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.

This work is a part of investigations supported by Svenska Sällskapet för Medicinsk Forskning (G.B.), and Statens Medicinska Forskningsgråd (K.G.P.).

G. BLOOM and K. G. PAUL

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.

of inositol occurs in corn-steep liquor used for penicillin fermentation, and its role in mycelial formation and penicillin biosynthesis under different conditions has also been studied by GOTOVTSEVA[4].

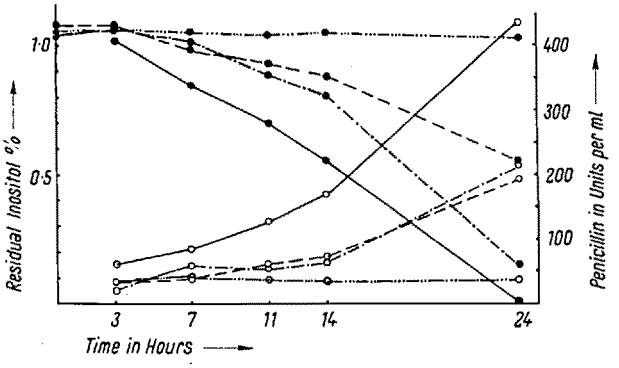

Fig. 1.—Effect of inhibitors. ○ Penicillin concentration; ● residual inositol; —— PA(0·05%) + Inositol (1·0%); --- PA (0·05%) + Inositol (1·0%) + DNP (0·0006 *M*); -·- PA (0·05%) + Inositol (1·0%) + KCN (0·005 *M*); ····· PA (0·05%) + Inositol (1·0%) + $NaAsO_2$ (0·005 *M*)

The experiments were conducted as previously described[1,2]. The stimulatory effects of 0·2, 0·5, and 1·0% of inositol were found to be about the same up to about 14 h, but the effects of 1·0% inositol persisted longer. The estimation of residual inositol in the medium by the method of HIRST and JONES[5] showed that inositol disappeared in the three cases within 7, 11, and 24 h respectively. The inhibitory effects of 2,4-dinitrophenol (DNP),

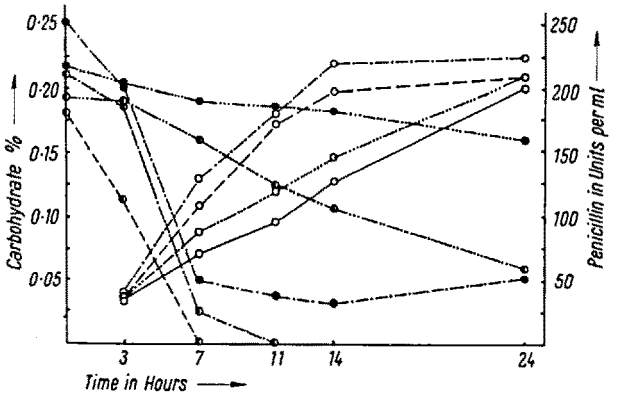

Fig. 2.—Effect of inositol, inosose-2, and glucuronolactone. ○ Penicillin concentration; ● residual inosose-2 or glucuronolactone estimated by method of SOMOGYI (reducing sugar); ◦ residual inositol, inosose-2, or glucuronolactone estimated by oxidation with periodate. —— PA (0·05%); --- PA (0·05%) + Inositol (0·2%); -·- PA (0·05%) + Inosose-2 (0·2%); ····· PA (0·05%) + glucuronolactone (0·2%)

cyanide (KCN), and arsenite in concentrations of 0·0006 *M*, 0·005 *M*, and 0·005 *M* respectively in PA plus inositol system, are given in Figure 1. It can be seen that penicillin biosynthesis is proportional to the degree of disappearance of inositol. In concentrations of 0·00006 *M*, 0·0001 *M*, and 0·0002 *M* respectively, DNP, KCN, and arsenite did not show any significant inhibition of penicillin biosynthesis, as was the case with glycerol[2].

[4] V. A. GOTOVTSEVA, Antibiotiki (USSR) *2*, 9 (1957).
[5] E. L. HIRST and J. K. N. JONES, J. chem. Soc. *1949*, 1659.

