SOUTH AFRICAN SOCIETY OF PATHOLOGISTS

SUMMARIES OF SCIENTIFIC PAPERS*

1. FEATURES OF THE FINE STRUCTURE OF THE KAPOSI SARCOMA

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The source of the Kaposi sarcoma rests with the identification of the predominant spindle cell.

Under the light microscope it has a rounded or oval, vesicular nucleus, an amorphous cytoplasm and indistinct cell boundaries. The cells surround clefts, not lined by endothelium, but usually containing red blood cells. It has been postulated that this cell is either a modified Schwann cell, a primitive mesenchymal cell, a pericyte or a supporting cell of the glomus body.

The ultrastructure shows the typical cell to have a large nucleus, often with a prominent nucleolus. The cytoplasm contains numerous vacuoles and a moderate number of mitochondria. Occasional lysosomes are present. The endoplasmic reticulum is moderately well developed. In addition to this an amorphous material, which we identify as a polysaccharide, is found between the cells, conforming to the histochemically demonstrated extracellular pool of mucopolysaccharide. This polysaccharide is also found in the cytoplasm and there is little doubt that the cell synthesizes it. The nucleus is fairly regular, varying from the rounded, notched type to the oval type in those areas where the cells are more crowded and the matrix less obvious. The plasma membrane is constant, but often difficult to trace, and this is why a syncytial appearance is seen under the light microscope.[2] The vacuolated cytoplasm is a prominent feature. Many of the vacuoles are surrounded by double membranes. In primitive mesenchymal cells the vacuoles are, in fact, cisternae surrounded by a single membrane of endoplasmic reticulum.

Schwann cells form myelin by whorling about an axon, and as they do so they form laminated membranes 150° A apart until the sheath is complete. The Schwann cells that embrace the non-myelinated fibres have double membranes surrounding nerve fibres and connected to the plasma membrane by mesaxons. Proliferating Schwann cells have a very similar appearance.

The cytoplasmic vacuoles of our cells have double membranes and in some places have membranous connections with the plasma membrane suggestive of mesaxons.

The typical tumour cell has a large and vesicular nucleus and where this cell lies alongside an endothelial cell the differences are easily demonstrated. The nucleus of the latter is smaller, while the cytoplasm is attenuated and contains small vesicles, scanty endoplasmic reticulum and fibrils.

We are unable to confirm 2 points made by Pepler and Theron.[1] Basement membranes are by no means constant.[2] The well-laminated structures which they demonstrated have not been seen in any of our tumours. On the other hand numerous double-membraned structures with apparent connections to the plasma membrane have been observed, a typical nucleus and a general morphological picture closely approximating the Schwann cell.

Prof. G. Causey[3] was consulted and he kindly sent us two photographs. The one of an experimentally induced fibrosarcoma with a fine fibrillar cytoplasm and different in many respects from our cell, and the other of a malignant intraneural tumour which he classifies as Schwannian in origin. He concludes by saying that he has picked this picture because it shows major similarities to our cells. Collagen is present but limited, and there are inclusions of cell processes within cells, producing the double-membrane appearance. The cytoplasm is granular rather than fibrillary.

In conclusion, the ultrastructure confirms the histochemical findings and excludes cells such as fibroblasts, smooth-muscle cells and endothelial cells. Furthermore, the features are unlike those of pericytes. Similarities to primitive mesenchymal cells have been noted,[2] but the double-membrane structures in particular have not been described in this type of cell. The ultrastructure of the supporting cell of the glomus body has not yet been studied.

The origin of the spindle cell of the Kaposi sarcoma has not been proved, but further support is lent to the contention that this cell is a modified Schwann cell.

I am grateful to Prof. G. Causey for his suggestions and for the photographs which he sent. I also acknowledge with gratitude the constructive criticism offered by Prof. D. C. Pease. Finally, my thanks to Prof. B. J. P. Becker for initiating this small study and for his guidance.

1. Pepler, W. J. and Theron, J. J. (1962): J. Path. Bact., **83**, 521.
2. Pease, D. C.: Personal communication.
3. Causey, G.: Personal communication.

2. ENZYME HISTOCHEMISTRY AND FLUORESCENCE MICROSCOPY OF KAPOSI'S SARCOMA, MALIGNANT HAEMANGIO-ENDOTHELIOMA AND POST-MASTECTOMY LYMPHANGIOSARCOMA

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The activity of hydrolytic enzymes has been studied in frozen sections of lesions from proved cases of Kaposi's sarcoma,

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post-mastectomy lymphangiosarcoma and malignant haemangio-endothelioma.

Alkaline phosphatase was demonstrated in the endothelium of blood capillaries. Lymphatics and all the spindle cells in Kaposi's sarcoma and the endothelial cells in the other two tumours were negative for this enzyme.

