SOUTH AFRICAN SOCIETY OF PATHOLOGISTS : ABSTRACTS OF PAPERS

The following are abstracts of papers presented at the Annual Congress of the South African Society of Pathologists and the Southern African Society for Haematology, held at the Civic Centre, Bellville, CP, on 4 - 6 July 1968.

TRITIUM INCORPORATION INTO LIVER HAEMIN FROM SUITABLY LABELLED HYDROGEN DONOR SUBSTRATES IN EXPERIMENTAL PORPHYRIA

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A primary action of porphyrinogenic drugs is considered to be interference in terminal mitochondrial oxidation in the liver, as evidenced by increased liver intramitochondrial NADH/NAD+ ratios following administration of porphyrinogenic drugs. It has been proposed that the increased redox status of the liver mitochondria induces increased porphyrin synthesis by initiating increased succinate synthesis through reverse TCA cycle activity. This proposal implies reductive synthesis of succinate from fumarate as substrate, with a direct transfer of hydrogen from reduced cofactor to fumarate. To establish the validity of this hypothesis, it is necessary to demonstrate that reversal of the succinate to fumarate reaction is possible in the liver of the intact animal and that intramitochondrial hydrogen transfer from cofactor to fumarate takes place in the intact liver. Accordingly, the incorporation ^{3}H from succinate-2-2′-^{3}H, ethanol-1-^{3}H, malate-2-^{3}H and glutamate-2-^{3}H into liver haem was studied in normal rats, and rats given allylisopropylacetamide (AIA). It was consistently found that in AIA-treated rats ^{3}H incorporation from these precursors was decreased, despite increased δ-aminolaevulinate synthetase activity and a known increase in liver haem synthesis. These results suggest that the succinate substrate pool for haem synthesis does not equilibrate with the tritiated precursors. This interpretation is in direct conflict with existing views on haem synthesis and necessitates a reconsideration of the mechanisms postulated to control liver haem synthesis.

THE OCCURRENCE OF CYTOPLASMIC FIBRILS IN NORMAL RAT LIVER CELLS, PRE-NEOPLASTIC CELLS AND EXPERIMENTAL HEPATOCELLULAR CARCINOMAS

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Cytoplasmic fibrils have been described in a variety of non-neoplastic and neoplastic tissues including human liver cancer. In normal rat liver cells, fibrils are seen in relation to junctional complexes and rarely in the cytoplasm where they occur as short bundles of 4 - 6 fibres. Prominent bundles of fibrils have also been observed in experimentally induced liver carcinomas and appear to resemble their counterparts in the human neoplasms. They have also been found in the hepatomas induced in our strain of albino rats fed the carcinogen, p-dimethylaminoazobenzene. After the experimental animals have been fed the carcinogenic dye for some months and before the tumours arise, the pre-neoplastic cells show a remarkable increase in the number and length of the cytoplasmic fibrils. They are found mainly in three situations: (*i*) along the cell membranes where some of them may be related to the normal fibrils of the junctional complexes; (*ii*) in the cytoplasm itself where they occur as bundles of straight fibres and (*iii*) rarely along the outer nuclear membrane. With the possible exception of those in (*i*) they appear to be free in the cytoplasm and do not

have any other particles or structures attached to them. The fibrils described bear some similarity to the cytoplasmic filaments which are characteristic of squamous epithelial cells. The main interest at the moment concerns their appearance in increased numbers in liver cells exposed to a carcinogen while they persist in the neoplasms after the carcinogenic stimulus is withdrawn.

EFFECT OF PORPHYRINS ON RED CELL SURVIVAL

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In erythropoietic porphyria the basic biochemical disturbance is an imbalance between porphobilinogen deaminase and isomerase. This leads to the synthesis by erythroid tissue of uro- and coproporphyrin type I isomer as well as type III. The type I isomer cannot be utilized for haem synthesis and accumulates in red cell precursors in the marrow and in mature red cells. Porphyrin crystals have been observed in the cytoplasm of red cell precursors in the marrow. To determine if excessive amounts of porphyrins bring about haemolysis, rabbit red cells were incubated with δ-aminolaevulinic acid increasing the level of porphyrins in the cells. These cells were then labelled with ^{51}Cr and their life-span was measured. No shortening of red cell life-span was found even with high porphyrin levels in the cells. These results differ from those found in human cases. Red cells from a normal subject and a case of erythropoietic porphyria were incubated with δ-aminolaevulinic acid and labelled with ^{32}DFP. A control population of cells was labelled with tritiated DFP. The cells with increased porphyrins both from the normal subject and from the patient with erythropoietic porphyria had a shortened life-span as compared with the control population of cells. In erythropoietic porphyria, however, high porphyrin levels in red cells may be found with normal red cell survival and vice versa. The above observations suggest that while high levels of porphyrins in human red cells may cause haemolysis this is unlikely to be the cause of shortened red cell survival in erythropoietic porphyria. The type of porphyrin isomer also is unrelated to haemolysis since the same degree of haemolysis was produced in the normal subject and in the case of erythropoietic porphyria, although in the former type III isomer was formed whereas in the latter both type I and type III were produced.

BLOOD AND URINARY FOLATE LEVELS IN LIVER DISEASE, AND IN ASSOCIATION WITH SURGICAL TRAUMA

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Folate is poorly bound to plasma proteins, and it is known that renal conservation of folate is limited. In the present study the effect of active liver disease (virus hepatitis) and abdominal and extra-abdominal surgical procedures on serum and urinary folate levels (*L. casei* assay), was investigated. At the onset of hepatitis, serum folate remains unchanged or rises slightly; urinary folate excretion, however, is markedly increased (more than 30 μg./day). With convalescence the urinary folate levels rapidly return to normal, while serum folate may become temporarily decreased. Liver damage probably releases large amounts of storage folate, which is rapidly lost in the urine because of poor plasma binding. In the first 5 days after major surgical trauma urinary folate loss is similarly increased, while serum folate may become slightly decreased. The cause of this is uncertain but possibly relates to liberation of tissue storage folate during the non-specific catabolic reaction which follows trauma. Folate is, *inter alia*, essential for nucleoprotein synthesis, and an adequate supply would thus seem important to meet the increased metabolic needs associated with post-operative healing. It is unlikely that the degree of urinary folate loss detected in this study could rapidly drain normal folate stores, but it might be of importance when tissue stores are already low.

FOLATE METABOLISM DURING LACTATION

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Severe folate-deficient megaloblastic anaemia in Bantu adults is seen most frequently in association with lactation, suggesting that the physiological requirement for folate during lactation may be considerable. The breast-fed infants of these mothers do not usually show evidence of folate deficiency, suggesting that secretion of folate into breast milk takes precedence over the mother's needs. The sequence of events attendant on low dietary folate intake by lactating mothers was studied. All items of food in the hospital diet were assayed for folate activity, and a diet was devised containing 10 - 15 μg. of folic acid daily. The diet was adequate in all other respects. On feeding this diet to a lactating subject, the serum folate concentration fell significantly within 7 - 10 days, but breast milk folate remained constant over a 2-week study period. Administration of 100 μg. of folic acid daily failed to halt the fall in serum folate level, but this was accomplished by 300 μg. daily. These results demonstrate the priority which breast milk holds over maternal folate stores during lactation, and suggest that the physiological requirement for folic acid during lactation may be of the order of 4 - 5 times higher than the 50 μg. daily established by Herbert for non-lactating females.

THE RATE OF GENERATION OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE AND REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE IN THE LIVERS OF NORMAL AND PORPHYRIC RABBITS

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In addition to δ-aminolaevulinate synthetase control of haem biosynthesis, there are other metabolic changes which accompany or precede haem synthesis. A drug-induced stimulation of haem biosynthesis has been shown to be accompanied by altered succinate and fumarate metabolism. An inducible form of the mitochondrial enzyme, succinyl coenzyme A synthetase has been observed in porphyric liver. This presumably serves to supply additional substrate, together with glycine-pyridoxal for δ-aminolaevulinate synthetase. In rat-liver homogenate, fumarate-1-4-^{14}C was shown to be incorporated via succinate and succinyl CoA into haem at an accelerated rate. Fumarate reduction to succinate requires reduced NAD. In addition, porphyria-inducing drugs have been shown to inhibit NADH-oxidase. In this study, an attempt has been made to demonstrate an altered reducing climate in the livers of porphyric rabbits. The rabbits were made porphyric by daily intubation with 200 mg. allylisopropylacetamide per kg. body-weight, for 7 - 10 days. Nicotinamide-^{14}C was injected into the rabbits, which were then sacrificed at appropriate time intervals, the livers being rapidly removed and placed into liquid nitrogen. NAD, NADP and their corresponding reduced forms were isolated by acid and alkali extraction respectively, and separated on Dowex formate columns. The specific activity of each fraction was measured with a Packard Tricarb liquid scintillation counter. The ratio of the specific activity of reduced to oxidized nucleotide was calculated, and plotted against time. The results show a striking increase in the rate of generation of reduced NAD and NADP in the livers of porphyric rabbits. It is therefore suggested that there is an alteration in the reducing potential of porphyric liver that leads to an increased rate of formation of haem precursors, and ultimately haem biosynthesis. The overproduction of haem precursors would explain the rise of δ-aminolaevulinate and porphobilinogen in the urine of AIA-induced porphyric rabbits.

FREE AND TOTAL FOOD FOLATE: EFFECT OF FOOD INTAKE ON URINARY FOLATE EXCRETION

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Food folate consists partially of polyglutamate compounds unavailable to *L. casei*, as folate assay organism. Although extensively studied, little is known about the availability of food folate to the human body. The free and total folate content of raw and cooked food was assayed before and after treatment with chicken pancreas conjugase. Cooking caused a marked fall in free folate activity, as is well known (100% of the original values, mean 39·1%), but total folate was much less affected (10·9 - 100% of original values, mean 73·9%). An attempt was made to assess the absorption of food folate by measuring urinary folate excretion after ingestion of food items with known free and total folate content. A healthy 34-year-old male was kept on a normal but constant diet and his urinary folate excretion assessed. For a period of approximately 8 months the 24-hourly urinary folate remained fairly constant (1·0 - 4·2 µg./day). During this period measured amounts of PGA were taken by mouth and urinary folate excretion was assayed. When 5 µg. PGA/kg. body-weight was taken, urinary excretion was 7·8 - 16·6 µg./first 24 hours; with 10 µg. PGA/kg. body-weight, excretion was 18·8 - 50·5 µg./24 hours. Test foods with measured folate content were then eaten and urinary folate was assayed. The excretion pattern suggested that total folate rather than free folate was utilized from most foods tested.

