxygen. All experiments with homogenate were carried out with and without addition of an excess of cytochrome *c*. The addition of an excess of cytochrome *c* always resulted in an increase of the succinoxidase activity of the homogenate. No effect of this addition to the slices was found; this result is no doubt due to the fact that cytochrome c does not penetrate through intact cell membranes.

Forty milligrams of wet tissue per vessel gave oxygen uptakes which were not influenced by additions of aluminium and calcium, the use of which has been suggested by Horecker and his co-workers (8) and

TABLE I: ARRANGEMENT OF THE EXPERIMENT AND COMPOSITION OF THE DIET

Diet	Sex of animals	No. of animals	Body weight during the experiment	Caloric value of the diet: Total calories in 100 gm. of food	Caloric value of the diet: Percentage caloric value of the diet in: Carbohydrate	Protein	Fat
Rice, carrots + 2% arachis oil	♂	10	maintained				
" " " " "	♀	10					
Rice, carrots + 2% arachis oil + 0.06% *p*-DAB[1]	♂	10	slightly decreased	412	86.9	6.8	6.3
" " " " " " "	♀	20	" "				
Rice, 6% casein + 2% arachis oil + carrots	♀	10	maintained				
Rice, 6% casein + 2% arachis oil + carrots + 0.06% *p*-DAB	♀	30	"	436	82	12	6
Rice, 10 ml. milk + 2% arachis oil	♂	30	increased				
" " " " "	♀	20					
Rice 10 ml. milk + 2% arachis oil + 0.06 % *p*-DAB	♂	30	"	477	78.6	8.7	12.7
" " " " " " " " "	♀	20					
" " " " " " " " "	♂	10	"				
" " " " " " " " "	♀	20					
Rice, 10 ml. milk only	♂	20	"	460	81.6	8.9	9.5
" " " "	♀	10					
Rice, carrots	♀	10	maintained	396	90.5	7.1	2.7
Rice, carrots + 2% arachis oil + 0.06% azobenzene	♀	10	"	412	86.9	6.8	6.3
17% Protein, 30% fat	♀	10	increased				
17% Protein, 30% fat + 0.06% *p*-DAB	♀	20	"	480	53	17	30
" " " " " "	♂	10					

[1] *p*-DAB = *p*-dimethylaminoazobenzene.

Axelrod and his group (1) (Table II). By using less tissue the oxygen uptake per unit weight of homogenate was in many cases less than that found when 40 mgm. was used. On addition of aluminium and calcium the relation of oxygen uptake to the amount of tissue became generally linear, but in some cases when 10 mgm. of tissue was used with the addition of these ions the oxygen uptake per unit weight was much higher

TABLE II: EFFECT OF THE ADDITION OF 0.2 ML. 4×10^{-3}M $AlCl_3$ AND 0.2 ML. 4×10^{-3}M $CaCl_2$ ON THE SUCCINOXIDASE ACTIVITY OF 40 MGM. RAT LIVER HOMOGENATE

μl Oxygen uptake per gram wet tissue per hour

No. of Experiment	No cytochrome *c* added		Cytochrome *c* added	
	Without $AlCl_3$-$CaCl_2$	With $AlCl_3$-$CaCl_2$	Without $AlCl_3$-$CaCl_2$	With $AlCl_3$-$CaCl_2$
1	5,500	5,100	7,450	7,100
2	10,000	10,300	11,000	11,000
3	8,150	8,150	10,100	10,100
4	6,400	6,350	7,800	8,000
5	7,400	7,400	8,400	7,800
6	4,890	4,900	6,200	6,200
7	7,000	7,300	....	....
8	10,000	10,500	11,800	10,000
9	6,800	7,200	7,800	8,000

than that obtained with 40 mgm. (Table III). It seems that in some cases this addition increases the activity, in some other cases it counteracts only the dilution effect. The use of 40 mgm. of wet weight of tissue without addition of Ca and Al furnishes a reliable basis of comparison as long as the dry weight of the tissue is nearly the same. This is the case with normal livers, and also livers of animals fed *p*-dimethylaminoazobenzene, with or without development of liver tumors. They mean dry weight of livers from over 200 rats was 29.4 (range 26 to 36) per cent of wet weight. But with tumor tissue the dry weight varied

TABLE III: EFFECT OF THE ADDITION OF 0.2 ML. 4×10^{-3}M $AlCl_3$ AND 0.2 ML. 4×10^{-3} M $CaCl_2$ ON THE SUCCINOXIDASE ACTIVITY OF VARYING AMOUNTS OF RAT LIVER HOMOGENATE

