

EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

The following are summaries of the proceedings of the 10th meeting of the Experimental Biology Group (EBG) held on 16 October 1963:

THE INFLUENCE OF TRACE ELEMENT DEFICIENCIES UPON THE WILTING RATE OF WHEAT

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The effects of deficiencies of boron, copper, manganese and zinc upon the rate of wilting of *Triticum vulgare* var. Vernal, were investigated. The plants were grown in sand culture and irrigated with the appropriate nutrient solution.

The plants grown on deficient media all wilted appreciably more rapidly than the controls. The behaviour of the boron deficient plants was unusual in that the wilting pattern of

12-day-old plants is quite different from that of 24-day-old ones.

This investigation also indicates the complexity of the role played by trace elements in the water relations of plants, as other work has shown that deficiencies result in a significantly decreased transpiration rate in plants. Hence, it seems unlikely that the rapid wilting is due to excessive water loss alone.

RETROGRADE INJECTION OF DYE SOLUTIONS INTO THE RAT BILE DUCT

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Some time ago it was noticed that bile collected from rats immediately following the retrograde injection of dye solutions into the bile duct sometimes appeared to contain dye at concentrations higher than that of the injected solution. This phenomenon has been further investigated because, while reabsorption sites are believed to exist in the biliary tree, and their distribution is thought to be uniform throughout most of the tree,^{1,2} nothing is known about the nature of the reabsorption mechanism. It was found that under certain condi-

tions, and with some dyes only, the rat biliary tree showed this concentrating effect, and reasons will be given for the tentative belief that this may be due to saturation of part of the reabsorption mechanism.

This work was aided by grants from the Dr. C. L. Herman and the M. G. Fourcade Bequests, University of Cape Town.

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EFFECT OF SURGERY AND ANAESTHESIA ON STABILITY OF CHOLESTEROL

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The addition of small amounts of total serum lipids to a supersaturated cholesterol solution in a triglyceride oil increases the stability of the solution and depresses the rate of crystallization of cholesterol.¹ The serum phospholipids apparently account quantitatively for this effect of the serum lipids.¹

The crystallization rate of cholesterol is used as the basis of a routine laboratory test. After cooling to 26°C. the rate of crystallization is measured by periodic determinations of the optical density. From the time-density graph the maximum crystallization rate (CR value) is computed.^{1,2}

Male subjects with ischaemic heart disease and rabbits that have received adrenaline have higher CR values than matched controls.^{1,2}

The effect of surgery and anaesthesia on the CR value has been investigated. Surgery and anaesthesia have been shown to produce a wide variety of metabolic disturbances, including a state of hypercoagulation of the blood³ (this also occurs after the administration of adrenaline⁴) and the possible release of a lipid mobilizing hormone from the posterior pituitary.⁵

Cats and rabbits of both sexes were anaesthetized with intraperitoneal pentobarbitone. Respiration was maintained artificially via a tracheal cannula. Blood samples (4-5 ml. each) were obtained from a femoral artery. The first sample was obtained as soon as possible after anaesthesia, and samples were taken at intervals thereafter. The CR values of the blood were computed as described elsewhere.^{1,2}

Among the results obtained were the following:

- (1) Different animals have markedly different basal CR values.
- (2) Within the first 2 hours of surgery animals have a significantly higher CR value. The CR value tends to rise progressively thereafter.
- (3) Associated features noted include a significant tendency for the blood pressure to fall, for the peripheral circulation to fail, for no shivering to occur, and for hypothermia to develop.

To exclude the possibility that the increase in CR values was due to repeated loss of blood, CR values in conscious non-traumatized rabbits were investigated. Rabbits were bled once, then 3 hours later. No significant change in CR value was found.

Four equal blood samples were removed from rabbits at 2-hourly intervals. The first 3 samples had CR values which did not significantly differ. The CR value of the last sample was higher than the first obtained, but not significantly so, and did not equal the rise obtained in most animals during surgery.

