

## TRANSVAAL SOCIETY OF PATHOLOGISTS

## SUMMARIES OF SCIENTIFIC PAPERS\*

## 1. THE PRESENT POSITION OF THE IMMUNOLOGICAL CONCEPT OF THE PATHOGENESIS OF SILICOSIS

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After reviewing the different aspects of this concept, the work which supported it was summarized.

Although the early results from South Africa indicated that some type of antibody reaction might be occurring in silicosis, subsequent work has not confirmed this. Certain basic immunological problems have been found during this study:

(a) The unexplained inhibition of a haemolytic system made up with silicic acid by the sera of miners attending the Pneumoconiosis Bureau requires further investigation.

(b) The reactions between silicic acid and silicates and certain fractions of complement also require elucidation.

(c) Animals exposed to a silica dust cloud show a well-marked drop in properdin levels between the 6th and 14th weeks of dusting.

Although the well-known variation in the reaction of individual miners to the inhalation of dust was considered to be evidence of an immunological factor, it is now suggested that this may well be a variation in the dust-clearing mechanism of individual lungs.

## 2. GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN SOUTH AFRICA: INCIDENCE AND CLINICAL IMPLICATIONS

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Acute haemolytic anaemias caused by aromatic drugs and certain vegetable extracts may be associated with a metabolic defect in mature erythrocytes. The susceptibility is hereditary, owing to a defect in the gene located on the X chromosome. Thus the male can be either normal or mutant; on the other hand, the female, with two X chromosomes, may be homozygous normal or homozygous mutant or heterozygous, so that intermediate degrees of the abnormality may appear in heterozygous females.

The red-cell defect is characterized by an absence, partial or complete, of glucose-6-phosphate dehydrogenase; this produces a low reduced glutathione level and, on *in vitro* incubation with drugs such as acetylphenylhydrazine, an increased methaemoglobin and Heinz-body formation and marked instability of reduced glutathione.

A demographic survey among the racial groups of South Africa included Caucasians (148), Indians (150), Cape Malays (50), Cape Coloured (159), Bantu females (253), and over 1,000 Bantu males. The defect was absent or negligible in all groups except the Bantu, where it was present in 2-4% of most Bantu tribes and up to 8% of certain groups. The occurrence of glucose-6-phosphate dehydrogenase deficiency is thus useful in detecting subjects sensitive to drug-induced haemolysis and the drugs liable to precipitate haemolytic episodes, and as a possible cause in acute haemolysis of unknown origin.

## 3. HAEMAGGLUTINATION INHIBITORY SUBSTANCES

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As first described by Keogh, North and Warburton,<sup>1</sup> indirect bacterial haemagglutination depends on the ability of certain bacterial antigens to be attached to the surface of the erythrocytes. Such cells, often referred to as modified erythrocytes, are readily agglutinable by homologous bacterial antibodies.

Employing enterobacterial lipopolysaccharides, Neter *et al.*<sup>2</sup> showed that certain antibiotics like polymyxin B, neomycin,

and streptomycin interfered with the attachment of the antigen to the erythrocytes. Treatment of the red blood cells with these antibiotics before or after modification did not prevent haemagglutination by the bacterial antibodies. The inhibitory effect was not shown by bacitracin, penicillin and erythromycin.

Further studies by Neter *et al.*<sup>3,4</sup> showed that the low-molecular serum-protein fractions, egg yolk, protamine, and histone showed a similar inhibitory effect, which was only moderately reduced by heating to 75°C. or 100°C. for 30 minutes. Modification was not inhibited by egg white and gelatin.

These investigations were extended, and it emerged that the action of the inhibitor was to slow down the attachment rate. Under experimental conditions, using fixed incubation time and temperature, the effective concentration of inhibitor was directly proportional to the concentration of antigen in the solution. Fractionation of egg yolk showed the presence of inhibitors both among ether-soluble components and the lipid-free residue.

It was concluded that inhibitors are different from antibodies, that they are present in normal serum in limited amounts, that the effect may be associated with a number of different compounds, and that they act on the antigen resulting in a reduced but not abolished affinity for the surface of the red-blood cell.

1. Keogh, E., North, E. and Warburton, M. (1947): *Nature (Lond.)*, **160**, 63.
2. Neter, E., Gorzynski, E. A., Westphal, O. and Lüderitz, O. (1958): *J. Immunol.*, **80**, 66.
3. Neter, E., Zak, D. A., Zalewski, N. J. and Bertram, L. F. (1952): *Proc. Soc. Exp. Biol. (N.Y.)*, **80**, 607.
4. Neter, E., Gorzynski, E. A., Westphal, O., Lüderitz, O. and Klumpp, D. J. (1958): *Canad. J. Microbiol.*, **4**, 371.