μl. Oxygen per gram wet tissue per hour (Excess of cytochrome *c* added)

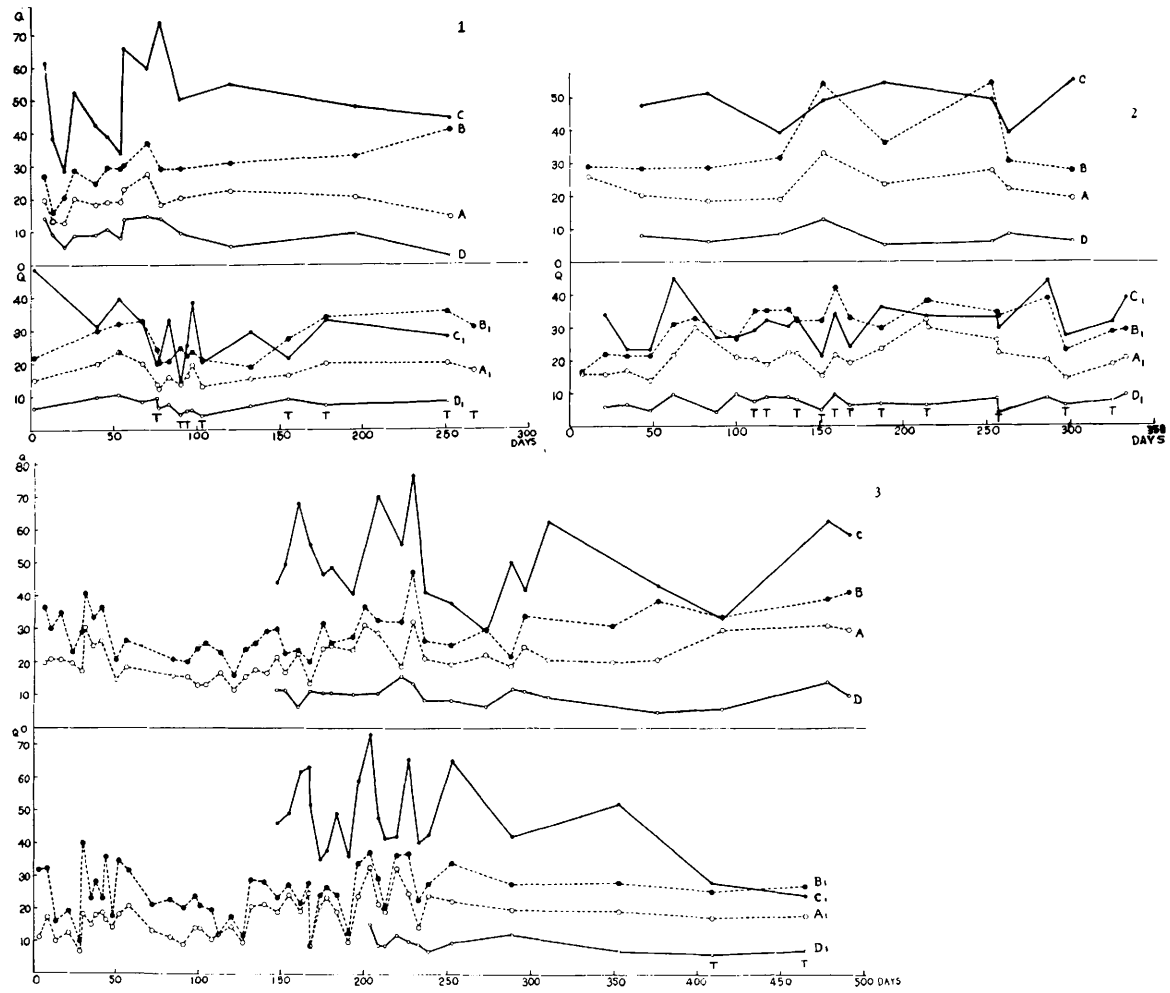
No. of Exper.	Amount of tissue employed, mgm.	Without $AlCl_3$-$CaCl_2$	With $AlCl_3$-$CaCl_2$
1	40	10,000	9,800
	20	3,700	9,600
	10	0	10,300
2	40	11,800	12,000
	20	11,100	14,200
3	40	7,600	8,400
	20	7,800	11,200
4	40	7,100	7,100
	20	4,750	9,200
	10	2,000	9,800
5	40	11,000	10,000
	20	9,400	12,000
	10	5,100	20,000
6	40	5,800	5,800
	20	1,650	5,600
7	40	10,350	10,350
	20	7,100	11,200

