Effect of Hepatocarcinogens on Rat Serum *para*-Phenylene Diamine Oxidase and Xanthine Dehydrogenase

Recently[1], it was shown that the serum para-phenylene diamine (PPD) oxidase level of adult albino rats was markedly suppressed after the intraperitoneal injection of some powerful azohepatocarcinogens. On the other hand, it was found that injections of the arachis oil vehicle alone or of solutions of non-hepatocarcinogenic azo dyes as well as of various other carcinogens (which attack organs other than the liver) and non-carcinogenic substances led to a progressive increase in the PPD oxidase activity of serum samples obtained at intervals during the first few days following injection. Each substance was applied at a dose level corresponding on a mole for mole basis to a convenient standard injection of 16·5 mg of 3'-methyl-4-dimethylaminoazobenzene (m. w. 239) in 0·6 ml of arachis oil per 100 g of body weight (b.w.).

At equimolar dose levels, unless otherwise stated, intraperitoneal injections of some other kinds of rat liver carcinogen have now been found to produce similar suppressive effects (see Table). Thus DL-ethionine[2] (but not DL-methionine; a convenient reference substance with no known carcinogenic activity) was active, and tannic acid[3] (5 mg/100 g b.w.) powerfully suppressed rat serum PPD oxidase.

[1] W. J. P. NEISH, Exper. *14*, 287 (1958).

[2] E. FARBER, Proc. Amer. Ass. Cancer Res. *2*, 15 (1955); Cancer Res. *16*, 142 (1956).

[3] B. KORPÁSSY and M. MOSONYI, Brit. J. Cancer *4*, 411 (1950).

Carbon tetrachloride, which is apparently not hepatocarcinogenic for rats[4], depressed rat serum PPD oxidase but only when applied at levels several times greater than our standard dose. At the level of 0·02 ml/100 g b.w. (approximately half as much again as the standard level), carbon tetrachloride caused only a steady increase in PPD oxidase activity.

The tranquilizer drug, chlorpromazine, when applied at half the standard dose (a level at which this compound is highly toxic), strongly suppressed PPD oxidase, but it was inactive at 2·5 mg/100 g b.w. This substance is said to enhance the carcinogenic activity of 4-dimethylaminoazobenzene[5] and our results suggest that it might be carcinogenic in its own right.

The rather feeble liver carcinogens, thioacetamide[6], and *p*-ethoxyphenylurea[7] failed to suppress PPD oxidase activity under our experimental conditions.

As we had found with the strong azohepatocarcinogens[1], serum copper levels were markedly depressed in rats which exhibited low levels of serum PPD oxidase due to injections of tannic acid or carbon tetrachloride.

In our earlier studies with azo dyes, it was noted that some strong azohepatocarcinogens not only suppressed

[4] J. L. HARTWELL, *Survey of compounds which have been tested for carcinogenic activity*, Suppl. I, p. 29 (1957), U.S. Public Health Service.

[5] K. PETERS, W. KRAIS, and H. DORN, Arch. exp. Path. Pharmak. *229*, 182 (1956).

[6] O. G. FITZHUGH and A. A. NELSON, Science *108*, 626 (1948).

[7] J. L. HARTWELL, *Survey of compounds which have been tested for carcinogenic activity*, Suppl. I, p. 102 (1957), U. S. Public Health Service.

Table. Effect of various hepatocarcinogenic and related substances injected intraperitoneally on the serum paraphenylene diamine (PPD) oxidase and xanthine dehydrogenase (XD) levels of pairs of adult albino rats