## 4. MUCOUS METAPLASIA IN THE STRATIFIED SQUAMOUS EPITHELIAL LININGS OF DENTAL CYSTS

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Dental cysts are of inflammatory origin and develop at the apices of non-vital teeth. They consist of a fibrous cyst wall, lined by a non-keratinized, stratified squamous epithelium of varying thickness.

In this study of histological sections of 200 cases, it was found that, although all the cysts were lined in part by stratified squamous epithelium, ciliated epithelium was also present in 20 cases (10%). Goblet cells were found in 36 cases and in 19 of these they were forming an orderly secretory layer on the surface of stratified squamous epithelium. In all, 39 cysts (19.5%) were partly lined by secretory epithelium. Of the 20 cysts containing ciliated epithelium, 14 are known to have come from the maxilla and 4 from the mandible. Of those containing goblet cells, 24 are known to have come from the maxilla and 9 from the mandible.

The presence of mucous cells in cysts occurring in the maxilla can be explained theoretically on grounds other than metaplasia. Those in the anterior region might in fact have been nasopalatine cysts. Those elsewhere in the maxilla may have communicated with either the nose or the antrum, but although in a few cases there was radiological evidence of close apposition between the cysts and antrum, in only 2 cases was there clinical evidence of communication.

As far as the mandibular cysts are concerned, however, the occurrence of secretory epithelium is less easily explained on grounds other than metaplasia. Photomicrographs were presented which illustrate the presence of secretory epithelium in mandibular dental cysts. Attention was drawn to the work of Fell,<sup>1</sup> who produced mucous metaplasia in explants of skin of chick embryo in tissue culture under the influence of excess vitamin A.

\* Read at a meeting of the Society held at Pretoria on 17 March 1962.

1. Fell, H. B. (1956): *Proc. Roy. Soc. B.*, **146**, 242.

## 5. MIXED-DUST PNEUMOCONIOSES IN SOUTH AFRICA AS DEMONSTRATED IN AN IRON MOULDER

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The difference between the classical silicotic islets and the nodule of a mixed-dust pneumoconiosis was demonstrated. Although the latter nodules had been described mainly in people working in ferrous and non-ferrous foundries, such lesions have been found in South African gold miners. Typical examples of mixed-dust lesions are also to be found in the lungs of coal miners and South African iron miners.

The possible interaction of silicates with silica in producing these lesions in South African gold miners was discussed.

## 6. A CHANGE IN PHENOTYPE ASSOCIATED WITH THE BACTERIOPHAGE CARRIER-STATE IN A STRAIN OF *PROTEUS MIRABILIS*

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Some properties of an endolysin liberated by lysing complexes of a strain of *Proteus mirabilis* and a virulent bacteriophage were described. The endolysin affects the surface structures of uninfected organisms of this strain, so that they do not absorb the phage, and also lyses versene-treated organisms.

The particular strain is also cryptic as regards sucrose fermentation, i.e. it possesses the necessary enzyme systems, but is impermeable to the substrate. Phage-carrier colonies of the strain ferment sucrose, but once these colonies are passed through phage antiserum they are all sucrose-negative again. This is interpreted to mean that the endolysin liberated by lysing complexes in a colony protects surviving cells from phage infection and also, through its action on surface structure, renders these protected organisms permeable to sucrose, which they then promptly ferment.

## 7. THE ENDOPLASMIC RETICULUM

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The unit membrane consists of two protein mono-layers with a bimolecular phospholipid layer. This is the structure of all cell membranes and is probably the structure of all organelle membranes, including the endoplasmic reticulum. The fact that it is not always seen as this triple unit may be due to either the methods of fixation, the physiological state of the cells, or the angle of the membrane to the plane of section.

The endoplasmic reticulum appears in many shapes or profiles. Besides the variety of shapes, this membrane has two fundamental textures. It may be smooth, that is, it is relatively free of attached particles, or it may be almost obscured by particles (ribosomes). We believe that the particles are closely attached to the membrane by fine stalks.

It will be seen that the endoplasmic reticulum consists of two phases: an intracytoplasmic phase, and an extracytoplasmic phase. Possibly secretory products form in the rough endoplasmic reticulum or ergastoplasm. From here the products pass down to the vesicular Golgi network where they coalesce and may come to be surrounded by a membrane. As granules they then pass out of the cytoplasm by travelling between smooth endoplasmic reticulum to the cell surface. Secretory granules may simply be discharged in this fashion or the membrane of the granule may fuse to the plasma membrane. The particle is then contained in something like an air lock. The outer membrane is pulled back and the free granules flow into the capillary lumen leaving behind two sides of the 'air lock' (emiocytosis). These are microvilli, but they should be called pseudo-microvilli. If there are enough microvilli, the border of the cell shows a digitate or cogwheel appearance, but true microvilli are not found. The endoplasmic reticulum serves as a circulatory system for 'export' and 'import'. Ingested particles may travel up to the nucleus, which is surrounded by a rim of endoplasmic reticu-

lum and separated from it by a moat. In many locations the nucleus may communicate with the endoplasmic reticulum through pores.