from 14 to 24 per cent of the wet weight. Thus using 40 mgm. of wet weight the amount of tumor tissue in each vessel was only about one half of that of the normal liver tissue when compared on the dry weight basis, and the addition of Ca and Al might seem necessary. In order to avoid a possible activation by these ions, which would make a comparison with normal liver tissue difficult, the tumor tissue was used in amounts double those of normal tissue (80 mgm.). Experiments on 40 mgm. of tumor tissue with addition of Ca and Al gave very similar values to those given by 80 mgm. of tissue alone.

TABLE IV: RELATION OF INITIAL TO FINAL DRY WEIGHT OF LIVERS FROM RATS FED ON A BASIC RICE DIET WITH OR WITHOUT ADDITION OF *p*-DIMETHYLAMINOAZOBENZENE

		Initial dry weight (a) as percentage of wet weight	Final dry weight (b) as percentage of wet weight	Mean	% Decrease on shaking $\left(\frac{a-b}{a} \times 100\right)$
Control diet		26.7	16.3, 16.7, 15.4, 15.4, 12.6	15.3	42.7
		30.2	16.5, 15.7, 16.5, 14.8, 16.0	15.8	47.7
		29.7	13.9, 12.7, 13.4, 12.9, 16.0	13.7	53.9
				Mean	48.1
Control diet + *p*-DAB	A	31.3	15.1, 15.7, 15.3, 15.1	15.3	51.1
		27.4	15.3, 15.3, 15.2, 14.8	15.1	44.9
		29.4	17.4, 17.9, 17.2, 14.7, 14.5	16.2	44.9
		28.6	12.2, 12.4, 11.4, 12.5, 14.8	12.5	56.3
				Mean	49.3
Control diet + *p*-DAB	B	29.0	14.1, 14.7, 14.7, 14.1, 12.8	14.1	51.4
		28.4	15.3, 15.3, 15.5, 15.5, 15.7	15.4	45.8
		26.8	13.2, 12.7, 14.8, 14.8, 15.5	14.2	47.0
				Mean	48.1
			MEAN	48.6	

A = livers of animals without tumors.
B = normal parts of livers of animals with liver tumor.



Oxidative activity of liver homogenates and slices.

Upper 4 curves in each figure represent oxygen uptake of livers from control rats; lower 4 curves, from rats on same diet with addition of 0.06 per cent azo compound. Values marked on any one ordinate were obtained from same liver. Oxidative activity is calculated as μl. oxygen per mgm. of initial dry weight per hour for homogenates and per mgm. of final dry weight per hour for slices.

A and A_1 O - - - O = Succinoxidase activity of homogenates without added cytochrome c. B and B_1 ● - - - ● = Succinoxidase activity of homogenates with excess cytochrome c added. C and C_1 ● —— ● = Succinoxidase activity of slices. D and D_1 O —— O = Oxygen uptake of slices without addition of succinate. A, B, C, and D represent values from control rats. A_1, B_1, C_1, and D_1 represent values from rats receiving *p*-dimethylaminoazobenzene. T = Tumor in liver. Estimations of oxidative capacity were carried out on tumor-free parts of liver.

FIG. 1.—Oxidative activity of liver homogenates and slices from rats on rice and carrots with and without addition of *p*-dimethylaminoazobenzene.

FIG. 2.—Oxidative activity of liver homogenates and slices from rats on rice, 6 per cent casein, and carrots with and without addition of *p*-dimethylaminoazobenzene.

FIG. 3.—Oxidative activity of liver homogenates and slices from rats on rice and milk with and without addition of *p*-dimethylaminoazobenzene (first series).

All results of oxygen uptake are calculated on a mgm. dry weight basis. In order to compare the oxygen uptakes per unit weight of liver tissue in homogenates and in slices it was necessary to establish for the present experiment the relation of the dry weight of the slices after having been subjected to the shaking in the Warburg vessel for 1½ to 2 hours at a rate of 110 to 120 per minute (final dry weight) to the dry weight of the original liver tissue (initial dry weight). The latter was determined on tissue which had been cut into small pieces before being dried (5). It was found that the tissue loses 42.7 to 56.3 per cent (mean 48.6 per cent) of its original weight during the shaking in the Warburg apparatus (Table IV). In the literature the factor of 50 per cent is generally used for calculating the final dry weight from the initial. Since this loss is variable the calculation of the oxygen uptake on the basis of the final dry weight of the slices, as is generally

