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.

My thanks are due to Prof. H. N. GREEN for his interest and support and to the University of Sheffield for the James Morrison Fellowship in cancer research.

W. J. P. NEISH

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

Résumé

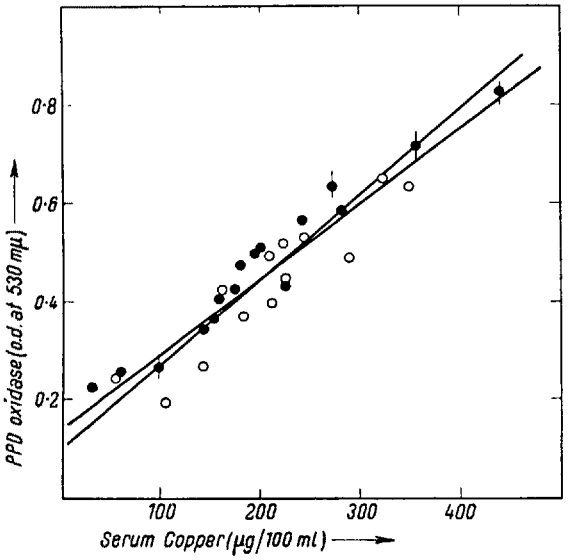
On a observé une diminution de la *para*-phénylènediamine-oxydase et parfois une augmentation de la xanthine déhydrogenase dans le sérum sanguin des rats traités avec quelques substances cancérigènes pour le foie.

[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).

It is of interest that HOLMBERG and LAURELL[5] found a strong positive linear correlation between human serum copper levels and the ability of the serums to oxidise PPD at pH 6 as determined manometrically.

In another connection, a small number of serums from untreated and X-irradiated cancer patients have been examined and we have again found a positive linear correlation between the copper content and PPD oxidase activity ($N = 10$; $r = +0{\cdot}87$; $t = 5{\cdot}13$ – just on the 0·1% level).

I should like to thank Mr. G. W. BLOMFIELD of the Sheffield Radiotherapy Centre for permission to study some of his cases and Mr. L. R. REEVES who collected the human serums. My thanks are also due to Misses J. A. OSBORNE and A. WILLIAMS for their excellent assistance and to the University of Sheffield for the James Morrison Fellowship in cancer research.

W. J. P. NEISH

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

Résumé

Il y a une corrélation très forte entre l'activité de la *para*-phénylène diamine oxydase du sérum sanguin du rat et de son taux en cuivre.

[5] C. G. HOLMBERG and C.-B. LAURELL, Scand. J. clin. Lab. Invest. *3*, 103 (1951).

Metrial Gland and Peroxidase Activity

The location of the metrial gland in the uterus of the pregnant rat[1] and the intimate relation between the granular cells and the blood capillaries suggest a functional connection between the two structures, e.g. a delivery of some substance into the blood. Such an interpretation has been given to the mast cell-capillary arrangement. Another possibility would be that the metrial gland acted as a protective barrier to free the blood from material harmful to fetus or placenta. Since peroxidases react *in vitro* with a large number of substances of various kinds and because of their possible role in some hydroxylations, this group of enzymes might participate in a detoxifying mechanism.

In one experiment of several with consistent results, a rat was exsanguinated a few days before the expected termination of pregnancy. The uterus was opened, the fetuses cautiously removed, and the uterine wall cut transversely into sections so that every second section contained a site of placental insertion rich in metrial gland cells, the pieces in between consisting of normal uterine wall. No. 1 was the section nearest to the vertex of a horn, No. 10 nearest to the other vertex. The pieces were weighed (98–169 mg), homogenised with four volumes of 0·15 *M* KCl, and the homogenate centrifuged for $1^1/_2$ min at 12000 r.p.m. To a Beckman cell (1 cm, 1·3 ml, room temperature) were added 1·0 ml of a 10:1 v/v mixture of acetate buffer (0·1 *M*, pH 4·9) and mesidine hydrochloride (0·2 *M* in water), 0·1 ml of the supernatant to be assayed and 0·05 ml of 0·10 *M* hydrogen peroxide. The increase in light absorption at 490 mμ[2] between 50 and 150 s after the addition of the peroxide was taken as a measure of the activity ($\Delta D = 0{\cdot}11$–$0{\cdot}36$). The supernatants were also assayed for nitrogen (micro-Kjeldahl, 1·9–3·0 mg N/ml). The results, however, lend no support to the assumption that the metrial gland contains significant amounts of peroxidase (Table).

Table

Peroxidase activity in the uterus wall

Section	ΔD/mg N in specimen from	
	placental insertion (incl. metrial gland)	interposed sections
1	0·58	
2		1·17
3	0·49	
4		0·83
5	1·25	
6		0·66
7	0·73	
8		1·04
9	0·79	
10		0·62
Average	0·77 ± 0·30	0·86 ± 0·24

Frozen pieces of the uterus wall were sectioned and stained with benzidine-hydrogen peroxide. The cells of the metrial gland showed no particular colour, whereas the epithelial cells as well as some scattered elements (leukocytes, and possibly others) in the underlying tissue exhibited a deep blue colour.

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Department of Cell Research and Genetics and Department of Biochemistry, Nobel Medical Institute, Stockholm, August 11, 1958.

Zusammenfassung

Mit Hilfe des Mesidintests wurde in der Mucosa, nicht aber in den metrialen Drüsen des trächtigen Rattenuterus eine Peroxydase gefunden.

[1] H. SELYE and T. MCKEOWN, Proc. R. Soc., London, [*B*] *119*, 1 (1935).

[2] K. G. PAUL and Y. AVI-DOR, Acta Chem. Scand. *8*, 637 (1954).

myo-Inositol in the Biosynthesis of Benzylpenicillin by the Mycelial Suspensions of *Penicillium chrysogenum*

Since the biosynthesis of benzylpenicillin by the mycelial suspensions of *Penicillium chrysogenum* in phosphate buffer plus phenylacetate (PA) is stimulated by a variety of carbohydrates[1], it is of interest to understand the mechanism of such a stimulation. With this object, the effects of a variety of carbohydrates and their metabolic pathways under these conditions are being studied. In continuation of our work reported with glycerol[2], we have now studied the effect of *myo-inositol*, a compound of considerable biochemical interest[3]. The phosphorylated derivative

[1] V. N. DESHPANDE and K. GANAPATHI, Exper. *13*, 475 (1957); J. sci. industr. Res. *17c*, 59 (1958).

[2] R. J. IRANI and K. GANAPATHI, Exper. *14*, 329 (1958).

[3] H. A. LARDY, *The Vitamins*, vol. II (Ed. by W. H. SEBRELL and R. S. HARRIS, Academic Press, New York 1954), p. 323.