CARBON SOURCES FOR INCREASED HAEM PRECURSOR AND HAEM SYNTHESIS IN EXPERIMENTAL PORPHYRIA

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In the classical conception of haem synthesis, succinate constitutes one of the substrate pools. Since it has been demonstrated that TCA cycle activity is unchanged in the livers of animals with allylisopropylacetamide (AIA)-induced porphyria, the problem arises as to how the succinate pool is augmented to support the increased haem precursor and haem synthesis in the porphyric animals. One hypothesis proposes that fumarate acts as substrate for non-constitutive TCA cycle succinate synthesis and that substrate fumarate is derived from pyruvate. To test this hypothesis pyruvate carboxylase (pyruvate : carbon-dioxide ligase EC 6.4.1.1) activity, acetyl-CoA concentration, 'malic' enzyme (L-malate : NADP oxidoreductase (decarboxylating) EC 6.4.1.1), 'fumarate reductase' and δ-aminolaevulinate synthetase (succinyl-CoA; glycine N-succinyl transferase EC 2.3.1.13) activities were measured in the livers of rats given AIA. It was found that pyruvate carboxylase activity and acetyl-CoA concentration were unaffected by AIA administration. Hence oxaloacetate synthesis by this route seems unlikely to give rise to augmentation of the fumarate pool. 'Malic enzyme' activity was also unaffected by AIA administration and malate as the carbon source for fumarate synthesis by this route is therefore unlikely. 'Fumarate reductase' activity in livers could not be consistently demonstrated in either the normal group or the experimental group of rats, and the existence of a 'fumarate reductase' in mammalian tissue as an entity may be questioned. Despite the demonstrated unresponsiveness of the enzymes which could support an increased fumarate pool for subsequent haem synthesis, δ-aminolaevulinate synthesis was increased. These findings suggest that the source of succinate for haem synthesis is unlikely to be controlled by the carboxylating enzyme systems operative with pyruvate as substrate.

SPECIFICITY OF THE PORPHOBILINOGEN DEAMINASE-ISOMERASE ENZYME COMPLEX

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Previous studies from this laboratory have suggested that the cause of the shortened red cell survival in erythropoietic porphyria is related to the basic enzyme disturbance in the disease, i.e. an imbalance between porphobilinogen deaminase and uroporphyrinogen isomerase. In effect there is a relative or absolute excess of porphobilinogen deaminase. A possible cause for the haemolysis is that the excess porphobilinogen deaminase may be active against closely related substrates causing non-specific deamination and therapy impairing cell structure. To test this hypothesis the PBG deaminase-isomerase enzyme complex was isolated from fowl and human red cells and also from a patient with erythropoietic porphyria. The enzyme complex was incubated with porphobilinogen in a Conway flask and ammonia was produced. The enzyme was also incubated with substrates closely related to porphobilinogen; i.e. glycine, glycol-proline, tryptophane and cysteine. In no case was ammonia produced. The uroporphyrinogen isomerase enzyme was then destroyed by heating, thus isolating the porphobilinogen deaminase enzyme. This enzyme was studied as for the enzyme complex. The results were similar. The findings do not support the hypothesis that PBG deaminase may be non-specific. The Michaelis constant for the enzyme complex from fowl normal blood and from the blood of the patient with erythropoietic porphyria was also determined. The results for fowl and normal human blood were similar. However, there was a 10 - 20-fold higher Km for the enzyme complex from the case of erythropoietic porphyria. There was thus a marked reduction in the affinity of the enzyme complex for its substrate porphobilinogen. The finding suggests that the basic defect in erythropoietic porphyria is a structurally altered porphobilinogen deaminase enzyme.

THE INCIDENCE OF FOETO-MATERNAL HAEMORRHAGE IN DIFFERENT RACIAL GROUPS IN DURBAN: A PRELIMINARY REPORT

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Using the Feldhaus modification of the Kleihauer acid-elution staining technique, blood samples from 291 Caucasian, 253 Coloured and 68 Bantu patients with ABO-compatible infants were examined for the presence of foetal red blood cells. The blood samples were collected within three hours of the delivery of the placenta. An area of 50 sq.mm. of the blood film was scanned using a low power (× 10) objective. Using the interpretation suggested by Woodrow and colleagues that 5 foetal cells in an area of 34·5 sq.mm. are equivalent to a foeto-maternal haemorrhage of approximately 0·25 ml., it was assumed that a count of 7 or more foetal cells per 50 sq.mm. was equivalent to a foeto-maternal haemorrhage of 0·25 ml. or greater. The incidence of foeto-maternal haemorrhage of 0·25 ml. or greater did not differ significantly in the three racial groups, and was found to be as follows: Caucasians 12·0%, Bantu 11·7% and Coloureds 11·4%. A further 74 Caucasians, 37 Bantu and 75 Coloureds with ABO-incompatible infants were examined. The incidence of foeto-maternal haemorrhage of 0·25 ml. or greater was found to be 4·3% in the combined figures of the three racial groups, indicating the rapid elimination of the ABO-incompatible cells from the maternal circulation.

VITAMIN B_{12} AND THE LIVER

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A study of 60 patients with liver disease. Apart from the standard liver-function tests the following were performed: paper electrophoresis; assay of vitamin-B_{12} binding capacity; assay of serum vitamin B_{12} with *L. leichmannii*; assay of serum vitamin B_{12} by a radio-isotope technique; and assay of p-binder (a specific protein-binding hydroxocobalamin). In patients with ascites the ascitic fluid was also analysed. In addition, two subsidiary series of patients with kwashiorkor and with infec-

deficiency as assessed by serum folate estimations on infants followed-up till 16 months (± 12%). It was not associated with any measurable effect on infant health and was associated in only one instance with evidence of significant depletion of folate stores, i.e. a megaloblastic bone marrow. Evidence was presented that the low serum folate values were primarily related to suboptimal dietary intake of folate. Megaloblastosis developed where deprivation was severe and associated with recurrent gastro-intestinal and other infection. The necessity for prophylaxis of latent folate deficiency was discussed.

SCREENING TESTS FOR THE DETECTION OF ERYTHROCYTE GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY: A CRITIQUE

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The original tests, namely, Heinz body and glutathione stability, were found to be inadequate as screening methods. In particular, Bantu groups in poor protein nutrition and subjects with thyroid dysfunction gave anomalous patterns for the glutathione stability technique. For the visual dye-screening tests, the dichlorophenol-indophenol (DCPIP) linked method provided better distinction between normals, heterozygotes and homozygotes (enzyme-deficients) than the brilliant cresyl blue (BCB) or methylene blue tests. Samples of DCPIP were uniform in the time required for decolorization in specimens with normal red cell enzyme. On the other hand, BCB dyes gave different decolorization times; only those with a violet component (isolated by chromatography; absorption peak at 620 mμ) were satisfactory. Further, some BCB brands were photosensitive. The tetrazolium and fluorescent spot paper tests were more tedious, and the latter required a long-wave UV source to detect the native fluorescence of NADPH produced by normal erythrocytes. For field tests, where simple and stable reagents were important, the methaemoglobin reduction procedure proved to be more suitable than dye-screening tests. In general, the use of whole blood produced false results when there was marked anaemia or leucocytosis; standardization to a 50% saline erythrocyte suspension free of white cells was required. In cases of haemolytic anaemia with reticulocytosis, a true assessment of the glucose-6-phosphate dehydrogenase status of the mature red cells was obtained by testing the bottom layer (virtually reticulocyte-free) of a centrifuged blood sample. Results of screening tests indicated that periodic quantitative controls (spectrophotometric or colorimetric estimation of the ability of haemolysates to form NADPH or reduce DCPIP respectively in an assay system) were necessary. In particular, screening tests possessed a limited and variable ability for heterozygote detection in this X-linked enzyme defect.

PLEUROPNEUMONIA-LIKE ORGANISMS IN BURKITT'S LYMPHOMA

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Electron-microscopy has revealed the presence of pleuropneumonia-like organisms in species from 3 cases of Burkitt's multifocal lymphoma. Massive infection was found within the ovary, an abdominal lymph node and a peritoneal nodule collected 2 hours after death from a 3-year-old White female in Johannesburg. Frozen and thawed material from the liver and spleen of this case was inoculated into a 48-hour-old vervet monkey (*Cercopithecus pygerythrus aethiops*) and caused its death from bronchopneumonia within 13 days. A second monkey of the same age died from similar causes 57 days after inoculation with viable whole cells from the same liver and spleen. Electron-microscopy of postmortem specimens taken from the monkeys revealed massive PPLO infections in the spleen, lung and axillary lymph node, with fewer organisms in the liver. The human material was passaged in the hamster adult kidney line and caused a cytopathic effect. Cells in third passage were examined by electron-microscopy and found to contain isolated organisms resembling PPLO. The electron-microscope showed the presence of PPLO in two further biopsy specimens obtained from 7-year-old Bantu children; one from a maxillary tumour and the other from a tumour of the ovary.

AMINO ACIDS AND ANAEMIA IN KWASHIORKOR

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The role of amino acids in the pathogenesis of anaemia in kwashiorkor is equivocal. Controlled clinical trials, feeding amino acids while excluding other possible haemopoietic factors, are usually impractical. For this reason, the effect of added amino acids on haem and globin synthesis by erythroid precursors has been studied *in vitro*. Marrow samples were aspirated from anaemic protein-malnourished infants, with subnormal serum albumin concentrations. Marrow cultures were set up in autologous serum, and a mixture containing all amino acids necessary for haemoglobin synthesis, minus glycine, was added. Control cultures contained no added amino acids. Glycine ^{14}C was added as label to all tubes. After 4 hours' incubation at 37°C, KCN was added. The cells were washed and lysed, and the haemoglobin solution was filtered after removal of debris by centrifugation. The haem was crystallized, and the globin purified. The samples were combusted and the specific activity of the glycine ^{14}C in haem and globin was assayed. The addition of amino acids failed to enhance either haem or globin synthesis in the marrows studied. In the same way haem or globin synthesis was not augmented by added amino acids in the presence of a mixture of iron, folic acid, vitamin B_{12}, pyridoxin and ascorbic acid. By way of contrast, these haemopoietic factors did increase haem and globin synthesis, *without* added amino acids, in some patients. These results do not support the concept that amino acids may be a rate-limiting factor in haemoglobin synthesis in kwashiorkor.