The metabolism of inositol has been studied in *Aerobacter aerogenes*[6], in kidney extracts[7], and in the phlorhizinized rat[8], and has been postulated to follow three courses: (a) oxidation to *myo*-inosose-2, then further to the diketone which is split into C_3 and C_2 compounds and carbon dioxide[6]; (b) conversion into glucose and glycogen[8,9]; and (c) conversion into glucuronic acid[7], further metabolism of this not being clear. To understand the metabolism of inositol by *P. chrysogenum*, under the conditions of our experiments and its effects on penicillin biosynthesis, we added *myo*-inosose-2, D-glucuronolactone, and inositol in marginal concentrations of 0·2%, the results obtained being given in Figure 2. *myo*-Inosose-2 is as effective as inositol. Glucuronolactone in 0·2% concentration does not significantly increase the biosynthesis of penicillin but in 0·5% concentration has been found to have a definite stimulatory effect. It disappears from the medium only very slowly and it appears that it has to undergo some transformation before it becomes effective. Its metabolism is being further studied by isolation of the possible intermediates, to examine whether it follows the pathway suggested by MACCORMICK and TOUSTER[10] in rat and guinea pig.

Two percent *myo*-inositol as the sole carbon source supports the growth of *P. chrysogenum* in the synthetic medium of GITTERMAN and KNIGHT[11] in shake flasks, comparable to that obtained with glucose as the sole carbon source. From the mycelium obtained, cold water extracted 7·2% of reducing sugar as estimated by the copper reduction method of SOMOGYI[12]. There was also sugar alcohol present in this aqueous extract as indicated by the periodate oxidation method of estimation[5]. The residual mycelium contained about 22·5% of polysaccharides as estimated by the anthrone method[13], which is very much less than that reported by SHU and THORN[14] who grew the mycelium in glucose, galactose, and xylose. The hydrolysate of the polysaccharide with sulphuric acid was found to consist almost entirely of glucose as detected by the method of DEVOR, CONGER, and GILL[15] using unsulphonated and presulphonated resorcinol. Evidently, a mechaism exists in *P. chrysogenum* for the conversion of *myo*-inositol into glucose; this is being studied.

Full details will be published elsewhere.

We are grateful to Prof. TH. POSTERNAK for a gift of a sample of *myo*-inosose-2 and to Miss I. NALINI for the bioassays reported here.

ROSHAN J. IRANI and K. GANAPATHI

Antibiotics Research Centre, Pimpri (Poona District, India), August 18, 1958.

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[7] F. C. CHARALAMPOUS and C. LYRAS, J. biol. Chem. *228*, 1 (1957); F. C. CHARALAMPOUS, S. BUMILLER, and S. GRAHAM, J. Amer. chem. Soc. *80*, 2022 (1958).
[8] M. R. STETTEN and D. STETTEN, JR., J. biol. Chem. *164*, 85 (1946).
[9] V. D. WIEBELHAUS, J. J. BETHEIL, and H. A. LARDY, Arch. Biochem. *13*, 379 (1947). - T. POSTERNAK, W. H. SCHOPFER, and D. REYMOND, Helv. chim. Acta *38*, 1283 (1955). - H. O. L. FISCHER, Harvey Lectures *40*, 156 (1944-45).
[10] D. B. MACCORMICK and O. TOUSTER, J. biol. Chem. *229*, 451 (1957). - B. L. HORECKER and H. H. HIATT, New England J. Med. *258*, 177, 225 (1958).
[11] C. O. GITTERMAN and S. G. KNIGHT, J. Bact. *64*, 223 (1952).
[12] M. SOMOGYI, J. biol. Chem. *195*, 19 (1952).
[13] J. H. ROE, J. biol. Chem. *212*, 335 (1955).
[14] P. SHU and J. A. THORN, Canad. J. Botany *30*, 252 (1952).
[15] A. W. DEVOR, C. CONGER, and I. GILL, Arch. Biochem. Biophys. *73*, 20 (1958).

Résumé

Le *myo*-Inositol, en concentration de 0,2%, active la biosynthèse de la benzylpénicilline par des suspensions mycéliales de *P. chrysogenum* dans le tampon de phosphate et d'acétate de phényl. L'action stimulante est inhibée par le 2,4-dinitrophénol, cyanure ou arsénite en concentrations de 0,0006 *M*, 0,005 *M* et 0,005 *M* respectivement. Le *myo*-Inosose-2 en concentration de 0,2% est comparable à l'inositol en sa stimulation de biosynthèse de la pénicilline; la D-glucuronolactone ne présente aucun effet notable en concentration de 0,2%, mais à 0,5%, elle a un effet stimulant. Le *P. chrysogenum* est capable d'accroissement dans un milieu synthétique contenant 2% d'inositol comme seule source de carbonne et le polysaccharide du mycelium fournit après hydrolyse presque uniquement du glucose.

Immunology of Toxemias of Pregnancy
I. Findings of Organ-Specific Antibodies

An abundant literature on the pathogenesis of preeclamptic conditions appears to support the assumption that pathologic immunological mechanisms are participating in the genesis of these conditions. It was the purpose of the present investigation to establish whether antibodies directed against tissues of human body can be demonstrated during pregnancy and, if so, to assess their relationship to the clinical conditions of pregnant women.