done, involves some uncertainty. The use of the final dry weight for calculating the oxygen uptake is based on the assumption that this is the weight of the tissue active in the respiration (5). This holds as long as processes bound to intact cells are measured. But succinoxidase is active also in disintegrated tissue and the initial wet weight of the slices would be a better basis for calculation. On the other hand, the necessity for weighing the tissue slices before the experiment would prolong the time-interval between the killing of the animal and the immersion of the slices in the nutrient solution and affect the viability of the cells. In the tables the oxygen uptake of the homogenates is calculated on the basis of initial, that of slices on the basis of final, dry weight.

In comparing the succinoxidase activity of homogenates with that of slices it also has to be taken into account that the homogenates generally do not show any oxygen uptake without addition of substrate; while the slices have a definite oxygen uptake. Whether the succinoxidase activity found in the slices should be corrected for autorespiration has been questioned. Rosenthal and Drabkin (15) maintain that the oxidation of a substrate added in excess might completely suppress the autorespiration and so the two values are not superimposed.

TABLE V: OXIDATIVE ACTIVITY OF LIVER HOMOGENATES FROM RATS ON CONTROL DIETS AND ON DIETS WITH ADDITION OF *p*-DIMETHYLAMINOAZOBENZENE

			μl O_2 uptake with excess of Na succinate per mgm. initial dry weight per hour					
			No cytochrome *c* added			Cytochrome *c* added		
Supplement to rice diet	No. estimations	Sex	Range	Mean	Standard error	Range	Mean	Standard error
Carrots	7	♀	15-23	19.6	1.1	29-41	31.9	1.6
"	7	♂	13-28	18.9	1.9	16-29	26.2	2.6
Carrots + *p*-DAB	A 8	♀	12-24	17.7	1.3	19-23	25.0	2.0
	B 8		13-21	16.4	1.1	21-36	27.6	1.9
6% Casein + carrots	9	♀	19-33	23.2	1.6	28-54	35.5	3.6
6% Casein + *p*-DAB	A 12	♀	14-32	21.1	1.3	17-39	29.0	2.1
	B 11		14-30	20.8	1.4	23-42	33.0	1.9
	C 1		....	3.7	..	....	6.2	..
Carrots†	10	♀	21-33	24.5	1.3	27-47	35.1	2.4
Carrots, azobenzene	9	♀	16-23	18.7	0.9	18-32	25.0	2.0
Milk†	*9	♀	26-36	28.6	1.0	30-45	36.2	1.0
	8		26-36	28.9	1.1	31-45	37.0	0.7
" †	*15	♂	11-27	18.6	1.1	19-38	24.8	1.4
	6		16-27	21.8	1.7	24-38	29.9	2.0
"	*17	♀	15-31	21.9	1.3	20-41	29.0	1.6
	11		19-31	24.5	1.3	23-41	31.0	1.9
"	*24	♂	11-32	20.3	1.2	16-47	29.1	1.6
	11		13-32	22.0	1.7	20-37	30.4	2.5
Milk + *p*-DAB Series 1	A *18	♀	11-33	19.7	1.3	12-37	28.8	1.6
	13		19-33	21.8	1.2	21-37	28.0	1.6
	B 2		17-18	17.7	..	25-27	26.2	..
	A *25	♂	7-32	15.7	1.2	9-40	24.4	1.7
	6		9-32	19.3	3.7	9-36	23.1	4.3
Milk + *p*-DAB Series 2	A 12	♀	19-32	24.9	1.2	25-44	34.2	1.7
	B 1		....	23.5	..	....	34.2	..
	C 1		....	2.7	..	....	6.2	..
	A 6	♂	18-27	23.5	1.3	28-46	36.3	2.6
30% Fat, 17% protein, carrots†	9	♀	8-25	17.1	2.0	19-33	27.3	1.7
30% Fat, 17% protein, carrots + *p*-DAB†	A 1	♀	....	18.6	..	....	27.8	..
	B 5		12-20	14.7	1.4	25-42	32.3	4.2
	C 5		0.5-4	2.6	0.6	0.8-28	13.0	5.5
30% Fat, 17% protein, carrots + *p*-DAB†	A 2	♂	11-21	15.8	..	16-35	25.4	..
	B 3		5-17	12.9	..	7-25	17.8	..
	C 2		3-5	3.8	..	5-7	6.1	..