Substance (mg or ml/100 g bodyweight) injected as suspension or solution in arachis oil	PPD oxidase activity[8] (expressed as optical density at 530 mμ)			XD activity** (expressed as tome (min, s) for 100% reduction of methylene blue)		
	of serums obtained:					
	24 h before injection	24 h after injection	48 h after injection	24 h before injection	24 h after injection	48 h after injection
Nil; ♂	0·302	0·380	0·414	11 min	11 min 15 s	11 min 30 s
	0·366	0·505	0·560	10 min 45 s	10 min 30 s	11 min 30 s
Tannic acid*	0·356	0·219	0·183	11 min 45 s	7 min 15 s	6 min
(5 mg) ♀	0·660	0·428	0·232	12 min 45 s	3 min 30 s	4 min 15 s
DL-Ethionine	0·479	0·348	0·287	11 min	10 min 15 s	11 min.
(11·3 mg) ♂	0·334	0·262	0·212	12 min 30 s	10 min 45 s	10 min 15 s
DL-Methionine	0·412	0·497	0·435	14 min 30 s	11 min 15 s	12 min
(10·3 mg) ♂	0·285	0·389	0·412	13 min 15 s	13 min 15 s	12 min 30 s
Carbon tetrachloride	0·356	0·301	0·210	9 min 30 s	3 min	—
(0·1 ml) ♂	0·278	0·248	0·189	10 min 30 s	3 min 30 s	2 min 15 s
Carbon tetrachloride	0·345	0·450	0·506	11 min	7 min 15 s	4 min
(0·02 ml) ♀	0·406	0·543	0·554	10 min 30 s	5 min	4 min
Thioacetamide	0·351	0·353	0·360	12 min	6 min 45 s	9 min 15 s
(5·2 mg) ♂	0·272	0·330	0·358	13 min 45 s	7 min	9 min 45 s
p-Ethoxyphenylurea	0·528	0·590	0·510	11 min 45 s	12 min 15 s	11 min 45 s
(12·5 mg) ♂	0·454	0·510	0·486	13 min 45 s	11 min	12 min 15 s
Chlorpromazine HCl	0·405	0·328	died	11 min 15 s	9 min	—
(12·3 mg) ♂	0·344	0·296	0·197	9 min 45 s	7 min 30 s	—
Chlorpromazine HCl	0·252	0·293	0·335	10 min 45 s	10 min 30 s	9 min 30 s
(2·5 mg) ♂	0·348	0·367	0·428	11 min	12 min 15 s	9 min 45 s

* Similar results were obtained with male rats.

** 1 ml of aqueous methylene blue solution (50 mg/l) + 1 ml of hypoxanthine solution (4 mg of hypoxanthine in 25 ml of full strength Sörensen buffer, pH 8) in evacuated Thunberg tube (3 min at water pump) was mixed with 0·2 ml of serum at zero time and incubated at 38°C.

[8] H. A. RAVIN, The Lancet *1956*, 726.

PPD oxidase activity but also increased the levels of rat serum xanthine dehydrogenase (XD). This latter effect has again been obtained with some of the substances mentioned in this report (see Table).

Although DL-ethionine failed to augment serum XD activity, appreciable increases occurred after the application of tannic acid and, in confirmation of the work of AFFONSO *et al.*[9], following carbon tetrachloride injections. Since XD was strongly stimulated by levels of carbon tetrachloride which failed to suppress PPD oxidase activity, it would seem that there is no direct relationship between PPD oxidase suppression on the one hand and XD stimulation on the other by a particular substance. Again, although both thioacetamide and *p*-ethoxyphenylurea failed to suppress PPD oxidase, the former but not the latter substance led to a rise in rat serum XD activity 24 h after injection. It may be noted that the carcinogen, 4-dimethylaminostilbene, highly active in suppressing PPD oxidase, had little or no effect on serum XD, while the less active PPD suppressor, 2-aminofluorene, increased XD to some extent. The non-carcinogen, 4-aminofluorene, neither increased XD nor depressed PPD oxidase.

The majority of rat hepatocarcinogens which have been examined up to date have been found to suppress rat serum PPD oxidase activity and their effectiveness in this respect closely parellels their carcinogenic potencies. Some but not all of these carcinogens also augment rat serum XD activity. One weak hepatocarcinogen, thioacetamide, did not suppress PPD oxidase even when applied at twice the standard dose level, although it increased temporarily XD activity at both levels. Injections of non-hepatocarcinogenic substances failed either to suppress PPD oxidase or to elevate XD. We should like to remark that although the weak carcinogen, 4'-methyl-4-dimethylaminoazobenzene produced no decline in PPD oxidase when applied at the standard level[1], it did evoke a temporary suppression at twice this level.

Any compound which is found to suppress serum PPD oxidase and/or to elevate XD in the rat under our experimental conditions may be suspected as a potential hepatocarcinogen.

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Cancer Research Unit, The University, Sheffield (England), July 14, 1958.