ASSAY OF INFECTIOUS DEOXYRIBONUCLEIC ACID DERIVED FROM POLYOMA VIRUS

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The infectious deoxyribonucleic acid (DNA) prepared from polyoma virus causes malignant transformation of susceptible cells. However, on mouse embryo cells it has a cytopathic action and can therefore be titrated by a plaque count. The standard assay method for infectious DNA required the infection of monolayers of mouse embryo cells in 0·55 M NaCl. Damage caused by hypertonic NaCl renders subsequent plaque counts difficult and inaccurate. A preferable assay method is the infectious centres technique whereby cells suspended in hypertonic solutions are infected and are allowed to settle on normal monolayers after suitable dilution in isotonic medium. Unsuccessful attempts to use this method in the case of polyoma DNA have been reported. Since this method has obvious advantages, further investigations seemed justified. This infectious centres technique proved consistently successful when a sufficiently high concentration of cells was infected in suspension in 0·55 M NaCl and allowed to settle on sparsely seeded monolayers. This method gave plaque counts which were approximately $\frac{1}{3}$-$\frac{1}{2}$ of the counts obtained when the direct plating method was used. An attempt was made to enhance the sensitivity of the infectious centres assay method by adding various concentrations of dimethyl sulphoxide (DMSO). This substance had no enhancing effect in hypertonic 0·55 M NaCl but when used in an isotonic solution a striking increase in plaque count was obtained. Maximal enhancement was found with 15% DMSO. With this concentration the infec-

tious centres technique is found to give up to a 100-fold increase in plaque count as compared with the standard direct plating technique.

GLOMERULAR 'PORE SIZE' ESTIMATION BY AN AUTOMATED MOLECULAR-SIEVING TECHNIQUE

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Recent work has shown that the renal clearance of a range of serum proteins permits an estimate of glomerular 'pore size' or permeability. A molecular-sieving method (column 100 cm. × 1 cm.) was used and fractions were analysed by the Folin-Ciocalteau method on a Technicon auto-analyser. The results were calculated by computer after the calibration curve had been analysed algebraically. A bi-directional phase shift was introduced into the calculations to achieve optimal statistics for a linear least-squares regression on the graph of the logarithm of the urine/serum ratio against fraction number. The angle between this graph and the perpendicular is an estimate of glomerular 'pore size'. The results have shown that the method is highly reproducible on the same and on different columns and over long periods in 2 patients who have remained static. Of the 14 patients tested, only 2 have shown a response to steroids, and these have had the smallest estimates of 'pore size'. The residual proteinuria in one case was associated, however, with a much larger 'pore size'. Four cases of orthostatic albuminuria tested, apart from having smaller amounts of urinary protein, have not been distinguishable from ordinary cases of nephrosis and have shown a range of 'pore sizes'. The two cases of 'healed' pyelonephritis with persistent proteinuria have had 'pore sizes' indicating virtually unobstructed passage of serum proteins through the kidney. Estimates of normal 'pore size' are hard to obtain as cystic and other proteins not of serum origin cause interference at low protein excretion rates. The infusion of albumin into nephrotic subjects causes only slight changes in the estimates of 'pore size'. There is no correlation between 'pore size' and quantitative proteinuria in different subjects although in an individual case there is a moderate degree of correlation at different stages of the disease.

A NEW GROUP OF HUMAN RESPIRATORY VIRUSES MORPHOLOGICALLY RESEMBLING THE INFECTIOUS BRONCHITIS VIRUS OF CHICKENS

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In the last few years a new group of human respiratory viruses has emerged, the members of which are medium-sized and ether labile, and contain RNA. They have a characteristic morphology, resembling infectious bronchitis virus (IBV) of chickens, and have fastidious growth requirements necessitating the use of organ cultures of human embryo ciliated respiratory epithelium for their primary isolation. There are periods when the rate of isolation of the usual causative viruses from patients with the common cold syndrome drops sharply. Working in collaboration with McIntosh in Chanock's laboratory, nasopharyngeal washings from patients during such a period were re-examined using organ cultures instead of standard cell cultures. From 23 specimens 8 viruses were isolated: 2 were rhinoviruses, and 6 were similar to IBV morphologically. The last 6 virus isolates were studied further. Attempts to adapt them to cell cultures were unsuccessful but two of the six were adapted to a selected strain of white mice which was free of mouse hepatitis virus (MHV) infection. It was shown that the 6 isolated viruses were apparently the same strain antigenically and that 18 of 59 consecutive patients with the common cold showed a specific antibody response indicating that they had been infected with this strain. Morphologically the virus resembles but is antigenically distinct from IBV, MHV, and another human respiratory virus isolated by other workers (strain 229E). Electron-microscopy confirmed that these viruses are distinct from the myxoviruses.

RESULTS OF CYTOGENETIC INVESTIGATION OF INTERSEX STATES

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The chromatin pattern in suspected cases of intersexuality is of value in the diagnosis. In chromatin-positive cases ovarian tissue has invariably been found. In chromatin-negative cases, 46XY or 45XO rudimentary or absent gonads are noted. Low percentage chromatin bodies may indicate mosaicism. Cases investigated in the unit have been classified under chromatin-positive and chromatin-negative groups. Forty-two chromatin-positive cases were classified as follows: Of true hermaphrodites 46XX (26 reared as males, 5 reared as females), one presented as a female with 46XX/46XY mosaic. Tumours were present in 3 cases: bilateral cystadenocarcinoma, dysgerminoma and seminoma. Female hermaphroditism in 4 cases was due to exogenous hormones during pregnancy. Adrenogenital syndrome was not found in the Bantu. Two cases of XX males with testes had ambiguous genitalia. Two cases without a vagina and one case without a uterus were 46XX. In 44 males with hypogonadism, only 18 were true Klinefelter's syndrome 47XXY and 4 were mosaics 47XXY/46XY, one of whom was a Bantu. This syndrome is rare in the Bantu. Gonadal aplasia — Turner's syndrome were chromatin positive in 8 cases, all mosaics. Six were 46XX/45XO (one case was the mother of a 46XX child). Two were 47XXX/46XX/45XO, one case was 46XX with an isochrome X. Thirty cases with varied phenotypes were chromatin negative. Five male hermaphrodites 46XY had ambiguous genitalia. Eleven showed testicular feminization syndrome with female phenotye, 46XY, 3 of whom had Sertoli-cell adenomata. Four cases were Bantu. There were 3 cases with agonadism 45XO/46XY. The gonadal aplasia — Turner's syndrome with chromatin-negative sex were 45XO. Of 4 cases, 45XO was diagnosed as Bonnevie Ulrich syndrome in a Bantu infant, and as 46XY in one case; mosaic 45XO/45/XO+ fragment was found in twins. Mosaic pattern 46XY/45XO was found in 2 cases of male Turner syndrome. One case of XYY male was investigated because of offspring with multiple congenital abnormalities.

A CAPSULE DEPOLYMERASE ASSOCIATED WITH AN *ALCALIGENES FAECALIS* PHAGE-HOST SYSTEM

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A virulent *Alcaligenes faecalis* bacteriophage A6 lyses its encapsulated host, *A. faecalis A6*, to produce plaques surrounded by halos. Organisms in the halos are devoid of capsules, but remain viable. The loss of capsules is due to the production of an enzyme present in high-speed supernatants of phage lysates prepared on A6 or A6T, a non-capsulated variant of organism A6. The enzyme occurs free as well as in a phage-bound form which could not be separated from the phage particles. These enzymes are possibly identical. Acid hydrolysis of the polysaccharide substrate of the enzyme yielded D-ribose, D-glucose, D-galactose, glucuronic acid and three other components which could not be identified. The enzyme hydrolysate contained no sugars or reducing substances and retained the antigenic properties of the capsule, but sedimented more rapidly in the ultracentrifuge and exhibited an increased rate of diffusion through agar. These results indicate that the action of the enzyme is limited, probably only resulting in internal cleavage of the polysaccharide with relatively large end-products. The efficiency of plating of the phage was unaltered by the presence or absence of the capsule, or the addition of excess polysaccharide or phage-free enzyme. Phage adsorption was diminished on the non-capsulated strain A6T or enzyme-treated cells of A6. These results may be interpreted to mean either that the capsule and the cell wall possess phage receptors, or that only the capsule has these receptors and that A6T is not completely devoid of capsule. Attempts to isolate other phages or mutants of phage A6 lacking enzyme activity were unsuccessful. This, and the inability to separate bound enzyme from the phage, made it impossible to define the role of the enzyme during phage infection.

SPONTANEOUS POLYOVULATION IN THE HUMAN AND ITS EFFECT ON THE ENDOMETRIAL PATTERN

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The endometria and corpora lutea of 8 cases with polyovulation were carefully dated according to accepted criteria. Six of the patients had 2 corpora lutea and two had 3 corpora lutea each. The corpora lutea were variously distributed between the two ovaries. None of the patients had received any exogenous gonadotrophins, and a history of twin pregnancy was obtained in one case only. In 6 of the 8 cases the morphological features in the endometrium consisted of an accentuation of those found in a normal progestational phase, namely glandular secretion, oedema and pseudodecidual reaction. These morphological features should be borne in mind in the differential diagnosis of a normal or ectopic pregnancy, a persistent corpus luteum or a persistent corpus luteum cyst, and it may occasionally be observed as a result of psychological disturbance such as is found in cases with pseudocyesis. In the majority of the cases the more mature and morphologically poorly developed corpus luteum was accompanied by a far better developed second or third corpus luteum with an age discrepancy of no more than 2 - 3 days. The physiological basis of polyovulation could be due either to insufficient progesterone production by the first corpus luteum which does not inhibit FSH and LH secretion, or to the so-called 'rebound phenomenon' following anovulatory cycles.