Strong non-specific esterase activity was exhibited by histiocytes (phagocytes), multinucleated cells and monocytes in all three lesions. Some spindle cells in Kaposi's sarcoma gave a weak reaction for this enzyme. The distribution of acid phosphatase was very similar. No cholinesterase activity was observed in the spindle cells of Kaposi's sarcoma.

The absence of alkaline phosphatase activity in the spindle cells of all cases of Kaposi's sarcoma investigated, is considered to be strong evidence against their origin from blood-vessel endothelium and from fibroblasts. The inability to demonstrate activity of cholinesterase in these cells is taken as evidence against their neural origin. The presence of only minimal non-specific esterase and acid phosphatase activity in some of the spindle cells is insufficient evidence of their derivation from reticulo-endothelial cells. That they may be pluripotent mesenchymal cells does not warrant Kaposi's sarcoma being classified as a neoplasm of reticular origin.

It is postulated that multicentric neo-formation of lymphatics is a primary process in the histogenesis of Kaposi's sarcoma, that the predominant localization of lesions corresponds to the normal distribution of lymphatics and that the pathological formation of lymphatico-venous anastomoses may account for many of the clinical manifestations of this disease.

The absence of alkaline phosphatase activity in the endothelial cells of the post-mastectomy lymphangiosarcoma is in keeping with the finding in normal lymphatics. However, the absence of this enzyme in endothelial cells of the so-called malignant haemangio-endothelioma suggests the possibility that this tumour is also of lymphatic rather than blood-vessel origin.

Fluorescence microscopy of the post-mastectomy lymphangiosarcoma and so-called malignant haemangio-endothelioma shows brilliant red-orange cytoplasm and yellowish-green nuclei of the endothelial cells, indicating a high content of cytoplasmic RNA and nuclear DNA respectively. By contrast the spindle cells of Kaposi's sarcoma appear to contain much smaller amounts of these nucleic acids.

3. ON THE ENZYME HISTOCHEMISTRY OF MENINGIOMATA

J. C. E. KAUFMANN, *Neuropathology Department, South African Institute for Medical Research*

This communication was about a study of 14 meningiomata, using histochemical methods for 3 enzymes. The results were:

1. That almost all the tumours contained alkaline phosphatase to a greater or lesser degree.
2. That in the few cases in which the cerebrospinal fluid was examined biochemically, alkaline phosphatase was, for practical purposes, absent from this fluid.
3. That in about half the tumours there were a few xanthoma cells, foamy cells, or large histiocytic cells which gave a positive reaction for acid phosphatase and non-specific esterase.

Some of the results were illustrated by colour transparencies. This work was compared with the results of studies by earlier workers, using histochemical techniques for the demonstration of alkaline phosphatase in these tumours. The demonstration of a rich alkaline phosphatase content in pure fibrous meningiomata, which does not seem to have been mentioned before, is in keeping with recent electron microscopic studies by Kepes (1961). He showed that the ultrastructure of meningothelial cells is the same as that of cells from fibrous meningiomata, in that both contain fine intracytoplasmic fibrils which are not present in ordinary fibroblasts.

4. LÖFFLER'S ENDOCARDITIS

H. W. WEBER, *Department of Pathology, University of Stellenbosch*

Three cases of Löffler's endocarditis were described, one of which was in the chronic and two in the acute phase. In the acute stage Löffler's endocarditis is characterized by eosinophilic infiltrates in the parietal endocardium and by an eosinophilic arteritis in various organs.

In later stages the heart shows intertrabecular thrombi, fibrosis and fibro-elastosis of the parietal endocardium and fibrotic foci in the myocardium. The eosinophilia of the blood is a phasic symptom which may be absent in the first and last stages of the disease.

5. ASBESTOSIS HYALINE

J. G. THOMSON, *Department of Pathology, University of Cape Town*

In active pulmonary asbestosis a histiocyte containing hyaline is readily seen in sections stained by haematoxylin and well-differentiated eosin. The histiocyte is very large, and the nucleus and cytoplasm are displaced to the edges by a large eosinophilic hyaline mass, delimited by a narrow pale-staining zone. The suggested interpretation is that substances dissolved from the asbestos fibre interfere with the nutrition of the histiocyte and with one of its functions, since signs of phagocytosis are absent in these cells.

6. SOME ASPECTS IN THE DIFFERENTIAL DIAGNOSIS OF VIRUS HEPATITIS AND BILIARY ATRESIA IN THE NEWBORN

D. MCKENZIE, *Department of Pathology, Red Cross War Memorial Children's Hospital, Rondebosch*

1. The problem of neonatal jaundice.
2. Features which make the differential diagnosis difficult histologically.
3. Aids to diagnosis.
4. A workable approach to the problem.