With the animals receiving *p*-dimethylaminoazobenzene, A = Liver of animals without tumors; B = Normal parts of livers of animals with liver tumor; C = Tumor of the liver.

* Including experiments carried out on homogenate only. Lower values are comparable with the values given in Table VI.

† With exception of groups so marked, all diets contain 2% arachis oil.

RESULTS

(*a*) *Homogenates.*—Figs. 1 to 6 show the succinoxidase activities of all rat livers estimated in slices and in homogenates. Table V summarizes the findings. Considering the values for oxygen uptake of homogenates of rats on different control rice diets without addition of *p*-dimethylaminoazobenzene there is a slight increase in the oxygen uptake with increasing protein content of the diet, both with and without addition of an excess of cytochrome *c*. This seems to point to an increasing succinic dehydrogenase content of livers with increasing protein in the diet. But in all the animals on control diets without addition of arachis oil the succinoxidase and cytochrome c content of the homogenates is higher than that with diets containing arachis oil. In the semisynthetic diet with much higher protein content, but also much higher fat content, the succinoxidase activity is the lowest. It seems that, keeping the fat values constant, the succinoxidase activity varied with the protein in the diet, but that a high fat content in the diet diminished the succinoxidase activities even of livers from rats receiving fairly high (17 per cent) protein. The tumors grew much more quickly on the fat-rich diet; the time before their appearance however was not shortened.

Eighty to 100 per cent of the rats which had received *p*-dimethylaminoazobenzene with the rice, rice and casein, or the fat-rich diet, developed tumors in the liver after 90 days. The tumors which developed in animals on rice diet were generally too small for separate estimation of the succinoxidase content. During the whole course of the experiment the succinoxidase activities of the liver homogenates of these rats were slightly lower than those of animals on the same diet without addition of the dye. The difference however was in no case statistically significant. Similarly the decrease of the succinoxidase activity in the healthy parts of the livers from animals developing tumors was not significant. But the tumor tissue itself showed always a very low oxygen uptake without addition of cytochrome *c*. On the addition of cytochrome *c* the succinoxidase activity increased and in two cases reached a value as high as found in normal liver tissues.

A curious behavior was observed in the first series of animals (50 rats receiving *p*-dimethylaminoazobenzene in the rice diet with addition of milk. The oxygen uptake in the presence of an excess of succinate without addition of cytochrome *c* dropped to a very low level 3 days after the beginning of the experiment (Fig. 3) and remained low until the 150th day. At that date the oxygen uptake rose to normal values and remained normal until 460 days. The succinoxidase activity of the homogenate with excess of cytochrome *c* was about the same during the whole course of the experiment. Thus the percentage increase of the oxygen uptake on addition of cytochrome *c* was much higher in the first 150 days. These rats did not develop hepatic lesions until the 430th day. The 2 rats killed on the 430th and 460th days respectively showed incipient cystic cholangiomas. In a second series of 30 rats this initial fall in the succinoxidase activity was not observed. Four of these animals developed tumors of the liver (but only in one case was the succinoxidase activity estimated).

No other differences could be observed between the 0_2 uptake with the added Na succinate of homogenates of livers from rats on control diets and on the same diets containing *p*-dimethylaminoazobenzene, irrespective of whether the animal developed tumors or not. Changes observed in the succinoxidase activity of liver homogenates from rats receiving 0.06 per cent azobenzene with a rice carrot diet were not significant.

(*b*) *Slices.*—The succinoxidase activities of liver slices from animals on rice, or rice and casein diets dropped on addition of *p*-dimethylaminoazobenzene. A further drop was found in normal parts of the livers from animals bearing tumors, and in tumors very low figures were obtained. In the first series of animals on the rice and milk diet, where the rats were protected considerably from tumors of the liver, the succinoxidase was not estimated in slices during the first 150 days of the experiment. After the 150 days, when the succinoxidase activity of the homogenates suddenly rose, the succinoxidase activities of the slices were within the normal range. The two rats which developed tumors had low succinoxidase activity in the slices. In the second series of rats on rice and milk diet with *p*-dimethylaminoazobenzene the succinoxidase activity of the slices was depressed.

The great discrepancy between homogenate and slices in the response of the succinoxidase activity to the feeding of *p*-dimethylaminoazobenzene could suggest that the basis of comparison might be inadequate in these cases. Since the values for the slices are calculated from the final dry weight one might assume that during the shaking in the Warburg vessel the liver tissues from animals receiving *p*-dimethylaminoazobenzene lose less weight and consequently the values for the oxygen consumption divided by abnormally high values for the dry weight will give very low figures for succinoxidase activity. The histological evidence of cirrhosis in these livers would give support to this assumption. To test this, initial wet weights and final dry weights of livers from control rats and rats receiving *p*-dimethylaminoazobenzene were measured. No differences in the different livers were found (Table IV).

