Serum para-Phenylenediamine Oxidase and Xanthine Dehydrogenase Levels during Liver Carcinogenesis in the Rat

Recently it was reported that the levels of serum para-phenylenediamine (PPD) oxidase and xanthine dehydrogenase (XD) may be temporarily lowered and raised respectively following single intraperitoneal injections of powerful hepatocarcinogens into albino rats[1]. We have now studied the changes in the levels of these enzymes during the course of induction of liver tumours by oral administration of 3'-methyl-4-dimethylaminoazobenzene (3'-MeDMAAB) to rats.

Serum samples were obtained by tail-bleeding 10 young adult male albino rats and the enzymes were determined as previously described[1]. On the following day (15. 7. 58) the rats were divided into two equal groups, one of which received a daily feed of 100 g of pulverised Diet No. 86 (prepared in pellet form by the North Eastern Agricultural Cooperative Society of Aberdeen) containing 0·06% W/W of 3'-MeDMAAB and made into a paste with tap water (60 ml). The other group received the same regimen but without the azo dye. Both groups were provided with tap water *ad libitum*. The diets were supplied for 155 days until 8. 12. 58 after which both groups were fed Diet No. 86 until the experiment was terminated on 24. 3. 59 when the last surviving azo-fed rat had to be killed because of a large liver tumour. At intervals, serum PPD oxidase and XD were determined and on several occasions blood haemoglobin and methaemoglobin levels were estimated by the method of VANDENBELT *et al.*[2].

A statistical analysis of the changes in serum PPD oxidase activity is given in Table I, but data for one of the azo-fed rats has not been included in the analysis. Although this rat responded initially to dyefeeding by a drop in serum PPD oxidase level the decrease was less than that shown by the other 4 rats. Also it behaved abnormally in two other respects. Thus after about 1 month on the diet, the growth of the animal became severely retarded and remained so until it died suddenly on 19. 1. 59 with a moderately large liver tumour. Moreover, the rat developed an anaemia which was more intense than that exhibited by other members of the azo group (see below).

[1] W. J. P. NEISH, Exper. *14*, 287 (1958); *15*, 20 (1959); Naturwissenschaften *46*, 175 (1959).

[2] J. M. VANDENBELT, C. PFEIFFER, M. KAISER, and M. SIBERT, J. Pharmacol. exp. Therap. *80*, 31 (1944).

Table I

Serum PPD oxidase levels in groups of rats kept on a diet containing the azo dye 3'-MeDMAAB and on a normal diet

Group		1 day before diet commenced	Days after diet commenced			
			21	62	120	154
Azo (n = 4)	mean weight (g)	163 ± 19+ (139)†	182 ± 13 (155)	224 ± 11 (186)	262 ± 16 (194)	269 ± 22 (197)
	PPD range	0·295 – 0·632	0·238 – 0·287	0·161 – 0·222	0·311 – 0·358	0·309 – 0·442
	PPD mean ($\bar{x}_A$)	0·502 (0·623)†	0·258 (0·488)	0·184 (0·451)	0·332 (0·574)	0·367 (0·572)
	PPD σ_A^2*	0·022346	0·000467	0·000714	0·000378	0·003478
Normal (n = 5)	mean weight (g)	153 ± 12	190 ± 12	228 ± 19	263 ± 23	273 ± 21
	PPD range	0·318 – 0·592	0·311 – 0·456	0·318 – 0·459	0·377 – 0·471	0·330 – 0·612
	PPD mean (x_N)	0·425	0·381	0·361	0·401	0·439
	PPD σ_N^2	0·011091	0·004211	0·003232	0·001543	0·009020
	PPD t**	0·9 (< 5% level)	4 (> 1% level)	6·1 (> 0·1% level)	3·4 (1% level)	1·5 (< 5% level)

+ Standard deviation † Bracketed values refer to the rat omitted from the analysis.

* Best estimate of variance, $\sigma^2 = \frac{\Sigma(x-\bar{x})^2}{n-1}$.

** t test, $t = \frac{\bar{x}_A - \bar{x}_N}{\sqrt{\frac{\sigma^2_A}{n_A} + \frac{\sigma^2_N}{n_N}}}$

Table II

Group	Rat	Days after diet commenced				
		154	189	202	223	251
Azo Diet	1*	13m45s	19m	14m	7m45s	–
	4+	13m15s	20m30s	–	–	–
	5++	not done	12m30s	14m	14m45s	16m30s
Normal Diet	range	8m30s–11m	8m30s–14m30s	9m30s–12m45s	9m30s–12m15s	9m–10m30s
	mean values for	9m45s	11m	11m	11m	10m
		5 rats		4 rats		

* Killed at 223 days; total weight of liver + tumour = 31 g.

+ Killed at 190 days; total weight of liver + tumour = 40 g.

++ Killed at 251 days; total weight of liver + tumour = 44 g.