Résumé

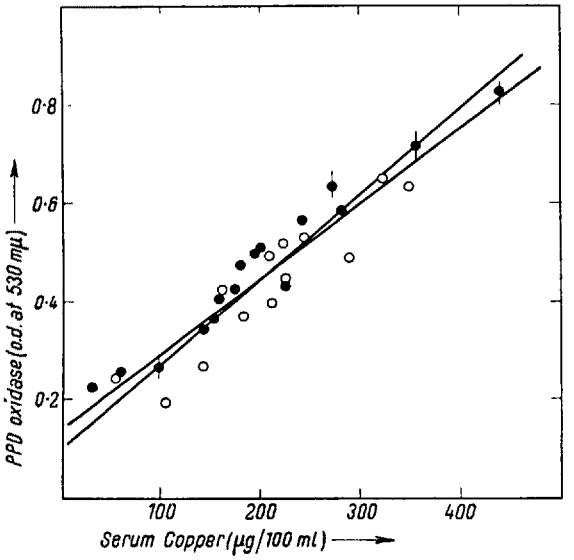
On a observé une diminution de la *para*-phénylènediamine-oxydase et parfois une augmentation de la xanthine déhydrogenase dans le sérum sanguin des rats traités avec quelques substances cancérigènes pour le foie.

[9] O. R. AFFONSO, E. MITIDIERI, L. P. RIBEIRO, and G. G. VILLELO, Proc. Soc. exp. Biol. Med. *90*, 527 (1955).

Correlation between *para*-Phenylene Diamine Oxidase Activity and the Copper Content of Rat Serum

Intraperitoneal injection of some powerful hepatocarcinogens into albino rats results in an appreciable reduction of the level of the copper-containing enzyme, *para*-phenylene diamine (PPD) oxidase[1], as determined by RAVIN's method[2], and also of the total serum copper as estimated colorimetrically with sodium diethyldithiocarbamate[3]. Injections of certain other substances, however, stimulate serum PPD oxidase activity and at the same time produce high levels of copper in the serum. Again, apparently normal rats[4] may sometimes exhibit abnormally high levels of PPD oxidase and here, too, high levels of serum copper are encountered.

Evidently, there is a strong positive linear correlation between the copper level of rat serum and its PPD oxidase activity. This is clearly shown by the scatter diagram, in which is collected data from various experiments.



Relationship between rat serum copper levels and *para*-phenylene diamine oxidase activity.
Chest blood was obtained at death from all male rats ○ and from female rats ⧫. Tail blood was obtained from female rats ● under ether anaesthesia.

We found for 29 (N) serums, a correlation coefficient of + 0·94 (r) for which the t-test value $\left(t = r\sqrt{N-2}/\sqrt{1-r^2}\right)$ was 14·8. Since this value greatly exceeds the 0·1% probability level, the correlation is highly significant.

Putting y = serum copper level (μg/100 ml)
and x = PPD oxidase level (expressed as optical density at 530 mμ) we found the following mean values:

$$\bar{y} = 203{\cdot}93 \quad \text{and} \quad \bar{x} = 0{\cdot}4539$$

and standard deviations:

$$\text{S.D.}_y = 92{\cdot}991 \quad \text{and} \quad \text{S.D.}_x = 0{\cdot}152.$$

From these values we obtained the regression equations:

$$y = 575{\cdot}93x - 57{\cdot}499$$
$$\text{and} \quad x = 0{\cdot}001546y + 0{\cdot}138654$$

which are shown in the Figure.

The standard errors of estimate are:

$$\text{S.E.}_y = \text{S.D.}_y\sqrt{1-r^2} = 30{\cdot}7811$$
$$\text{and} \quad \text{S.E.}_x = \text{S.D.}_x\sqrt{1-r^2} = 0{\cdot}050433.$$

[1] W. J. P. NEISH, Exper. *15*, 20 (1959).
[2] H. A. RAVIN, The Lancet *1956*, 726.
[3] C. J. GUBLER, M. E. LAHEY, H. ASHENBRUCKER, G. E. CARTWRIGHT, and M. M. WINTROBE, J. biol. Chem. *196*, 209 (1952).
[4] Hypercupremia frequently accompanies both acute and chronic infections, see e.g. C. J. GUBLER, M. E. LAHEY, G. E. CARTWRIGHT, and M. M. WINTROBE, Amer. J. Physiol. *171*, 652 (1952).