RESTRICTION WITHOUT MODIFICATION OF PHAGE 34/13 IN A STRAIN OF *PROTEUS MIRABILIS*

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Phage 34/13 prepared on its host strain *Proteus mirabilis* 13at does not form plaques on *P. mirabilis* strain N6 when serial dilutions are spotted on a lawn of the organism. Low dilutions show areas of clearing due to bacterial lysis but no phage capable of forming plaques on N6 is obtained from these zones. Phage 34/13 adsorbs to an extent of 99% on strain N6 within 15 min. With the use of ^{32}P-labelled phage 34/13 DNA it is shown that within 15 min. of adsorption to N6, 57% of the label is in the medium in the form of acid-soluble nucleotides. This figure is reduced to 11% when the phage adsorbs to its normal host 13at and 0·9% when the phage is mixed with a strain of *P. mirabilis* to which it does not adsorb. This indicates that the DNA of phage 34/13 is restricted by strain N6. The phage DNA which escapes restriction is not modified as no plaques are formed. Phenotypically strain N6 behaves as if its genotype is r^+m^-. It may be argued that r^+m^- strains should degrade their own DNA and be nonviable. Strains of this genotype have never been isolated after mutagen treatment of wild strains. Many bacteria which restrict foreign DNA owe this property to a prophage. Strain N6 carries a prophage which on UV induction produces a defective phage which manifests itself as a bacteriocin which kills strains of *P. mirabilis*, and this plasmid may contribute to the restrictive process. This has not been proved. Attempts to isolate mutants of phage 34/13 which escape restriction have been unsuccessful. Fruitless attempts have also been made to isolate r^-m^- or r^-m^+ mutants of strain N6 which would allow plaque formation by the phage.

THE ALPHA-FETO-PROTEIN TEST FOR PRIMARY CANCER OF THE LIVER

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Embryo-specific alpha-feto-protein was detected in the sera of 4 patients with primary carcinoma of the liver by Russian workers. A project on malignant hepatoma occurring in Bantu mineworkers has been in progress on the Witwatersrand, South Africa, for the past 4 years and during this period sera have been collected at monthly intervals from suspected cases referred to a mine hospital. The collected sera were tested for the presence of alpha-feto-protein by a standard Ouchterlony immunodiffusion technique, using an antiserum prepared by injecting rabbits with serum from a 5-month-old stillborn foetus and absorbing the rabbit antiserum with pooled normal adult human serum. Immuno-electrophoresis studies confirmed the presence of a single antigenic component in the reacting sera tested. Sera from 194 cases, a total of 405 samples, were available for study. Of the cases, 132 were proved to have a malignant hepatoma by biopsy alone (19 cases) or by postmortem (113 cases). The remainder were not considered to be cases of malignant hepatoma after detailed testing or actual postmortem examination. One hundred cases of primary cancer of the liver out of a total of 133 (i.e. 75%) showed a positive alpha-feto-protein test. In addition there was one positive in the case considered to have congestive cardiac failure clinically. No positive result was confuted by a negative postmortem. No false-positive result has been obtained in an adult serum up to the present time, although sera being submitted for liver-function tests and electrolyte determinations are being screened as a routine. Neonatal serum samples are often found to be positive to the test. The alpha-feto-protein test, even in its present relatively insensitive form, appears to be a valuable specific test for primary cancer of the liver.

DYSGAMMAGLOBULINAEMIA IN THE BANTU

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Since June 1965, routine electrophoresis has been carried out at the chemical pathology laboratory of the Pretoria General Hospital on all serum protein investigations requested. Up to March 1968, 58 Bantu cases and 99 White cases were discovered with one or more abnormal bands in the electrophoretic pattern. Immuno-electrophoresis with antiserum against immunoglobulins IgG, IgA and IgM was carried out on 49 of the Bantu cases and 72 of the White cases. Of the Bantu cases 54% showed IgG dysgammaglobulinaemias, with 12 of the 26 cases showing an abnormal band concentration of more than 1·0 G/100 ml. Of the 11 cases of IgA dysgammaglobulinaemia (23%), 7 had an abnormal band concentration of more than 1·0 G/100 ml., and of the 3 cases of IgM dysgammaglobulinaemia (6%), one had a concentration of more than 1·0 G/100 ml. Myeloma cases proved clinically, radiologically and by bone-marrow examination all fell in the groups in which the concentration of the abnormal protein band was greater than 1·0 G/100 ml. The IgM dysgammaglobulinaemia with a concentration greater than 1·0 G/100 ml. was a Waldenström macroglobulinaemia with Bantu porphyria. Eight cases, 3 of which were proved cases of myeloma, displayed no dysgammaglobulinaemias. These 3 cases showed advanced clinical, radiological and bone-marrow manifestations of the disease, with high concentrations of Bence-Jones proteinuria and a marked decrease in all three types of immunoglobulins.

TRANSFECTION IN PROVIDENCE AND PROTEUS SPECIES

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The first step in phage-bacterial interactions is adsorption of the bacteriophage to a specific receptor on the bacterial cell wall. The presence of the bacteriophage receptor is necessary for phage to inject its genome. It was decided to examine the infectious properties of phage DNA in systems where this condition does not apply. Spheroplasts of different strains of providence, *Proteus mirabilis*, *P. morganii* and *P. vulgaris* were prepared by penicillin treatment. DNA was obtained by phenol-extraction of bacteriophage PL25, which is normally

infective for providence strain P29. Band-sedimentation in CsCl solution showed this DNA to be homogeneous with a molecular weight of 26 × 10^6 and a S_{20w} of 32·3. This DNA was not infective for intact P29 organisms. Spheroplasts could not be infected by intact phage. Infectivity of the phage DNA was examined by its addition to the different spheroplast preparations. Samples of the mixtures were assayed for infectious centres on providence strain P29. In all the systems infectious centres could be detected after approximately 35 minutes. Infectious centres increased slowly to reach a maximum at approximately 110 minutes. Maximal efficiency of DNA infection of 10^{-1} was obtained in spheroplasts of the homologous host, while lower efficiencies of about 10^{-9} were demonstrated in the other strains which are not normally susceptible to the phage. These results may be interpreted to mean that phage sensitivity is just another phenotypic character which strains may have in common. The low efficiencies of phage DNA infection might result from the unfavourable circumstances of interaction between the DNA and the spheroplasts, since entrance to the spheroplasts presumably occurs only by way of the scattered lesions in the cell wall resulting from spheroplast formation. Net DNA synthesis could not be demonstrated, so that it cannot be decided whether infectious DNA primes new DNA synthesis via a DNA polymerase or acts only as a template for the synthesis of messenger RNA concerned with capsid formation.

THE STRUCTURE OF DNA FROM A PROVIDENCE-TRANSDUCING PHAGE

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The temperate providence bacteriophage PL26 has been shown to contain a single duplex DNA molecule. Band-sedimentation proved this DNA to be homogeneous with a calculated molecular weight of 25 × 10^6. Determination of molecular weight and length of bacteriophage DNA is hampered by the great length of intact DNA. Direct study of the anatomy of the phage DNA may verify results obtained with physical studies and yield information regarding its physical nature. Phenol-extracted DNA was diluted to 2 μg./ml. in 1M ammonium-acetate. Cytochrome C was added to the DNA to give a final concentration of 0·01%. The protein-DNA mixture was spread on a clean air-water interface. The surface film was transferred to a carbon-coated electron-microscope grid. Contrast was produced by small angle (8°) vacuum deposition of palladium-gold from two directions at an angle of 90°. Electron-microscopy showed the presence of linear fibres of DNA with no circular forms. The contour length of the fibres was measured. Assuming a linear density of 196 daltons/Å for the B configuration of the Watson-Crick DNA model at ionic strengths between 0·14 and 0·50, the molecular weight of the DNA was calculated. Heating of concentrated DNA to 75°C followed by slow cooling did not produce circular forms. This suggests the absence of 'sticky' end as described for phage lambda DNA. The DNA was denatured by treating with 0·20M NaOH followed by annealing at 65°C for 40 minutes. This treatment also produced no circular forms of the DNA. A similar result was obtained with denaturation by heating in the presence of ClO_4^- and annealing at 25°C in the presence of 7·2M $NaClO_4$. These results suggest that the collection of DNA molecules of phage PL25 is unique and not permutated like T-even phage DNA.

PROBLEME IN DIE VROEË DIAGNOSE VAN DIABETES MELLITUS

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Omdat die sogenaamde komplikasies van diabetes mellitus dikwels die optrede van die klassieke simptome en tekens van hierdie siektetoestand voorafgaan, word die relatiewe waarde van sekere chemiese patologiese ondersoeke as hulpmiddels in die korrekte vroeë diagnose bespreek. In 'n reeks van meer as 500 Blanke volwassenes en 50 Blanke kinders was die orale glukose toleransieproef nog ons beste hulpmiddel om 'n leidraad van die moontlike abnormaliteit, wat aanwesig is, te verskaf. Sekere kliniese simptome en tekens het tesame met die orale glukose toleransiekurwe die beste leidraad verskaf. Insulienantagonisme as gevolg van verskillende oorsake speel 'n belangrike rol in die ontstaan van verminderde verdraagsaamheid teenoor glukose. Hipokalemie met sy oorsake en lewerfunksie stoornisse, is van die belangrikste minder bekende insulienantagoniste. Hipotireose, hipertireose, hiperkortikoadrenalisme, al die oorsake van reaktiewe hipoglukemie, en gebruik van geboortebeperkingtablette, kan almal verwarring in die vroeë diagnose van diabetes mellitus veroorsaak. Seruminsulien bepalings as sulks het ons min gehelp om 'n korrekte diagnose te maak. Maar seruminsulien bepalings, saam met natrium en kalium bepalings en urienondersoeke vir glukose tydens die orale glukose toleransiekurwe was van groot waarde. Dit was soms nodig om die intraveneuse tolbutamiedproef, orale leusienproef, intramuskulêre glukagonproef en aminosuur chromatografie op die urien in twyfelagtige gevalle uit te voer.

AN ISOLATED RECEPTOR FOR A *PROTEUS VULGARIS* BACTERIOCIN

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Bacteriocins derived from *Proteus vulgaris* strains induced by ultraviolet light have been shown to consist of sheathed phage tail-like particles which lack DNA. Their sheaths are contractile and they adsorb specifically to, and kill, sensitive bacteria. To investigate the specificity of this adsorption, lipopolysaccharide was extracted with 45% (w/v) phenol at 70°C from acetone-dried cells or cell wall preparations of sensitive indicator strains, and also from strains resistant to these bacteriocins. Receptor activity of the extracts was measured by their neutralization of bacteriocin activity when mixed with the latter and incubated at 37°C for 5 min. Mixtures of bacteriocin and lipopolysaccharide from the sensitive strains showed marked reduction of bacteriocin titres. Control experiments with extracts from the resistant strains showed no loss of activity. The mixtures were also negatively stained with neutral potassium phosphotungstate and examined in an electron-microscope. This revealed adsorption of bacteriocin particles to lipopolysaccharide of the sensitive strain. The adsorption caused contraction of the sheaths and also resulted in the breakdown of most of the particles. No adsorption occurred with the heterologous lipopolysaccharide and the bacteriocin particles remained untriggered and undamaged. The site of specific adsorption of bacteriocin 45 thus appears to be located in a lipopolysaccharide fraction of the cell wall.