In embryonal cells there is little, if any, endoplasmic reticulum present. As cell specialization matures so the endoplasmic reticulum develops. Consequently, rapidly proliferating cells or undifferentiated neoplastic cells are poor in endoplasmic reticulum, while differentiated neoplastic cells may have an elaborate endoplasmic reticular pattern. In some malignant cells vesiculation with smooth membranes surrounding the vacuoles may be observed, or the endoplasmic reticulum may be whorled. These changes are found in induced hepatic carcinomas where the well-organized endoplasmic reticulum becomes disorganized both in profile and in its relation to enzymatic particles. The change is probably related to the alteration of the metabolic processes at the particle surface. Consequently, the synthesis of products for the normal metabolism of the endoplasmic reticulum is deranged and the endoplasmic reticulum becomes disorganized.

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Palade, G. E. (1961): *Electron Microscopy in Anatomy*, pp. 176-193. London: Arnold.  
Robertson, J. D. (1959): *Biochem. Soc. Sympos.*, 16, 3.  
Yodaiken, R. E. (1962): *S.Afr. J. Med. Sci.* (in press).

## 8. THE ULTRASTRUCTURE OF TWO INSULIN-SECRETING ISLET-CELL ADENOMAS OF THE PANCREAS

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Islet-cell adenomas from two patients with spontaneous hypoglycaemia have been studied. In the first patient the tolbutamide tolerance curve was abnormal, but reverted to normal postoperatively. In the second one, on the other hand, the tolbutamide curve remained abnormal postoperatively.

The major features of the ultrastructure of the first tumour were as follows: The nuclei were large, and the cytoplasm contained a dense network of endoplasmic reticulum. A few granules were present and most of these were peripheral and in the luminal phase of the endoplasmic reticulum. The granules conformed in part to the structure and size of human insulin granules, but nevertheless there was considerable variation in size and even in shape. The mitochondria were few in number. Between the cells and the capillaries a double basement membrane was present with a variable amount of fibrillar material in the intervening spaces. Occasionally this space was filled with a thicker material resembling in some respects hyaline material. In a few cells the endoplasmic reticulum was not well developed and was represented by an ill-formed tubular structure. Some cells contained both a well-developed endoplasmic reticulum and, in parts, a poorly formed endoplasmic reticulum such as has just been described.

The ultrastructure of the second tumour showed differences from that of the first tumour. The cells tended to be oval in shape, with oval nuclei and prominent nucleoli. The plasma membrane between the cells interdigitated at the lower pole of the cells. The cytoplasm was agranular, but contained numerous mitochondria, which were not prominent in the first tumour. These mitochondria varied considerably in size and shape. The endoplasmic reticulum comprised poorly formed tubular structures with considerable numbers of ribosome-like particles lying 'free' in the cytoplasm. These cells approximated more closely the scattered cells seen in the first tumour.

The reasons for the difference in these two tumours may be found possibly in the histogenesis of islet-cell tissue.

In conclusion, two islet-cell tumours were demonstrated. The one showed a mixed-cell type which possibly arose from acinar tissue and ductules. The second tumour showed a less differentiated endoplasmic reticular pattern and probably consisted of either rapidly proliferating cells or less mature cells. There was no evidence of acinar participation in this tumour. The histological differences appeared to be correlated to some extent with the clinical differences.

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Zagury, D., Brux, J. de, Micheline, A. and Leger, L. (1961): *Presse méd.*, **69**, 887.

## 9. CHROMOSOME PATTERNS OF MALIGNANT AND NORMAL CELLS IN TISSUE CULTURE

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The chromosome pattern of normal cells in any one species is fairly constant. Man has 23 pairs, making a total of 46 chromosomes. This investigation has confirmed that the vervet monkey of South Africa, *Cercopithecus aethiops pygerytheus*, has a total of 60. The study of the chromosome pattern in man has proved invaluable in a number of congenital anoma-

lies such as mongolism, and Turner's and Klinefelter's syndromes.

The chromosome patterns of a variety of human and monkey cells grown in tissue culture have been studied. Normal cells retain their normal chromosome pattern, while malignant cells in tissue culture, and cells established in continuous line culture, show abnormal patterns. This abnormality of the chromosome pattern appears to be an important, perhaps fundamental, difference between malignant and normal cells.

The acridine-orange staining technique was applied to chromosome preparations in tissue culture. The clear picture presented suggests that it may be possible to develop histochemical techniques for the study of the finer structures of the chromosomes.