The values of the oxygen uptake of the slices from

livers of rats on the control diets divided by the ratios of initial to final dry weight give exactly the values obtained with homogenates without the addition of cytochrome *c*. This seems to suggest that the effective succinoxidase activity of the intact tissue is equal to the succinoxidase activity of homogenates to which no cytochrome *c* is added; which would also imply that, in rats under the described dietary conditions, not all the succindehydrogenase is active *in vivo*. In slices of livers from rats receiving *p*-dimethylaminoazobenzene the oxygen uptake is lower than that calculated from the oxygen uptake of homogenate and the ratio of initial to final dry weight.

DISCUSSION

The absolute values for the succinoxidase activity for the various organs even in the same species of animal given in the literature vary widely; *e.g.* for rat liver slices Roskelley and his co-workers (16) give Q suc from 18.5 to 23.1 and Rosenthal and Drabkin (15) Q suc 46.7 ± 1.51 as a mean. For liver homogenates Elliott and Greig (5) give the values 66μ 0_2 and Schneider and Potter (18) 87.7 (from 76.8 to 101μ 0_2) for 1 mgm. of dry tissue for 1 hour. The relative order of the succinoxidase content of different tissues is generally found the same. The experiments presented here and other experiments on the influence

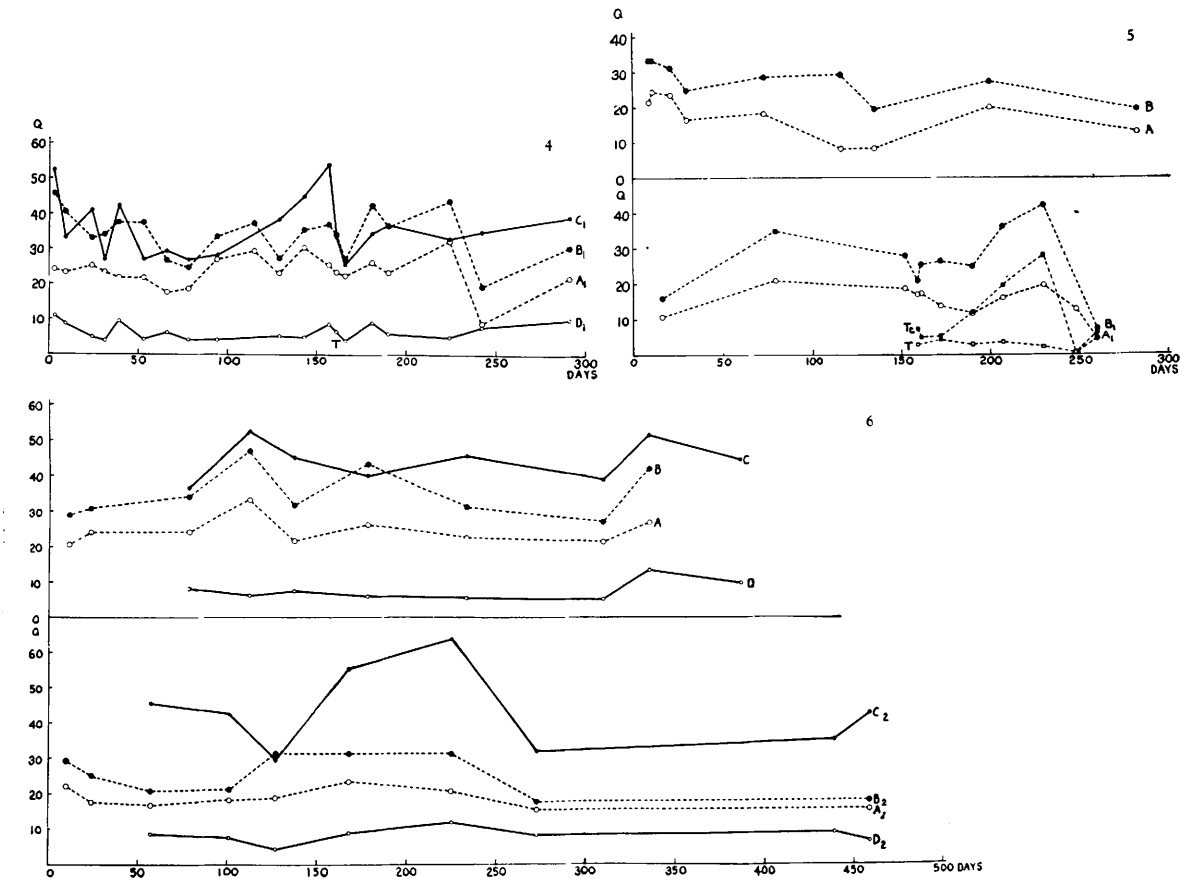


FIG. 4.—Oxidative activity of liver homogenates and slices from rats on mice and milk with addition of *p*-dimethylaminoazobenzene (second series).

FIG. 5.—Oxidative activity of liver homogenates from rats on diet containing 17 per cent protein and 30 per cent fat with and without addition of *p*-dimethylaminoazobenzene. T □ - - - □ = Succinoxidase activity of tumor tissue homogenates without addition of cytochrome c. T_c ● - - - ● = Succinoxidase activity of tumor tissue homogenates with excess cytochrome c added. The values (A_1 and B_1) above T and T_c are obtained in tumor-free parts of livers.