7. PATHOLOGY OF CARCINOMA OF THE OESOPHAGUS IN THE THREE ETHNIC GROUPS IN CAPE TOWN

M. C. BERMAN *and* J. G. THOMSON, *Deparment of Pathology, University of Cape Town*

A study of 292 cases of carcinoma of the oesophagus, based on biopsies, surgical excisions and autopsies in White, Coloured and African patients, showed no significant difference in duration, site, infrequency of metastases, histological structure, mitotic activity or degree of differentiation.

African patients were one decade younger than White and half a decade younger than Coloured patients, a feature of most internal malignant tumours in this area. The only other difference noted was that the cancers in Africans evoked more desmoplasia.

In all three racial groups adenocarcinomas were absent, except for those resulting from extension of a gastric cancer. Two were mixed adeno-acanthomas. In all groups pleomorphism was a feature, and it was concluded that all biopsy could do was to permit the diagnosis of the presence of a cancer. In 20% of the autopsy cases, no secondary deposits were demonstrated, and in another 26% they were confined to limited regional lymph-node spread.

In 10% of the cases the presence of intra-epithelial cancer was noted; in half of these this was regarded as secondary infiltration by underlying cancer. In the remaining 5% the lesion was judged to be cancer-*in-situ*, though this was only noted in patients with existing infiltrating cancers. Out of 523 oesophageal biopsies, 400 showed cancer and in none of the 123 negative biopsies was cancer-*in-situ* seen.

8. SICKLE HAEMOGLOBIN: TESTS AND STUDIES OF THE SICKLE-CELL TRAIT

R. E. BERNSTEIN *and* M. C. SALKINDER, *South African Institute for Medical Research*

Sickle cells may be detected in slide preparations by various techniques; a simple method providing an immediate result was described. Confirmation of screening tests by quantitative estimation of haemoglobin-S is recommended. A standardized

solubility procedure has been used to measure the amount of sickle haemoglobin quantitatively. Different methods of electrophoresis have been employed to separate and measure the various haemoglobins quantitatively; adequate separation can be achieved in 2 hours or less. Aspects of the incidence and clinical implications of the sickle-cell trait in Southern Africa were discussed.

9. THE USE OF RADIOACTIVE IRON IN THE DEMONSTRATION OF SPLENIC SEQUESTRATION OF YOUNG POPULATIONS OF ERYTHROCYTES

J. METZ,* S. KRAWITZ *and* D. HART, *South African Institute for Medical Research*

The prediction of the outcome of splenectomy in various types of haemolytic anaemia remains a serious and common problem in haematological practice. The introduction of radioactive chromium (^{51}Cr) techniques with surface counting over the spleen to detect increased splenic sequestration of erythrocytes has marked a major advance in the elucidation of this problem. By means of ^{51}Cr, the circulating red cells are labelled *in vitro*, and the labelled cells are aged from 1 - 130 days. Providing then that, at the time of labelling, the abnormal cells comprise a significant proportion of the circulating red cells, ^{51}Cr labelling will demonstrate a more rapid fall of circulating radioactivity and accumulation of radioactivity over the spleen where there is increased splenic sequestration of red cells. However, young populations of abnormal red cells, rapidly removed by the spleen, are significantly diluted in the circulation by the longer-lived normal cells, and therefore inadequate numbers might be labelled to enable their sequestration in the spleen to be detected.

As opposed to ^{51}Cr, radioactive iron (^{59}Fe) is a 'cohort label', i.e. the label is taken up by the developing erythroid precursors in the bone marrow over a period of some 7 - 10 days, and therefore a single population of red cells is labelled. In this way the distribution of label between short-lived and long-lived cells would be expected to approximate their true numerical ratio.

Investigations have been carried out with patients suffering from various types of haemolytic anaemia using a combined ^{59}Fe and ^{51}Cr technique. Increased splenic sequestration has been demonstrated with ^{59}Fe in some patients where there was no significant splenic accumulation of ^{51}Cr. The differentiation from other causes of ^{59}Fe accumulation in the spleen were discussed.

It was concluded that splenic sequestration of young populations of erythrocytes is best demonstrated by use of a cohort label, such as ^{59}Fe.

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10. FIBRINOLYTIC ACTIVITY IN SOUTH AFRICAN BANTU AND WHITE MALE SUBJECTS

B. A. BRADLOW, I. BERSOHN *and* A. ANTONIS
Department of Chemical Pathology, University of the Witwatersrand and the South African Institute for Medical Research, Johannesburg

11. THE CHARACTERIZATION OF A SERIES OF LYTIC LACTOBACILLUS BACTERIOPHAGES

H. C. DE KLERK, J. N. COETZEE *and* J. J. THERON, *Department of Microbiology, University of Pretoria*

A series of 15 bacteriophages active on strains of Lactobacillus have been grouped according to microscopic morphology, host range, plaque morphology, growth and thermal inactivation constants, citrate sensitivity and serological characteristics.

