EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

Abstracts of papers read at the 20th Scientific Meeting of the Experimental Biology Group (EBG):

THE GEL IMMUNE DIFFUSION TECHNIQUE AS A METHOD FOR STUDYING THE ANTIGENIC RELATIONSHIPS AMONG RHIZOBIA

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The gel immune diffusion technique has not been widely used for studying the serology of rhizobia. It was therefore thought of interest to compare the results obtained by this method with those of the classical agglutination technique, performed as described by Loos and Louw.[2] Antisera were prepared against a number of rhizobial isolates, isolated from uninoculated clovers collected in the Stellenbosch district.

The antigen suspensions used in the gel immune diffusion tests were washed, using 0·85% saline, and concentrated by two successive centrifugations (10,000 r.p.m. for 10 min.). The pellets were resuspended in small volumes of saline to obtain heavy cell suspensions which were standardized to a cell content of approximately 100 mg. dry weight per ml. (dried at 80°C to constant weight).

The gel immune diffusion plates were prepared in petridishes (diameter 9 cm.) using 0·75% agar, 0·85% saline and 0·025% sodium azide. The plates were poured using 20 ml. of agar. Wells were cut in a hexagonal pattern around a central well. The wells had a diameter of 7 mm. and were spaced 4 mm. apart. Undiluted serum was placed in the central well and antigen suspensions in the surrounding wells. The precipitation bands were recorded after 3 days at room temperature (Dec.-Jan. 1965, aver. approx. 20°C) in a glass container having a moist atmosphere. Whole cells were used as antigens in this study, since Dudman[1] found that concentrated suspensions of washed whole cells of rhizobia gave rise to the same number and pattern of precipitation bands as concentrated solutions of extracellular diffusible antigens. The medium used for obtaining the antigens was also tested against all the antisera, but no bands were formed.

It was found that the isolates always produced strong, wide bands when tested against their homologous antisera. Strong, wide bands were sometimes also formed when isolates were tested against sera prepared against other organisms. The production of strong identical bands was always correlated with identical somatic agglutinating properties. This was not so in the case of the flagellar agglutinating properties.

Isolates which showed strong somatic agglutination (at dilutions higher than 1/200) were always found to share extracellular diffusible antigens with the antiserum-producing antigen. When weak agglutination occurred, however, common extracellular diffusible antigens were not necessarily found. Agglutination sometimes occurred even though the isolates shared only a single extracellular diffusible antigen, while in other cases no agglutination occurred although the isolates shared as many as 3 bands.

It can be concluded that when using only the homologous antiserum to identify a particular rhizobial strain among rhizobial isolates, the gel immune diffusion technique is a much faster method than the agglutination technique. The identification is not absolute, however, since the results obtained by means of the gel immune diffusion technique are not always correlated with the flagellar agglutinating properties. However, the somatic antigens have been shown by Vincent[3,4] to be highly strain specific.

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OXIDATIVE PHOSPHORYLATION IN HEREDITARY CARDIOMYOPATHY

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The oxidative phosphorylation process in hereditary cardiomyopathy was studied in the Syrian hamster, *Mesocricetus auratus*. These animals spontaneously develop a muscle dystrophy together with cardiomyopathy which becomes progressively worse, and they finally die of massive cardiac necrosis.[1,2] Oxidative phosphorylation (P/O ratio) in the heart sarcosomes was determined, using α-ketoglutarate, malate and pyruvate as substrates.[3] A significant depression in the P/O ratio of the myopathic hamster heart sarcosomes was obtained. The uncoupling of oxidative phosphorylation was substantiated by the findings that the adenosine triphosphate and creatine phosphate contents of these hearts were depressed, while the inorganic phosphate content was elevated. Further studies to determine where in the electron transport chain uncoupling occurs, indicated that the phosphorylation in the cytochrome oxidase region was significantly depressed.

These results are in concomittance with those obtained in experimental cardiac failure where uncoupling of oxidative phosphorylation as well as a decrease in the high energy phosphate content have been demonstrated.[4,5]

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THE EFFECT OF ACUTE ETHANOL INTOXICATION ON THE METABOLISM OF PYRUVATE AND PALMITATE IN THE PERFUSED RAT HEART

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In order to determine if any biochemical abnormality exists in alcoholic cardiomyopathy,[1,2] the effect of alcohol on substrate metabolism in the isolated perfused rat heart was studied.

The effect of different concentrations of ethanol (100mM, 200mM) on the metabolism of pyruvate-C^{14} and palmitate-1-C^{14} was studied, using a closed recirculation system.[3] It could be demonstrated that the higher concentration of ethanol in the perfusate depressed the metabolism of pyruvate, while having no effect on the uptake of palmitate. The incorporation of palmitate into tissue lipid, however, was enhanced, while less was converted to $C^{14}O_2$.[4] The effect of 100mM ethanol on both substrates was minimal.

It is suggested that the high level of ethanol may affect the permeability of the cell membrane or the osmolarity of the perfusion medium.[2]

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3-O-METHYL-L-RHAMNOSE AS A CONSTITUENT OF PLANT POLYSACCHARIDE GUMS

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3-O-Methyl-L-rhamnose (acofriose) is a component[1] of cardiac glycosides, bacterial glycolipids, and the hemicelluloses of Black spruce (*Picea nigra*) wood. We report finding this sugar as a minor component of a polysaccharide (I) found abundantly in radial canals, and also as an exudate from the stems, of *Encephalartos longifolius* Lehm. (female), a member of the botanically significant family Cycadaceae. 3-O-Methyl-L-rhamnose was shown as identical to a specimen from methylated *Khaya senegalensis* gum[2] by m.p. and mixed m.p., $[\alpha]_D$, paper- and thin-layer chromatography, i.r. spectrum and X-ray diffractometry. The derived glycitol gave CH_2O on IO_4^- oxidation, and the methyl glycosides of the sugar, like those of the authentic specimen, were only partly unaffected by IO_4^- during 6 days (checked by g.l.c. against an internal standard). De-O-methylation of the sugar gave rhamnose.