Materials and Method. Observations were made on 113 pregnant women of whom, in the course of their pregnancy, 66 were found to suffer from preeclampsia. The remaining 47 women were used as controls. Serological tests for antibodies were carried out in each case as soon as the symptoms of preeclampsia had become apparent as well as on occasion of any examination during pregnancy. Sera were obtained by centrifugation immediately after sampling and stored at – 25°C. Tests for the presence of antibodies were undertaken at the latest within 72 h after sampling by the method of collodion agglutination as described elsewhere[1,2]. The employed antigens were kidney, placenta, liver, and myocardium tissues taken from human bodies in the manner indicated *ibidem*.

Results. Correlation between the incidence of preeclampsia and the findings of antibodies is illustrated by Figure 1. Columns above the central line represent the numbers of positive findings. The results are given separately for the control group, for the mild form of preeclampsia and for its severe form. Statistical evaluations show an association between the incidence of preeclampsia and the findings of the different organ-specific antibodies, with the only exception of those to placenta tissue. This association is, of course, not absolute, as antibodies were detected also in several pregnant women without apparent symptoms of preeclampsia and *vice versa*. It may be pointed out, though, that an analysis of the 13 positive cases of women without apparent symptoms of preclampsia has shown that in six of them the pregnancy took a thoroughly normal course, only whilst the remaining seven developed pathological disorders, *viz.*: other types of toxemia of pregnancy (3 cases), albumin in urine with pathological casts (2 cases), hyperemesis (5 cases), the ratened abortion (1 case), premature labour (3 cases), delayed parturition (4 cases), still birth (4 cases). These complications were combined indifferent ways in the seven pregnant women.

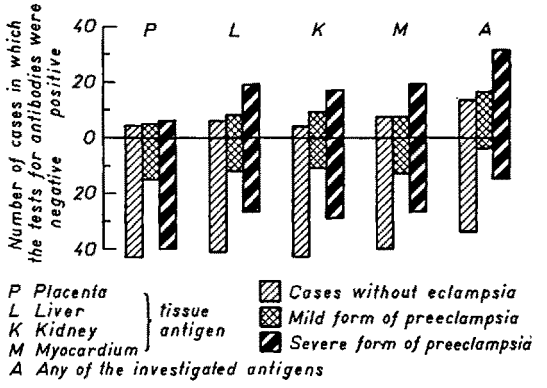

Fig. 1. – Incidence of Preeclampsia in Relation to Production of Autoantibodies

Figure 2 demonstrates a certain association between the occurrence of severe form of preeclampsia and the presence of a higher titre of antibody.

The investigation aimed further at establishing whether the presence of antibodies has any bearing upon the timing of the delivery and on live or still births. A statistically significant correlation between the findings of antibody to myocardium and kidney tissues and the occurrence of still births has been established; no such correlation has been found for the timing of delivery.

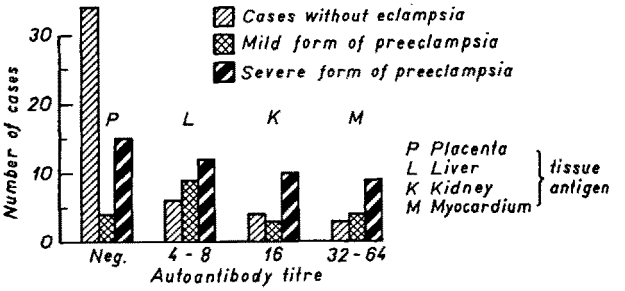

Fig. 2. – Severity of Preeclampsia in Relation to Titre of Autoantibodies

Discussion. Presence of autoantibody[3] has been demonstrated in 47 pregnant women out of a total of 66 preeclamptics. As, in the majority of cases, the test for autoantibody was carried out only once, the possibility cannot be precluded that a repetition of the test might have substantially increased the rate of positive autoantibody findings. This is borne out by the experience gained in separate cases where a repetition of the test was performed. While looking for rational explanations of negative findings in preeclampsia, the possibility should not be neglected that, at the time of collecting blood samples,

[1] V. WAGNER and J. ŠEBA, Dermatologica *112*, 25 (1956).

[2] V. WAGNER, V. REJHOLEC, and V. MALÝ, Ann. rheum. Dis. *15*, 364 (1956).

[3] From the data presented it is by no means clear whether the antibodies are produced following isoimmunization by antigens from the foetus, or by autoimmunization by changed antigenic composition of tissues of pregnant women. The label 'autoantibodies' is therefore tempting though representing only the personal opinion of the authors.