TRANSFER OF HAEM FROM HAEMOGLOBIN TO SERUM ALBUMIN

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The transfer of haem from human haemoglobin or carbonyl haemoglobin to serum albumin was demonstrated when the pure proteins were brought together in solution. Both haem transfer (rate and extent) and binding to albumin were increased by rise of temperature from the ambient to 37°C and by a pH in the solution of 7 or above, while autoxidation of HbO_2 or HbCO to methaemoglobin also favoured the exchange. Ferri(met)haemoglobin was much more unstable than ferrohaemoglobin in the presence of albumin, and the over-all synthesis of methaemalbumin was dependent on the formation of methaemoglobin as a haem donor. Dissociation and transfer of haem was dependent on the presence of albumin as a contiguous acceptor: haem transfer did not occur if haemoglobin and albumin were separated by a cellophane membrane.

Albumin appeared to play an active role in haem transfer. Serum albumin was also incubated with a colloidal solution of linoleate before adding ferrihaem to the mixture. Linoleate increased both the rate of formation and the yield of methaemalbumin, whereas in controls containing no albumin, coupled peroxidation was observed whereby the unbound ferrihaem underwent oxidative breakdown. Similarly, preincubation of albumin with linoleate augmented the transfer of haem from HbO_2 and the subsequent formation of methaemalbumin. Linoleate appeared to increase one or more of the following: the oxidation of HbO_2 to metHb, the dissociation of haem from the globin apoprotein or the binding affinity of albumin for haem. At the same time, the presence of albumin inhibited the coupled peroxidation which occurred in controls containing HbO_2 and linoleate. It was concluded that Hb could be prepared and kept in a state of purity only if it was not brought into contact with serum albumin.

PROPERTIES OF A *PROTEUS MORGANII* BACTERIOCIN

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Investigations of the chemical nature of bacteriocins have shown them to be a heterogeneous group of macromolecular substances ranging from simple proteins which may be complexed to carbohydrate and lipids to particles of high molecular weight which resemble phages or parts of phages. A bacteriocin produced by *Proteus morganii* strain MR336 was investigated. It can be obtained in high titre by ultraviolet induction of broth cultures of the organism and it differs from the other *P. morganii* bacteriocins in being very mobile on agar electrophoresis. This bacteriocin kills 31 of 94 different isolates of *P. morganii* but has no extra-species activity. Bacteriocin was purified by precipitation with 50% ammonium sulphate and the precipitate chromatographed on Sephadex G-200 and then on calcium phosphate gel columns to yield a pure preparation with a sedimentation constant of 4·0. The bacteriocin consists of protein 87·9% and carbohydrate 8·1%. Seventeen amino acids are present in the protein fraction, with the acidic amino acids predominating. The carbohydrate fraction contains four sugars: glucose, galactose, arabinose and xylose. Activity of the bacteriocin was lost upon oxidation with oxygen, hydrogen peroxide, bromine or potassium permanganate. Aqueous solutions of the bacteriocin lost all activity above 60°C for 30 min. Activity was destroyed by pepsin, pronase and trypsin but was unaffected by lysozyme. Separation of the protein moiety by treatment with phenol resulted in inactivity. This means that activity is dependent on integrity of the complex. No lipids, nucleic acids, hexosamine or phosphorus were detected. Acridine orange can eliminate bacteriocinogeny from 65·7% of cells under optimal conditions. This favours an autonomous cytoplasmic existence of the factor(s) which controls bacteriocin production in *P. morganii* strain MR336. Bacteriocinogeny could not be transmitted to other strains of *P. morganii*. Bacteriocin-resistant mutants of a sensitive *P. morganii* strain were readily obtained.

COMPARISON OF SOLUBLE AND PARTICULATE PEPTIDASES OF RAT LIVER AND KIDNEY

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Classical leucine aminopeptidase (LAP) is usually prepared from the microsomal particle fraction of swine kidney homogenates. We have recently purified a similar enzyme from rat liver which, although soluble and not particle-bound, appears to have similar properties. In order to exclude possible species differences, the intracellular distribution and enzymatic properties of rat liver and kidney LAP activity have been compared. Particulate fractions and a soluble phase were prepared from homogenates of the two organs. Particle-bound enzymatic activity was released by n-butanol and further purified by $(NH_4)_2SO_4$ fractionation. Hydrolysis of L-leucyl-p-NO_2 anilide was followed by increase in absorption at 405 mμ and fission of L-leucyl-beta-napthylamide by coupling of released beta napthylamine to tetrazotised-O-dianisidine. Hydrolysis of small peptides was followed in the pH stat and by release of ninhydrin-reacting material. Peptidase activity in kidney was recovered from the microsomal fraction as well as from the soluble phase. Activity was tested against a range of L-leucyl peptides, L-leucinamide, and the chromogenic substrates L-leucyl-p-nitroanilide and L-leucyl-beta-napthylamide. On the basis of substrate specificity, metal ion requirement and inactivation, it was concluded that the soluble enzymes from liver and kidney were similar. They had relatively low activity against chromogenic substrates but hydrolysed L-leucinamide, L-leu-gly and L-leu-gly-gly readily. These properties justify the label of true leucine aminopeptidase. The kidney microsomal peptidase readily hydrolysed chromogenic substrates, L-leu-gly and L-leu-gly-gly but had little or no activity against leucinamide. These correspond to properties of arylamidase. The different intracellular locations of liver and kidney peptidase activity can be explained on a basis of molecular species of the enzyme rather than difference in binding by intracellular particles. It is proposed that histochemically demonstrable LAP be renamed 'arylamidase'.

RESISTANT *SALMONELLA TYPHI* IN A HOSPITAL

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In March 1968, 3 convalescent typhoid patients in an infectious diseases hospital were found to be excreting multiresistant *Salmonella typhi* strains in their faeces. The resistance could be transferred to suitable donor bacteria at high frequency. During this time there were several other patients in the ward excreting infectious drug-resistant *Salmonella johannesburg* organisms, and from one of the 3 resistant *S. typhi* excretors a multiresistant *S. johannesburg* was subsequently isolated. From all 3 patients sensitive *S. typhi* strains were isolated before, simultaneous with and after the isolation of the multiresistant strains. In 2 patients the resistant strains were isolated after full courses of chloramphenicol treatment, i.e. during the convalescent period and after cessation of all antibiotic treatment. The third patient was of special interest as a mixed resistant and sensitive *S. typhi* population was isolated from the faeces, during a relapse, while at the same time a fully sensitive strain was recovered from a blood culture. In spite of the selective action of chloramphenicol favouring the resistant clone, the patient made the usual quick recovery on treatment with this antibiotic. It is likely that sensitive *S. typhi* organisms in extra-intestinal macrophages were responsible for the relapse, while the resistant organisms acquired their resistance in the gut but failed to spread beyond it. The sequence of events in this patient strongly suggests diminished virulence of the multiresistant strain. However, a rather stormy course with intermittent pyrexia of 15 days' duration during chloramphenicol and ampicillin therapy in one patient and another seriously ill patient on chloramphenicol treatment, suggests that *S. typhi* strains with infectious drug resistance can be virulent. The last 2 patients had no connection with the 3 convalescent excretors and their resistant strains were isolated respectively during treatment and before treatment was started.

OBSERVATIONS ON THE ORGAN SPECIFICITY OF LACTATE DEHYDROGENASE WITH SPECIAL REFERENCE TO OPTIMAL SUBSTRATE CONCENTRATIONS AND UREA INHIBITION

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Lactic dehydrogenase occurs in the form of 5 isoenzymes in human tissue. Erythrocytes, myocardium and kidney contain predominantly LDH_1 and LDH_2, whereas liver and skeletal

muscle are rich in LDH_4 and LDH_5. The present experiments were designed to establish the optimum conditions for differentiating between these isoenzymes using the properties of substrate affinity and urea stability. Representative samples from human organs were obtained at autopsy, usually within 24 - 36 hours after death. Homogenates were prepared in phosphate buffer pH 7·4 at 4°C and enzyme assays performed on the extracts. Erythrocyte haemolysates were similarly prepared from fresh samples of heparinized blood. LDH activity was measured using pyruvate as substrate at various concentrations. In addition, alpha OH butyrate dehydrogenase was determined using alpha keto butyrate $6{\cdot}67 \times 10^{-3}$M final concentration. Urea stable activity was measured in 2M urea. All determinations were performed at 25°C in a Beckman Model DB spectrophotometer with 3 ml. reaction mixture and 1 cm. light path. The optimum substrate concentration for LDH from heart muscle and erythrocytes was observed to be $0{\cdot}5 \times 10^{-3}$M pyruvate with significant inhibition at concentrations higher than $1{\cdot}0 \times 10^{-3}$M. Reaction rates were non-linear in pyruvate excess. LDH from liver and skeletal muscle exhibited less inhibition at high substrate concentrations. Reaction rates were depressed and non-linear in the presence of NADH + H+ excess. The inhibition of LDH of heart muscle and erythrocytes in high pyruvate concentrations was partially prevented by 2M urea with apparent activation of total LDH. The slower moving isoenzymes were markedly inhibited by urea. There was a fair degree of correlation between alpha OH butyrate dehydrogenase activity and urea stable activity. By proper selection of the conditions in the assay system, the tissue origin of LDH could be fairly accurately localized without determining actual isoenzyme patterns.

PRIMARY AND ACQUIRED DRUG RESISTANCE IN PULMONARY TUBERCULOSIS

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In a country-wide survey of 15,000 TB patients in 36 hospitals, the emergence, frequency and epidemiology of drug-resistant strains were studied. The significance of factors such as sex, age, weight, race, tribe, environment, occupation, place of treatment, number of hospital admissions, degree of pulmonary tuberculosis, length of previous and current therapy, and use and dosages of the different drugs, was investigated. All data were coded, computerized and analysed. Drug sensitivity of the strains was determined for 4 - 10 drugs on Loewenstein-Jensen medium by either the absolute concentration or the vertical diffusion method. The incidence of primary resistance was 10% to isoniazid (INH), 5% to streptomycin and 4% to PAS. Double and triple primary resistance occurred in 5% of strains. Advanced lung TB was caused by INH-resistant bacilli in 68% and INH-sensitive bacilli in 63% of fresh cases. Fifteen cases of pulmonary TB were caused by fully or partly catalase-negative strains. There were 4,000 strains from patients denying history of anti-TB chemotherapy, but after repeated questioning one-third admitted such therapy. Acquired resistance was related to the length of therapy and other contributing factors. About 3,500 strains were studied. Patients failing to revert to negative sputum after 8 months excreted strains resistant to INH in 70%, to streptomycin in 50%, and to thioacetazone and ethionamide in 40% of cases. The high incidence of primary drug resistance necessitates culturing and testing of every new case. Second-line drugs are indicated for patients still sputum positive after 4 months, but laboratory guidance is needed.