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STRUCTURE OF THE GLYCOSYL RESIDUES IN ORIENTIN AND HOMO-ORIENTIN

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Previous work¹ has shown that both orientin and homo-orientin possess aliphatic residues containing 3 vicinal hydroxyls. That the oxygen bridge is between C-1 and C-5 of the hexopyranosyl residue is further demonstrated by the fact that glycerol is liberated on ferric chloride treatment² of the borohydride-reduced, periodate-oxidized tetramethyl ethers of orientin and homo-orientin. Ferric chloride treatment of orientin and homo-orientin tetramethyl ethers resulted in each case in the production of a ca 1:1 mixture of glucose and arabinose, indicating that oxidative cleavage of the aliphatic

residue occurs not only between C-1 and C-2 of the glycosyl residue as in the case of barbaloin,² but also between C-1 of the glycosyl residue and the point of attachment of this residue to the flavonoid nucleus.

Orientin and homo-orientin are therefore both C-glucopyranosyl derivatives of luteolin (5:7:3':4'-tetrahydroxyflavone).

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THE INTERCONVERTIBILITY OF SELECTIVELY METHYLATED FLAVONE C-GLYCOSIDES

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The previous suggestion¹ that the difference in the rate of periodic-acid oxidation of orientin and homo-orientin tetramethyl ethers is due to a difference in the geometrical arrangement of their vicinal hydroxyls, is no longer valid in view of the evidence that both compounds possess glucopyranosyl residues.

The acid-catalyzed interconvertibility of these compounds is

now best explained in terms of ring isomerization of the flavone. Thus orientin and homo-orientin 7:3':4'-trimethyl ethers are interconvertible when refluxed in aqueous ethanolic 2N-hydrochloric acid whereas no interconversion of the 5:7:3':4'-tetramethyl ethers occurs under these conditions.

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GENETICALLY DETERMINED GOITRE IN CATTLE

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A herd of Afrikaner cattle with congenital goitre was selectively bred at the Onderstepoort Veterinary Research Institute. The mean serum protein-bound iodine (40.0 $\mu\text{g.}/100$ ml. serum), 24 hr. PB^{131}I conversion ratio (87.8), and ^{131}I plasma clearance (414.0 ml./min.) suggested hyperthyroidism, although the animals were clinically euthyroid.

The mean thyroïdal protein-bound iodine of goitrous cattle (37.1 $\mu\text{g.}/\text{G.}$ thyroid), was only a fraction of that of the control animals (605 $\mu\text{g.}/\text{G.}$ thyroid), although the total organic iodine in the thyroids of goitrous cattle was about the same as that of normal animals. This indicates that the goitre is compensatory.

Radio-autographic identification of iodinated compounds in thyroid and serum extracts showed the presence of mono- and di-iodotyrosine in the thyroid and serum of goitrous animals with only minute traces of thyroxine. Iodotyrosines also appeared in urine.

The thyroxine-binding proteins had a normal thyroxine-carrying capacity. However, electrophoretograms of serum samples of goitrous cattle that received ^{131}I showed radioactivity on the albumin fraction alone. These findings suggested the absence of a coupling enzyme or the absence of

an iodotyrosine de-iodinase in the thyroid of goitrous cattle.

Thyroid homogenates of goitrous cattle were tested against controls for their thyroglobulin protease and iodotyrosine de-iodinase activities. The findings show that the activity of both these enzymes was greater in the goitrous than in normal animals. In 4 goitrous animals of the same family tested so far, more than 90% de-iodination of both mono- and di-iodotyrosine occurred within 5 minutes of incubation of thyroid homogenates with ^{131}I -labelled iodotyrosines. In the controls hardly any de-iodination occurred under the same experimental conditions.

It is concluded that this goitre, which is genetically determined, cannot be classified under any of the types which have been described.¹ This abnormality seems to be due to the increased activity of an iodotyrosine dehalogenase, which de-iodinates iodotyrosines so rapidly that hardly any thyroxine can form.