Table I shows that ingestion of the azo carcinogen led at first to a decline in the serum PPD oxidase levels of the experimental rats. The difference between the mean levels of PPD oxidase activity for experimental and control groups, already pronounced at 21 days, was very marked at 62 days from the start of the feeding experiment. Thereafter, in spite of continued ingestion of the azo dye, serum PPD oxidase levels of the experimental group began to rise and by 154 days there was no significant difference between the mean levels of this enzyme in the experimental and control groups.

When large liver tumours were present in the azo-fed rats, serum PPD oxidase levels remained high (0·4–0·6) so there is no question of permanent suppression or deletion of the enzyme during hepatocarcinogenesis. MILLER and MILLER[3] found that the ability of rats to bind azo dye to their liver proteins began to decline after the rats had been fed for about 2 weeks on a diet containing 3'-MeDMAAB. ARCOS and ARCOS[4] observed that the swelling ability of liver microsomes decreased to a minimum at 4 weeks with the feeding of 3'-MeDMAAB and again increased until normal levels of swelling were attained after 20 to 24 weeks of dye feeding. A diet containing the non-carcinogen 2-methyl-4-dimethylaminoazobenzene failed to influence the swelling of rat liver microsomes after 4 weeks. ROY, MIYA, and CARR[5] found that the total non-protein sulphydryl content of the livers of rats fed 4-dimethylaminoazobenzene reached a minimum value after 12 weeks on the diet. After 20–24 weeks, however, the sulphydryl content had returned to normal levels. In the present experiment we have indications that yet another early effect of the azo carcinogen appears to be overcome as dye-feeding is continued.

Although single intraperitoneal injections of 3'-MeDMAAB as well as of other hepatocarcinogens may produce a temporary increase in serum XD, no significant difference between the mean XD levels of experimental and control groups was found after 21, 62, 120, and 154 days of dye-feeding. It was found however that XD levels of azo-fed rats began to decline within a few weeks after the end of azo dye-feeding. This trend is shown in Table II which also records XD values for control animals. Serum XD levels began to decrease during liver tumour growth and we have noted in another series of rats which had been on a diet of 4-dimethylaminoazobenzene that low serum XD values may be encountered even before liver tumours are palpable. It is of interest that the growth of a transplantable sarcoma Rd/3 in the same rat strain leads to a marked depression in serum XD activity.

Finally, reference is made to the anemic condition of rats fed 3'-MeDMAAB. After 120 days on the diet, hemoglobin levels (as measured by the method of VANDENBELT *et al.*[2] and expressed as an optical density difference at 630 mμ) ranged from 0·229 to 0·254 (4 rats) with a mean value of 0·242. The normal group (5 rats) had values in the range 0·276–0·283 with mean = 0·279. Application of the t test gave $t = 6{\cdot}8$ ($> 0{\cdot}1\%$ level) showing that the difference between the means is highly significant. The azo-fed rat excluded from our analyses had a hemoglobin level of 0·195. Essentially the same results were obtained after 154 days on the diet.

The azo carcinogen has a weak methemoglobinogenic action when given orally to rats (a mean value of 1·7% as compared with 0·2% for the control group at 120 days from the start of dye-feeding) which confirms our previous finding[6] of this condition following single intraperitoneal injections of 3'-MeDMAAB into rats.

It is noteworthy that all the azo-fed rats developed liver tumours which proved to be cholangiocarcinomata. Attempts to transplant several of these tumours met with no success.

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Zusammenfassung

Werden Ratten mit dem carcinogenen 3'-Methyl-4-dimethylaminoazobenzol gefüttert, so nimmt die Konzentration der p-Phenylendiaminoxidase im Serum anfangs rasch ab, um am Ende der Fütterungsperiode langsam wieder auf den Normalwert anzusteigen.

Eine Verminderung der Xanthindehydrogenase des Serums war nur zu Beginn des Wachstums von Lebertumoren zu beobachten. Mit Azofarbstoffen gefütterte Ratten wurden anämisch.

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Antibodies in the Course of Resorption of the Ehrlich Cancer Heterotransplant in Rats

Mice tumours heterotransplanted to newborn white rats[1,2] or to white rat embryos[3,4] at first grow at a high rate and attain a large size, while in more than two-week old rats they degenerate and are completely resorbed. Resorption of the tumour heterotransplants under these conditions is indicative of lack of acquired tolerance[4], although tolerance to skin homotransplants can be elaborated in less than 2-week old rats[5,6]. The mechanism of transplant resorption in 2-week old rats is still obscure.

In the present study, we have followed up the dynamics of antibodies against tumour tissue in sera of rats with resorbing Ehrlich cancer transplant. For this purpose, the agglutination reaction of tumour cells was tested as well as cytotoxic action of sera upon tumour cells in a test tube and *in vivo-in vitro* neutralization.

Methods. The Ehrlich ascitis adenocarcinoma was subcultured to non-inbred strains of white mice. The ascitis fluid was procured from the mice on the $7-12^{th}$ day after inoculation. The cells were thrice washed with Ringer and 0·25 ml of the suspension of tumour cells containing 160 million cells per 1 ml were injected under the skin of the back of 1–2-day old white rats.

A total of 112 young rats of 12 litters have been inoculated, while 46 normal young rats of 5 litters were used as

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