Paper chromatography indicated[3] that 3-O-methylrhamnose is also present in the gum exudates, (II) and (III) respectively, of *Encephalartos latefrons* Lehm. (female) and of *Welwitschia mirabilis* Hook f. (fam. Gnetaceae). It is known that the parent sugar L-rhamnose may be formed from D-glucose (as thymidine diphosphate derivative) without rearrangement of its carbon chain, and the methyl ether may arise similarly from 3-O-methylglucose (detected in a glycoside of *Streblus asper*).[1]

E. longifolius gum (I) forms a voluminous, fragile gel on solution in water, has $[\alpha]_D^{25} -28°$, and is an acid of equivalent weight 600. From an acid hydrolysate were isolated, in addition to 3-O-methyl-L-rhamnose, L-rhamnose, D-galactose, L-arabinose and D-xylose (characterized as crystalline derivatives), while there is evidence (paper chromatographic) for D-mannose, L-fucose, and uronic acid (probably D-glucuronic, linked $\beta 1 \rightarrow 6$ to galactose and $\beta 1 \rightarrow 2$ to mannose). Hydrolysates of II and III (III had $[\alpha]_D -45°$ and equivalent weight 1,500) showed a similar complexity of sugar components; they resemble also the hydrolysate of Black spruce wood.[4]

Portions of methylated I ($[\alpha]_D^{25} -23°$ in $CHCl_3$; OMe, 34·6%) and of methylated III ($[\alpha]_D^{25} -58°$; OMe, 38·6%; prepared by Dr. D. H. Shaw) were (i) hydrolysed to give a number of methylated sugar components which were separated and recognized by paper chromatography, and (ii) methanolysed and analysed semi-quantitatively[5] by gas liquid chromatography. The exceedingly complex chromatograms defy complete interpretation, but the following tentative assignments of some of the inter-sugar linkages in I and III are given in a first attempt to compare the main structural features of the two polysaccharides:

	Sugar residue	*Encephalartos longifolius gum*[6]	*Welwitschia mirabilis gum*
End groups	Rhamnopyranose→	+++	Trace
	Arabinofuranose→	++	++++
	Galactopyranose→	Trace	+
	Mannopyranose→	+	+
	Glucopyranuronic acid→	++++	+[a]
Chain units	→6Gal$_p$→	++	+
	→3Gal$_p$→	+	Trace
	→Ara$_{(p\ or\ f)}$→	Traces	++++[b]
	→Glu$_p$Ac→	+++	+
Branch points	→6Gal$_p$→ 3 ↑	+++	++
	→2Man$_p$→ 3 ↑	+	Trace
	↓ 4		
	→6Gal$_p$→ 3 ↑	+	++

a—In addition. an acidic component resembling 2, 3, 4-tri-O-methylglucuronic acid appeared on the g.l. chromatogram.
b—Several components involved.

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EXPERIMENTAL PULMONARY HAEMOSIDEROSIS AND PULMONARY OEDEMA

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In 1951 Magarey showed that when blood was given endotracheally to rats it was first distributed diffusely throughout the lung. Later focal aggregates of haemosiderin-laden macrophages were formed, similar to those found in mitral stenosis and other conditions giving rise to chronic passive venous congestion in the human lung. These findings confirmed that focal aggregates of siderophages could follow a diffuse haemorrhage and were not necessarily the result of focal haemorrhage from bronchioles as has been suggested by Lendrum, Scott and Park (1950) in patients with mitral stenosis. Most authors agree that in mitral stenosis the walls of the alveoli surrounding focal haemosiderin deposits show some interstitial fibrosis, and it has been shown that the elastica of the pulmonary vessels and the alveolar walls may show iron encrustation. The absence of interstitial fibrosis and other changes reminiscent of the haemosiderosis of mitral stenosis in Magarey's rat lung experiments is not clear; however, it has been suggested that it may be the result of a lack of chronic venous congestion in the lung or due to the relatively short duration of the experiment.

Experiments will be described where the thesis that pulmonary oedema may alter the reaction of the lung to haemosiderin was examined.

Three groups of male albino Wistar rats were used and the procedures relating to each group were repeated twice weekly for a period of 18 weeks. After a further period of 4 weeks all the animals were killed with ether and the lungs were examined.

Pulmonary oedema was produced in group I by means of the intraperitoneal injection of 0·4 ml./100 mg. body-weight of 6% ammonium chloride. In the ether-anaesthetized animals of group II, 0·75 ml. of heparinized rat blood was injected endotracheally. In group III the injection of 0·75 ml. of blood endotracheally was followed immediately by the intraperitoneal injection of ammonium chloride in the same dose as for group I.

A total of 70 rats were used for these experiments. Ten animals in each group were allowed to complete the full course, and 14 in each group were killed or died at intervals during the course of the study. Paraffin sections were cut at 5 μ and stained with haematoxylin and eosin, Perls' method for iron and the Gomori method for reticulin.

It was found that in animals given ammonium chloride-induced pulmonary oedema and endotracheal blood, the focal aggregates of siderophages took longer to form. Iron encrustation of the connective tissue fibres occurred in these animals. No interstitial fibrosis was observed in the alveolar walls, but the alveolar epithelium had a tendency to be cuboidal where it surrounded a collection of siderophages. The findings in these lungs will be compared with those in human cardiac pulmonary haemosiderosis.