This work was aided by grants from the Dr. C. L. Herman and M. G. Fourcade Bequests, University of Cape Town, and by the South African CSIR.

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ELECTRON MICROSCOPY OF ACIDIC POLYSACCHARIDES

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Sedimentation and diffusion measurements on the purified acidic polysaccharide exudate from *Virgilia oroboides* bark indicated¹ that the substance is filamentous in aqueous solution; chemical study of the sugar units and their modes of linkage, however, reveals a highly-branched structure.² It occurred to us that electron microscopy^{3,4} might give fresh information on the size, shape, and homogeneity of the macromolecules from this and similar sources. Acidic polysaccharides from the barks of *Virgilia oroboides*, *Acacia mearnsii* and other trees and from the sacs present in hibernating *Watsonia* corms were accordingly examined in this manner. A number of derivatives (carboxyl-reduced,⁵ acetylated,⁶ methylated⁷) of the *Virgilia* polysaccharide were included in the study in order to observe directly, if possible, the degradative effects of fairly extensive chemical treatment.

Samples were deposited on carbon film supports by evaporation of suitably diluted solutions in appropriate solvents. The samples were shadowed with tungsten oxide at an oblique angle of 10° and examined under an A.E.I. EM 3A (75 Kv) electron microscope. Selected areas were photographed to give an overall linear magnification of 50,000 on the prints.

All the electron micrographs revealed fibres of variable thickness. The appearance and dimensions of the fibrils of the polysaccharides themselves and of carboxyl-reduced *Virgilia oroboides* polysaccharide indicate that they are aggregates of intertwining linear polymeric material. *Watsonia*

corm polysaccharide gave good photographs, with the appearance of fraying rope. The aggregate of chemically derived products in general appear to be smaller than those of the polysaccharides themselves.

The clearest electron micrograph is of acetylated *Virgilia oroboides* polysaccharide. Acetylation practically eliminates hydrogen bonding and hence aggregation. Adhesion to the support is reduced and in places the strands can be seen to be detached from it. Most strands have one or two long branches. Shadow dimensions show that the diameter of the strands varies between 55A and 165A consistent with extensive short branching within a linear 'envelope'. Making certain assumptions, values of 10^6 - 10^7 can be deduced for a mean particle weight from the dimensions of the strands, agreeing with accepted values.

In all the electron micrographs a suggestion of finer detail can be seen which is unfortunately beyond the resolving power of both the instrument and the technique.

We are grateful to Mr. D. H. Shaw for a gift of purified *Watsonia* corm polysaccharide.

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BILATERAL CORTICAL NECROSIS, ADRENAL HAEMORRHAGES AND ECLAMPTIC-LIKE LIVER LESIONS IN RATS AFTER TOXIC DOSES OF MILK PROTEINS

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This paper describes the toxic reactions of milk proteins when they are given without added histamine liberators.

Following a single, massive fatal dose, myocardial necrosis fails to develop, but one encounters instead other widespread lesions, of which focal hepatic necrosis, adrenal haemorrhage, splenic infarction and bilateral cortical necrosis are the most important. Basophilic intravascular material derived presumably from the massive amounts of milk given are present in sinusoidal and capillary channels, particularly in spleen and renal glomeruli. These are thought to be responsible, at least in part, for the necrosis in the various organs.

When the milk is administered as 2 injections, separated by an interval of some 5 hours, a hepatic lesion of a different type develops and resembles that seen in human eclampsia with widespread periportal and midzonal haemorrhages. In these animals, however, basophilic material replaces the fibrin thrombi seen in the human lesion. The morbid anatomical features suggest an obstructed portal circulation with good hepatic artery blood flow as one possible explanation of the lesion. It is not yet clear whether bacterial contamination of milk protein is an important factor in causing intrahepatic haemorrhages.