ALBUMIN METABOLISM IN CADMIUM-POISONED ANIMALS

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An albumin of low molecular weight (20,000) has been isolated from the urine of men and animals (rabbits, dogs and monkeys) chronically poisoned by cadmium. Circulating minialbumins of MW 5,000 - 20,000 have also been observed in the serum of poisoned rats and monkeys. Their albumins were antigenically indistinguishable from normal serum albumin (MW 66,000), had similar electrophoretic mobility and were closely similar in composition with regard to most of the constituent amino acids, except that the content of lysine and ½-cystine was consistently less than normal. Minialbumins were clearly separated from molecules of normal size on Sephadex G-75 as long as the concentration of sodium chloride in the medium was 0·2 M or more, without the appearance of proteins of intermediate size. At lesser concentrations, as for example in 0·9% sodium chloride solution, aggregation occurred with the development of a grossly inhomogeneous mixture containing approximately 40% of albumin MW 70,000 and 60% of smaller fragments down to peptides of MW 5,000. Aggregated molecules of MW 170,000 have been detected in some experiments. The tryptophan content of albumins found in the serum and urine of cadmium-poisoned monkeys was determined by two methods and compared with that of serum albumin of normal animals. Normal serum albumin of the monkey was found to contain 2 residues of tryptophan per molecule of the protein, whereas all albumins in the poisoned monkeys, whether of normal size or low molecular weight, contained less tryptophan, this amino acid being absent entirely in the minialbumins of both serum and urine. Serum albumin of the usual molecular weight (66,000) in the cadmium-poisoned monkeys contained 30% less tryptophan than its normal counterpart in untreated animals. Of the albumin molecules circulating in the serum of cadmium-poisoned animals, 40 - 60% were abnormal in amino acid composition, molecular weight or in both.

ALBUMIN SYNTHESIS BY THE ISOLATED PERFUSED RAT LIVER

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The use of isolated perfused rat liver has contributed greatly to our knowledge of protein turnover. This system has given information concerning protein synthesis and in particular the synthesis of albumin, but very little work has been done on the kinetics of albumin synthesis using modern techniques. Previous attempts to measure albumin synthesis rate by the liver assumed that the intracellular specific activity of the labelled amino acid was similar to its specific activity in the plasma. This assumption is invalid. Accordingly we have studied albumin synthesis in the isolated perfused rat liver by means of the MacFarlane technique which is based on the simultaneous incorporation of the guanidine carbon atom of arginine into albumin and into urea within the same liver cell. Livers of male albino rats of the Wistar strain were perfused with undiluted heparinized rat blood for a period of 2 - 3 hours. The blood flow and the bile production rate were used as an index of liver viability during the perfusion. The synthesis rate of albumin was found to fall in the same range in this *ex vivo* system, as has been previously reported for *in vivo* studies from this laboratory.

LIPID PROTEINOSIS AND ERYTHROPOIETIC PROTOPORPHYRIA: A HISTOLOGICAL COMPARISON

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Skin changes resembling lipid proteinosis (LP) can be produced by erythropoietic protoporphyria (EPP), which can thus masquerade as light-sensitive lipid proteinosis. Histological similarity between the two conditions is striking. Thus both conditions are characterized by the deposition of hyaline material particularly around the blood-vessels. In both conditions the first change appears to be a hyaline thickening of the

small blood-vessels in the dermis, followed by hyalinization of perivascular connective tissue. In this way hyaline perivascular cuffs are formed which histochemically appear to consist of a lipoglycoprotein. Despite the similarity mentioned, there are distinct differences. Thus in EPP the lesions are virtually confined to the small vessels in the superficial dermis, whereas in LP vessels at a deeper level may be affected as well. The degree of hyalinization is more extensive in LP than in EPP, since the latter appears to be more readily self-limiting and affects only the exposed skin. Apart from the vascular and perivascular lesions, the hyaline change in LP (but not in EPP) also affects many other structures in the skin including the nerves, arrectores and the outer connective tissue sheath of hair follicles to some extent, as well as collagen and elastic tissue. The most striking and most constant difference between LP and EPP is the fact that the sweat glands in LP constantly reveal progressive hyalinization affecting basement membranes, connective tissue and capillaries, giving rise to a highly characteristic appearance. We have yet to see a case of LP without this feature. By contrast the sweat glands appear to be unaffected in EPP. A skin biopsy should allow differentiation of the two conditions in most instances. This study is based on the biopsy of skin from 16 cases of LP and 2 cases of EPP.

THE DEVELOPMENT OF KERATIN IN THE ORAL MUCOUS MEMBRANE

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The oral cavity of the adult human is lined by keratinized, parakeratinized and unkeratinized epithelium. The keratinized and parakeratinized epithelium is confined to the gingivae, the hard palate and the dorsal surface of the tongue. The mucosa covering the cheeks, lips, mouth floor, underside of tongue and the soft palate is unkeratinized. Although the keratin layer on these surfaces can be regarded as inferior to the keratin layer of the epidermis, in that disulphide groups are very difficult to demonstrate, it is nevertheless easily demonstrated by conventional staining techniques. To study the development of keratin in the mentioned sites, material from embryos, foetuses and infants was obtained and stained with haematoxylin and eosin, picro-Mallory, and with the DDD method which demonstrates sulphydryl groups. The last-mentioned can be regarded as keratin precursors because the sulphydryl groups are oxidized to disulphide groups in the process of keratinization. It was noticed that there was already an epithelial differentiation during the first few months of intra-uterine life. Future keratinized epithelium assumed a more flattened squamous appearance, comparing it with the future unkeratinized epithelium, which broadly speaking retains its morphology even in later life. During the later months *in utero* sulphydryl groups were observed and from then on signs of keratinization, recognized by an increase in sulphydryl groups and by the staining reaction to conventional staining methods, became progressively more pronounced until the established pattern was reached within the first few years after birth.

ISOTOPIC DETECTION OF GALACTOSAEMIA AND THE CARRIER STATE

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Galactosaemia is a rare autosomal recessive metabolic error, characterized by a defect in galactose-l-phosphate uridyl transferase enzyme in tissues, especially the liver. The inability to convert galactose to glucose leads to cellular accumulation of galactose-l-phosphate; this produces a failure to thrive, jaundice and hepatosplenomegaly, cataracts, galactosuria and an abnormal blood galactose tolerance, convulsions and mental retardation, and early death may occur in affected infants in whom galactose (mainly present in milk) has not been removed from the diet. Early diagnosis is thus essential to prevent physical and mental disability. Urine tests may not be conclusive and galactose tolerance is a dangerous procedure for a galactosaemic or carrier. Where liquid scintillation counters are available, incubation of red cells with galactose-1-^{14}C and isolation of the metabolic products of galactose metabolism by chromatography or collection of $^{14}CO_2$ could be performed as readily as the quantitative spectrophotometric enzyme assay. After short incubation of red cells or haemolysates, the reaction was stopped and the supernate run on paper chromatography with ethanol:acetate mixtures. The paper was cut into uniform strips from origin to solvent front for consecutive counting in a liquid scintillation counter. The successive sites of radioactivity corresponded to that for UDP galactose, galactose-1-phosphate and galactose, respectively. With longer incubation, $^{14}CO_2$ was evolved in a later stage of galactose metabolism, and absorption in ethanolamine : 2-methoxyethanol was accompanied by less scintillator quenching than the use of hyamine or other CO_2 absorbents. Nine cases of galactosaemia (including 3 Bantu subjects) and their families were studied. The galactosaemics had normal galactokinase activity, but showed no radioactivity at the first site and produced no $^{14}CO_2$, indicating an absence of transferase enzyme. The parents had half or less than half the transferase activity of normal adults. Some of the siblings of the propositi and their parents' siblings yielded results indicative of a heterozygous state.

PATHOLOGICAL CHANGES OCCURRING IN SIX PATIENTS UNDERGOING RENAL HOMOTRANSPLANTATION

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The pathological changes occurring in the kidney and other organs in 6 patients undergoing renal homotransplantation have been studied. Two of the patients died, one from overwhelming fungaemia and viraemia (4 months after transplantation) and the other from an acute diffuse haemorrhagic pancreatic necrosis (8 months postoperatively). Three patients, one of whom received two grafts, have rejected their transplants in a most unusual fashion — namely, by undergoing almost total necrosis and infarction of the kidney within a few days of the operation, necessitating removal of the transplanted organ. In one of these patients renal biopsy by light and electron-microscopy and fluoresence microscopy showed fibrin thrombi in the glomeruli and failed to reveal any immunoglobulins. This was also observed in one of the patients on electron-microscopy. It is felt that this constitutes a Schwartzmann-type reaction. The cause of this may be related to a low grade endotoxaemia from infection of the dialysis bath. Culture of the bath showed numerous Gram-negative organisms. As part of the Schwartzmann reaction a hypercoagulable state develops and this leads to intraglomerular and intravascular thrombi. This encouraged the use of anticoagulant therapy in the sixth patient at the time of transplantation. This patient bled excessively, but light and electron-microscopy of the kidney showed only mild tubular damage. Subsequent studies have shown that the kidney is functioning well.

MIXED MESODERMAL TUMOURS OF THE FEMALE GENITAL TRACT

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During an 11½-year period 20 cases of mixed mesodermal tumour were seen in this department, comprising 0·35% of gynaecologic admissions to Groote Schuur Hospital. They accounted for 34·5% of all uterine sarcomas seen during this period, occurring closely second to leiomyosarcoma. Average age of the cases was 64·4 years and the race distribution showed no Bantu cases to be present (Bantu comprise 10·5% of admissions to the hospital). A detailed analysis of the histo-

logy of these neoplasms was undertaken and findings were compared, where relevant, with findings in 25 sarcomas, other than mixed mesodermal tumours, occurring during this time period. Emphasis was placed on the importance of differentiating true rhabdomyoblasts from altered pre-existing smooth-muscle cells of myometrial origin. The non-specificity of strap and tadpole cells, and of areas of so-called primitive mesenchymal tissue was noted. The finding of a high incidence of tumours containing prominent cells with hyaline eosinophilic droplets was discussed, and tumours with features resembling reticulum-cell sarcoma, stromal sarcoma and mesonephric carcinoma were described, as well as a lesion showing linear tubular structures, possibly of nephrogenic origin. The value of diagnostic curettage was noted.