FIG. 6.—Oxidative activity of liver homogenates and slices from rats on rice and carrots without arachis oil, and same diet with addition of 0.06 per cent azobenzene in 2 per cent arachis oil. A and A_2 O - - - O = Succinoxidase activity of homogenates without added cytochrome c. B and B_2 ● - - - ● = Succinoxidase activity of homogenates with excess cytochrome c added. C and C_2 ● —— ● = Succinoxidase activity of slices. D and D_2 O —— O = Oxygen uptake of slices without addition of succinate. A, B, C, and D represent values from control rats. A_2, B_2, C_2, and D_2 represent values from rats receiving azobenzene.

TABLE VI: OXIDATIVE ACTIVITY OF LIVER SLICES FROM RATS ON CONTROL DIETS AND ON DIETS WITH ADDITION OF *p*-DIMETHYLAMINOAZOBENZENE

Supplement to rice diet	No. estimations	Sex	μl. O_2 uptake with excess of Na succinate per mgm. final dry weight per hour: Found Range	Found Mean	Found Σ	Corrected for autogenous O_2 uptake Range	Corrected for autogenous O_2 uptake Mean	Corrected for autogenous O_2 uptake Σ
Carrots	7	♀	34-74	51.5	5.5	26-60	42.4	4.7
"	7	♂	29-62	47.8	4.5	23-47	37.7	3.4
Carrots + *p*-DAB	A 8	♀	21-49	34.3	2.9	14-42	26.5	2.9
	B 8		15-33	23.8	2.2	10-26	16.9	2.0
6% Casein + carrots	9	♀	39-55	47.8	2.1	30-49	40.2	2.6
6% Casein + carrots + *p*-DAB	A 12	♀	23-45	32.4	2.3	17-36	25.5	2.0
	B 11		21-36	30.1	1.5	17-29	22.7	1.2
	C 1		....	13.5	..	....	5.9	..
Carrots*	10	♀	37-52	44.3	2.0	28-46	36.6	1.8
Carrots, azobenzene	9	♀	30-64	43.3	4.1	24-52	35.0	3.6
Milk*	8	♀	53-78	63.4	1.1	35-69	54.0	1.3
" *	6	♂	43-85	67.8	5.2	32-73	58.2	5.1
Milk	11	♀	33-70	50.3	4.2	27-62	41.5	4.0
"	11	♂	29-77	51.1	4.0	23-63	39.9	3.6
Milk + *p*-DAB Series 1	A 13	♀	35-73	51.6	3.4	25-58	41.4	3.3
	B 2		28-24	26.0	..	22-17	19.3	..
" "	A 6	♂	36-63	47.7	3.7	26-53	37.5	3.9
Milk + *p*-DAB Series 2	A 12	♀	27-45	35.4	2.0	22-40	29.3	1.8
	B 1		....	33.5	..	....	26.9	..
	C 1		....	12.4	..	....	....	..
" "	A 7		27-54	36.9	4.3	23-45	29.6	3.6

With the animals receiving *p*-dimethylaminoazobenzene, A = livers of animals without tumors; B = normal parts of livers of animals with liver tumor; C = tumor of the liver.