12. SUCROSE FERMENTATION BY *PROTEUS HAUSERI*

J. N. COETZEE, *Department of Microbiology, University of Pretoria*

Fifty-five of 63 wild strains of *Proteus mirabilis* were found to be cryptic with regard to sucrose and raffinose fermentation. They possessed competent enzyme systems, but did not normally ferment the sugars. No enzyme capable of cleaving these sugars could be extracted from the remaining 8 strains. Partially purified enzyme preparations from 2 strains of *Proteus vulgaris* and 2 cryptic *Proteus mirabilis* strains were investigated in detail. All 4 were constitutive β-D-fructofuranosidases.

Sucrose-uptake studies showed that the 55 cryptic strains and 3 of the remaining 8 strains (Nos. U18, F20 and 40) failed to accumulate sucrose from 1% (w/v) solutions of the sugar. The remaining 5 strains (Nos. 12, 13, 34, 55 and 63) accumulated large amounts of sucrose. None of the *Proteus mirabilis* strains were permeable to another disaccharide, maltose. The permeability barrier for sucrose could be overcome by increasing its concentration to 5% (w/v). Under these conditions the 55 cryptic strains fermented sucrose-peptone water within 36 hours. Sodium desoxycholate was also capable of changing the permeability barrier of some of the cryptic strains, enabling them to ferment 1% (w/v) sucrose promptly.

After 3 - 11 days in 1% (w/v) sucrose-peptone water all 55 cryptic *Proteus mirabilis* strains, as well as strains 12, 13, 34, 55 and 63, fermented sucrose. This fermentation was not by the wild types, but resulted from the selection of sucrose-positive mutants which arose from the former and were capable of prompt sucrose fermentation. The mutants of strains 12, 13, 34, 55 and 63 arose at lower rates than those from the cryptic strains.

It is concluded that selective permeability to sucrose and β-D-fructofuranosidase activity are genetically distinct properties of Proteus and a scheme for the classification of phenotypes of *Proteus hauseri* was presented.

13. PRIMARY PIGMENTATION

F. P. SCOTT, A. T. NESER *and* R. KOOIJ, *Bloemfontein*

A short discussion on the appearance of primary pigmentation was presented. Following this, the results of measurements of energy of the responsible wavelengths with the help of a Kipp Thermocell and interference filters were supplied.

14. SOME ASPECTS OF INTERMEDIATE CARBOHYDRATE METABOLISM IN AFRICAN PORPHYRICS

S. M. JOUBERT, *Department of Pathology, University of Natal*

The intermediate carbohydrate metabolism leading up to the Shemin cycle was studied in African porphyrics and controls, using a loading dose of intravenous pyruvate. The findings were presented and their significance was discussed.

15. GALACTOSAEMIA: STUDIES ON THE BIOCHEMICAL DEFECT AND DETECTION OF THE CARRIER STATE

R. E. BERNSTEIN, *Electrolyte and Metabolic Research Unit, South African Institute for Medical Research, Johannesburg*

The metabolic defect in galactosaemia, an autosomal recessive inherited condition, is due to the absence of tissue galactose-1-phosphate uridyl transferase. This leads to the accumulation of galactose, which is inhibitory to certain vital glycolytic enzymes, and is the basis of galactose toxicity. Severe symptoms may occur shortly after birth and may lead to a fatal outcome in infancy. Early diagnosis is important, since the provision of a milk-free and low galactose diet in early life may ameliorate or prevent pathological tissue changes.

Urine and blood investigations will be discussed for their diagnostic value. The recent development of tests for the specific enzyme defect permits the detection of heterozygotes in the siblings of galactosaemics and their parents' relatives. The biochemical findings in several galactosaemics and their families were presented.

16. THE SUBVIRAL INFECTIVE AGENT OF WEST NILE VIRUS

W. DU T. NAUDE, *Department of Pathology, University of Cape Town*

17. PURIFICATION OF A VIRUS INHIBITOR IN NORMAL ANIMAL SERUM

A. KIPPS, *Department of Pathology, University of Cape Town*

18. THE ULTRASTRUCTURE OF THE TERN VIRUS

W. BECKER, *Department of Pathology, University of Cape Town*

19. AN OUTBREAK OF CHIKUNGUNYA FEVER IN SOUTHERN RHODESIA

B. M. MCINTOSH *and* H. E. PATERSON, *South African Institute for Medical Research*