OBSERVATIONS IN ALLOTRANSPLANTED BABOON KIDNEYS TREATED WITH SUBCELLULAR KIDNEY FRACTIONS

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Untreated renal allotransplanted Chacma baboons had an average postoperative survival time of approximately 9 days. Other baboons were treated with different doses of subcellular kidney fractions (SKF). Pre-operative and postoperative treatment of baboons with daily injections of 0·02 cc. SKF intramuscularly did not influence the average postoperative survival time. Intramuscular injections of 0·2 cc. SKF on alternate days and daily intramuscular injections of 2 cc. SKF during the first postoperative week and thereafter on alternate days prolonged the average postoperative survival time to 16 and 20 days, respectively. SKF in doses of 20 cc. intramuscularly slightly accelerated the rejection of the allografted kidneys. A single injection of 2 cc. SKF into the renal artery caused an accelerated and vehement graft rejection. Baboons treated with 0·2 cc. and more SKF had a high incidence of intrarenal thrombosis. The morphological rejection reaction in baboons treated with 2 cc. and 0·2 cc. SKF was milder than in untreated control baboons. The titres of heterohaemolysins, heterohaemagglutinins were normal and the complement titre was less elevated than in other experimental groups. Attempts were then made to modify the SKF. This was at first done by centrifuging the SKF at 30,000g. The pellet was discarded and 10 cc. of the supernatant were injected IM on alternate days. This did not prolong the average survival time significantly, but there was less fibrinoid necrosis than in the control animals and no intrarenal thrombosis. Experiments with different doses are still in progress. In another series the SKF was subjected to prolonged high frequency sonic disintegration at pH 3. Two cc. and 20 cc. of this contra-antigen given intramuscularly on alternate days prolonged the average postoperative survival time to 14 and 16 days, respectively. Pre-operative treatment with 2 cc. contra-antigen for 4 weeks prolonged the average postoperative survival time to 20 days. The titres of complement, heterohaemolysins and heterohaemmagglutinins remained within normal limits. These experiments are also still in progress. The enhancing action of SKF is dose related, but its mode of action is not yet definitely known.

THE HISTOLOGICAL DISTRIBUTION OF LUNG CANCER IN CAPE TOWN

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This investigation follows on a previous but more limited study which determined the histological distribution of bronchial carcinoma in the 3 racial groups of Cape Town. The series consists of 685 cases of primary epithelial neoplasms of the lung. There were 375 (54·8%) tumours in Whites, 258 (37·7%) in the Coloured and 48 (7·0%) in the Bantu. These proportions parallel those of the total admissions to Groote Schuur Hospital for each racial group over a 25-year period and do not suggest a significant variation in incidence. Squamous carcinoma is the most frequent tumour type in each racial group, and adenocarcinoma the least frequent. In Whites small-cell anaplastic carcinoma is next in frequency, being well above that of large-cell carcinoma; in the Coloured and Bantu these two tumour types approximate one another and occupy an intermediate position. In all three groups the maximum age incidence is between 40 and 69 years, with Whites showing a definite peak between 60 and 69 years and the Coloured between 50 and 59 years, a decade earlier; the Bantu shows no well-defined peak within this period. The age distribution of each of the tumour types also closely follows this pattern, in each of the races. The definite preponderance of males over females exists to a proportionate degree in each of the races. This tendency is most marked in squamous carcinoma, in each of the races, diminishing in small-cell anaplastic carcinoma and large-cell carcinoma, and it is barely evident in adenocarcinoma. Considered in terms of Kreyberg's subdivision of tumour groups there is a notable preponderance of males over females in group I tumours in all races, whereas males and females are involved almost equally in group II tumours. The above findings are similar to those observed in the previous survey and suggest (*a*) that there is no significant difference in the pathology of bronchial carcinoma in the racial groups of Cape Town, and (*b*) that it parallels that seen in other high-incidence countries.

PERCUTANEOUS RENAL BIOPSY AS AN INDEX OF KIDNEY PATHOLOGY

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This paper deals with the results of more than 300 renal biopsies that have been submitted for diagnosis over the last 2½ years. Certain histological patterns seen on biopsy, such as focal and local proliferative glomerulonephritis and the end-stage kidney, require detailed clinical data in order to reach a final assessment of the aetiological agents responsible for the disease. In this regard the usefulness and importance of having a close liaison with the members of a renal unit is accentuated. This allows the pathologist to be more than a diagnostician and enables him to be in a position to advise a therapeutic approach to various kidney diseases. Biopsy of renal specimens from African miners suffering from heatstroke has shown mild to moderate tubular damage, mild glomerular basement membrane thickening and moderate interstitial oedema. Follow-up studies of these patients for up to 3 years have shown marked tubular atrophy with moderate interstitial fibrosis. Renal biopsy in conjunction with clinical history has enabled the outlining of criteria for the diagnosis of analgesic nephropathy, namely, interstitial fibrosis, tubular atrophy with hyaline cuffing, lipofuscin pigmentation and 'ghost' outlines of necrotic tubules.

CARCINOMA OF THE LARYNX

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Histopathological evaluation of carcinoma of the larynx is generally based upon small biopsy specimens taken through a laryngoscope or upon selected tissue blocks prepared from the excised larynx. An opinion regarding the nature of the neoplasm, its mode and paths of extension, and the effects of any prior radiotherapy must of necessity be based upon selected material which may be far from representative. With the availability of a large number of larynges which had been surgically removed over a period of some 30 years it was decided to investigate the nature, extent and distribution of the malignant neoplasm in each larynx as comprehensively as possible. After suitable preparation each larynx was embedded in paraffin wax and serially sectioned in the coronal plane.

During the course of preparation an average of 1,200 - 1,600 12-μ sections were obtained from each specimen. Every 14th section was mounted upon a 5 × 7½ cm. glass slide and every 90th section was stained with haematoxylin and eosin. Lantern slides (3¼ × 3¼) for projection purposes were prepared from approximately every 200th section. In this manner it was possible to build up a complete picture of each larynx and to determine the exact extent of the neoplasm in each case. The method was of particular value in demonstrating the planes along which an intrinsic neoplasm tends to spread towards and into the extralaryngeal tissues. It has also enabled the evaluation of the effect on the neoplasm of prior irradiation therapy and the characterization of the nature and cause of post-irradiation laryngeal ulceration and deformity.

CEREBELLAR ASTROCYTOMAS: A CLINICO-PATHOLOGICAL CORRELATION

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In a series of 28 patients suffering from cerebellar astrocytoma seen at the Neurosurgical Unit of Johannesburg Hospital, 82% of the tumours were classified as grade I (Kernohan and Sayre), 14% as grade II, and 3·5% as grade III. The microscopic appearances are either those commonly described and illustrated in the literature on cerebellar astrocytomas, or else of less distinctive solid neoplastic tissue similar to that seen in diffuse cerebral astrocytomas. Two-thirds of the tumours were cystic and the volume of fluid reached 100 ml. in one instance. The commonest situation was one or other hemisphere alone, followed by the vermis and one or both cerebellar hemispheres, and lastly the vermis alone. The postoperative prognosis emerges as years, probably decades, and even a virtual cure. Nevertheless, histological features are present which are regarded as ominous in slow-growing astrocytomas elsewhere in the central nervous system, i.e. invasion of the leptomeninges, foci of atypical cells, and hyperplasia of vascular endothelial cells. Clinically almost all the patients presented with the classical triad of increased intracranial pressure: headache, vomiting and papilloedema. Cerebellar signs and occasional cranial nerve palsies especially of the 6th and 7th nerves were other features observed on examination. The majority of patients had a total macroscopic removal of the tumour and a minority had a partial removal on account of widely infiltrating growth. Deep X-ray therapy was given to about one-third of the patients, but the results of this therapy are difficult to assess.

FURTHER OBSERVATIONS ON NODULAR HYPERPLASIA IN THE LIVER IN THE BANTU

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It has been postulated that the multinodular cirrhosis which accompanies about 60% of cases of hepatocellular carcinoma in the Bantu is due to a nodular hyperplasia and is not a true postnecrotic cirrhosis. A serious deficiency of this hypothesis is a lack of cases demonstrating the earlier steps in the pathogenesis of the lesion. Histological studies of a selected series of livers show changes which might be early stages of this lesion. The liver remnant after partial hepatectomy for primary liver carcinoma in the Bantu may show a changed regeneration pattern and a focal reactivity of parenchymal cells. This is interpreted as the earliest change. The results of a similar focal regenerative response may also be seen in livers of Bantu patients who have died sudden deaths. A nodular hyperplasia resembling that seen in hepatocellular carcinoma may be present in Bantu patients with widespread metastatic involvement of the liver. On the basis of these observations it is postulated that the hepatic neoplasia and hyperplasia are independent but related processes, that they may develop in parallel but that the neoplasia may precede and even be the final stimulus for the development of a nodular cirrhosis.

A STUDY OF THE PULMONARY CHANGES ASSOCIATED WITH RESPIRATOR THERAPY IN INFANTS

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The purpose of this paper is to relate the pathology of the pulmonary changes that occur when newborn, often premature, infants are artificially ventilated and exposed to very high concentrations of oxygen for long periods. This mode of therapy is essential to the management of hyaline membrane disease in premature infants and has been considered to be partly responsible for pulmonary insufficiency. A retrospective examination has been made of autopsy material from 3 groups of infants. The first group consisted of premature infants with hyaline membrane disease who survived beyond 4 days on intermittent positive-pressure respiration, supplemented by high concentrations of oxygen. The second group were cases of tetanus neonatorum (mainly mature infants) receiving similar respirator therapy and intermittent oxygen supplementation, and the third group consisted of newborn infants requiring major surgery and to whom a high concentration of supplementary oxygen was also administered. Alveolar collapse with alveolar duct and terminal bronchiolar wall thickening due to fibrosis and muscle hypertrophy and loss of bronchiolar epithelium with hyperplastic regeneration have been striking features in the group with hyaline membrane disease. Residual hyaline membrane, intra-alveolar oedema and emphysema were less constant features. In the tetanus and surgical groups fibrotic thickening of alveolar ducts with loss, and subsequent regeneration, of bronchiolar epithelium was seen, but was less constant and less obtrusive. It is postulated that a high oxygen concentration possibly associated with a low nitrogen concentration in infant lungs probably stimulates mild fibrotic change and could perpetuate a tendency to fibrosis that may already be present.