* With the exception of the groups so marked, all diets contain 2% arachis oil.

of different diets carried out in this Institute (Elson, L. A., in press) show that the succinoxidase content of liver can be very much depressed by dietary means. Workers in America report generally higher succinoxidase values for rats than do workers in England. Perhaps the standard diet of the American rats is better. In experiments with diets containing high percentages of fat low succinoxidase values in the liver of rats were found; values which were well within the range for hepatic tumors from rats. The figures for succinoxidase for homogenates of hepatomas produced by *p*-dimethylaminoazobenzene is given by Schneider and Potter (18) as Q suc 25.0 0_2. This figure is about a third of their values for normal livers but differs only by about 20 per cent from the values for normal livers as found here. On the other hand, not every hepatic tumor produced by *p*-dimethylaminoazobenzene has a low succinoxidase activity when measured as homogenate with an excess of cytochrome *c*; two tumor homogenate showed high values. The low oxygen uptake without added cytochrome *c* might indicate a low cytochrome *c* content of this tissue.

The phenomenon, that the succinoxidase activity of liver from rats receiving *p*-dimethylaminoazobenzene is lower than in the control when estimated in slices but about the same when estimated in homogenates opens several theoretical possibilities:

(a) It would seem that characteristic differences between the enzymatic behavior of livers from rats on a control diet and livers from rats developing tumors on the same diet with *p*-dimethylaminoazobenzene are connected with the intact cells. The slight differences in activity found with homogenates may be due to some intact cells still present. Enzymic activity is in many cases connected with intact cell structures. Yudkin (20) showed that the activity of glucose dehydrogenase of *Bact. coli* and lactic dehydrogenase of the *Micrococcus lysodeikticus* is linked with the structure of the cells. In the experiments presented here, however, the activity in the intact cells is lower than in the disintegrated cells. Since the ratio of initial to final dry weight in the livers which developed tumors is not different from that obtained with normal tissue, and since it is not likely that the permeability of the cell membranes to Na succinate is changed, a possible explanation of this phenomenon could be a changed mutual accessibility of the parts of the sucinic oxidase system in the intact cell. A varying accessibility of enzyme to its substrate was first suggested by Claude Bernard (2) to explain the simultaneous presence of both

glycogen and diastatic enzyme in the liver of the hibernating frog, without formation of larger amounts of glucose.

(b) The low activity of liver from animals fed *p*-dimethylaminoazobenzene may also be the result of one part of the enzymatic system being blocked by the carcinogenic substance or by a metabolite. Substances connected with the metabolism of *p*-dimethylaminoazobenzene are known to inhibit the succinoxidase activity of the liver. If such substances are present in the liver cells they might suppress the succinoxidase activity of the slices. On disintegration of the cells and dilution of the cell content the concentration of the inhibitory substance might become too low to be effective. It might be significant that in the groups of animals in which, by addition of fresh milk to the diet the formation of hepatic tumors was largely prevented, no depression of the succinoxidase activity in liver slices was found with the exception of 2 animals that had incipient cholangiomas.

SUMMARY

1. The succinoxidase activity of rat liver slices and homogenates was studied during the course of development of hepatic tumors due to feeding of *p*-dimethylaminoazobenzene. The diets of the animals consisted of rice and carrot, rice, casein and carrot, rice and milk, or a semisynthetic diet containing a high percentage of fat.

2. The succinoxidase activity of the homogenates of livers from the rats not receiving *p*-dimethylaminoazobenzene varied slightly with the diet; it was lowest with the high-fat diet and highest with the rice diet containing 6 per cent casein, where the percentage of fat was lowest.

3. After the addition of Na succinate the 0_2 uptake of homogenates of liver, with and without excess of cytochrome *c*, from rats receiving *p*-dimethylaminoazobenzene was only slightly lower than the control irrespective of whether the individual animal had developed a hepatic tumor or not. The tumors themselves showed generally a low succinoxidase activity.

4. Slices of liver from animals subsequently developing tumors on *p*-dimethylaminoazobenzene had lower succinoxidase activity than the controls. The succinoxidase activity was further depressed on the development of tumors and was very low in tumor tissue.

5. In one series of rats where the addition of milk largely prevented the development of hepatic tumors the succinoxidase activity of liver slices did not differ from that in rats on the control diet.

6. The addition of azobenzene to a rice-carrot diet had no effect on the succinoxidase activity of the homogenates or slices of livers.

7. The discrepancy between the values for succinoxidase found when using homogenates, or slices of the same liver from animals fed *p*-dimethylaminoazobenzene might be explained by a different accessibility of the parts of the enzymic system or by assuming an intracellular inhibition in these livers.

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