TRANSFORMATION OF HAMSTER CELL CULTURES WITH SIMIAN ADENOVIRUS SA7

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Studies on the biochemical basis of malignant transformation are complicated by the fact that the cells undergoing transformation are not usually identifiable during the critical latent period during which these changes occur. This communication describes a new *in vitro* virus transformation system which possesses certain advantages for studies of this type. Simian adenovirus SA7 was originally isolated by Malherbe and Harwin and was shown by Hull and co-workers to produce a high incidence of tumours when inoculated subcutaneously into newborn hamsters. Early passage cultures of newborn hamster skin fibroblasts grown on MEM-tryptose phosphate medium supplemented with 10% bovine serum were inoculated with SA7 virus and were then kept on serum-free medium for 2 days. At this time a cytopathic effect (cell rounding) could be observed and the cultures were returned to the serum-supplemented medium. A period of rapid growth followed, usually continuing until the 6th or 7th day after virus inoculation. The growth phase terminated abruptly and simultaneously numerous clusters of small refractile rapidly-growing cells made their appearance. These could be propagated continuously as cell cultures and produced large tumours in 8 out of 9 weanling hamsters within 40 days of subcutaneous inoculation. The cells had an abnormal karyology characterized by the frequent occurrence of a large unmatched chromosome.

ALLERGIC GRANULOMA AND OTHER EOSINOPHILIC CONDITIONS OF THE LUNGS

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The presence of eosinophils in lung lesions is sometimes a conspicuous feature and in the differential diagnosis bacterial, viral, fungal and parasitic infections, eosinophilic granuloma and conditions which are probably due to hypersensitivity must be considered. A 17-year-old girl who presented with an itchy skin rash, sore throat and painful swollen elbow-joints and thumbs was found on chest X-ray to have consolidation of the right lower lobe with cavitation. The lobe was removed and showed extensive consolidation with cavitation. Microscopy revealed areas of necrosis with a marked infiltration by neutrophils, lymphocytes, histiocytes and numerous eosinophils. The areas of necrosis were surrounded by granulomata consisting of loose connective tissue with numerous lymphocytes, plasma cells, histiocytes, neutrophils, eosinophils and giant cells of the Langhans and foreign-body type. There was a similar infiltration into the walls of the bronchioles, and in parts the mucous membrane was ulcerated. There was, however, no evidence of arteritis. A diagnosis of allergic granuloma was made, and this disease is apparently related to the group of conditions that include Loeffler's and Wegener's syndromes. There had been no response to antituberculous treatment and prednisone was added. Several months later she developed a severe relapse with haemoptysis, a haemolytic anaemia and bilateral lung opacities on X-ray. She responded to treatment with blood transfusions, steroids and 6-mercaptopurine.

CONTAMINATION OF THIN SECTIONS FOR ELECTRON-MICROSCOPY BY ASBESTOS FIBRES

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Because of the increasing recognition of the importance of the exposure of human beings to asbestos dust, it was decided to initiate a study, utilizing the electron-microscope, of the distribution of asbestos fibres in various tissues and their method of migration. Tissues were first examined from the diaphragm of a patient who at autopsy had many pleural plaques and asbestos bodies in the lung bases. Fibres which had all the physical characteristics of chrysotile asbestos were seen apparently lying in the tissue between the muscle fibres. It was subsequently found that sections of mouse liver and kidney occasionally showed identical fibres in the tissues. As this appeared to be unlikely, the possibility of contamination was considered. Investigations have shown that the distilled water used for flotation of sections and even dental wax appeared to contain asbestos fibres. In the case of the distilled water, asbestos is used for lagging purposes in the still. A method will have to be devised for distinguishing contaminating asbestos fibres lying on a section from those in the tissues themselves, although this source of artefact naturally does not always arise.

EMPHYSEMA AS DETERMINED BY WHOLE LUNG SECTION CORRELATED WITH THE INCIDENCE OF PEPTIC ULCERATION AND ADRENAL HYPERPLASIA

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One hundred and sixty-two whole lung sections available in the Department of Pathological Anatomy of the University of Pretoria and prepared according to a modification of the method of Gough and Wentworth were examined. Of these, 68 showed unequivocal emphysema. The emphysema was of panacinar type in 6 and of centrilobular type in 62. Fifty-eight of the patients from whom the lungs were obtained were White, of whom 51 were male and 7 female, and 10 were Bantu (8 male and 2 female). The ages of the patients varied from 38 to 83 years. Eighteen cases, all White, showed peptic ulceration: 13 in the stomach and 5 in the duodenum. In 8 of these cases death was due directly or indirectly to the presence of the peptic ulcer. Weights of the adrenal glands were available in 9 cases only. In 4 of these cases their combined weight exceeded 16·0 G. In 6 of the remaining cases adrenal hyperplasia was also considered to be present. In 1 case with adrenal hyperplasia an ulcer was also present. This study confirms the reports in the literature of the association of emphysema and peptic ulceration and also that peptic ulceration is a major complication of emphysema. It further confirms the association between emphysema and adrenal hyperplasia. Peptic ulceration and adrenal hyperplasia, however, do not seem to be associated.

SIGNIFICANCE OF 'MYELIN FIGURES' OF THE MYOCARDIUM: ELECTRON-MICROSCOPIC EVIDENCE OF MYOCARDIAL DEGENERATION

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Demonstration of structural alteration of the cardiac myofibre is an essential prerequisite towards the assessment of functional impairment and clinicopathological correlation. Conventional light microscopy reveals gross departure from the normal, such as coagulative necrosis or replacement fibrosis, but gives little information with respect to detailed intrafibrillar morphology. Furthermore, postmortem degeneration makes it difficult to interpret such changes as vacuolation or fragmentation with any degree of accuracy. In cardiac biopsies the picture may be confused by the presence of 'contraction artefacts' produced by excision of material from a beating heart. Both of these pitfalls can be avoided by the study of tissue removed at open-heart surgery. Electron-microscopy of the myocardium of rheumatic hearts has revealed the presence of laminated, osmiophilic, rounded structures. These have been named 'myelin figures', from their resemblance to the ultrastructure of the myelin sheath. On the same basis, it has been concluded that they contain phospholipids and represent the ultrastructural characteristics of fatty degeneration of the myocardium. The myelin figures vary in size and number; the smaller ones being found in close relationship to mitochondria and to the sarcotubular system, especially at the periphery of the fibre. Increase in number and size occurs at the expense of the specialized structures of the myofibre with progressive diminution and disappearance of them. The myofibres are affected individually, the most gross degeneration being confined to a particular muscle cell by the intercalated disc.

COMPLICATIONS OF MITRAL VALVE REPLACEMENT

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Thrombo-embolic complications of mitral valve replacement have been described and those found in the heart have been ascribed to interference of the Starr-Edward prosthesis, reduction of the size of the left ventricle, prolonged periods of hypotension or associated bacterial endocarditis. The 6 cases described show a similar cardiac pathology in different degrees, namely thrombosis of the wall of the left atrium and, from this, recent thrombus formation in the left atrium. This type of thrombus formation has not been found in the large series of hearts examined for the Miners' Medical Bureau over the last 10 years, and is ascribed to mitral valve replacement. At least one of the suggested pathogenic factors has been found in different cases. A common pathogenic factor of surgical trauma to the wall of the left ventricle or at the site of attachment of the valve replacement and a low output state is postulated to account for the pathology found in the left atrium. Although an associated bacterial endocarditis has been postulated by some workers as an aetiological factor, this was not found in this series. In 4 cases there was evidence of pneumonia in different degrees of severity, but the association of this with left atrial thrombosis is not clear. In 1 case there was an extensive pulmonary oedema, but in one this was minimal.

Emboli were found in other organs but in some cases these occurred before cardiac surgery.

FILAMENTOUS MATERIAL ASSOCIATED WITH CULTURED HUMAN SKIN FIBROBLASTS

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Fibroblasts were cultured from a biopsy specimen of macroscopically normal human skin removed at a distance of 1·3 cm. from a benign tumour of the vulva in a patient who had undergone previous treatments for 3 different carcinomas of the perineal area. Electron-microscopy showed that the cells were encased by an unusual filamentous material with a helical substructure. Similar material was found in the lumen of the endoplasmic reticulum and in the form of rod-like cytoplasmic elements which probably represented cross-sections through invaginations of the plasma membrane. The filamentous material was apparently not excreted through the Golgi apparatus, but possible precursors of it were found in membrane-bound vesicles which could presumably have discharged their contents to the exterior of the cell after fusion with the plasma membrane in the manner of collagen excretion from chondroblasts. An unusual type of vacuolar structure indented by ribosome-covered protuberances was observed in some of the cells secreting the helical filamentous material, and this was considered to represent a cross-section through the ruffled membrane seen in time-lapse films of cultured cells. Attempted differential staining of the Epon-embedded cells failed to identify the filamentous substance, which was not collagen but may have been mucopolysaccharide. The findings have been compared with two previous reports of 'spindle-shaped bodies' in the cytoplasm of mammalian and avian fibroblasts.

TRANSPLACENTAL FOETO-MATERNAL HAEMORRHAGE

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Maternal iso-immunization is caused by transplacental foeto-maternal haemorrhage occurring during pregnancy and labour. Foetal cells can be detected in the maternal circulation by the acid-elution slide technique described by Kleihauer and Betke. A method for the conversion of foetal cell scores into absolute volumes of transplacental haemorrhage was put to test in a biological trial in which nine mothers were injected with 1 ml. of cord blood immediately after delivery. The calculated volume of foetal blood in the maternal circulation varied from 0·70 ml. to 1·47 ml., with an average of 1·07 ml. This method of interpreting foetal cell scores was applied to the postnatal study of 612 ABO-compatible pregnancies, comprising 291 Caucasians, 68 Bantu and 253 Coloureds. Foeto-maternal haemorrhage of 0·2 ml. or more was deemed to be clinically significant, i.e. capable of causing primary sensitization to the Rh factor. The incidence of clinically significant foeto-maternal haemorrhage was 4·9%. There was no statistically significant difference in the incidence in the various racial groups. It is now generally accepted that maternal iso-immunization can be prevented by the intramuscular injection of an appropriate dose of Rh immune globulin into every Rh-negative woman after delivery of an Rh-positive infant. It has not yet been established whether a foeto-maternal haemorrhage of sufficient volume can occur during pregnancy to cause primary sensitization, and whether prophylactic inoculation with Rh globulin may be necessary during pregnancy in selected cases.