EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

The following are abstracts of papers read at the 29th Scientific Meeting of the Experimental Biology Group (EBG) held at Karl Bremer Hospital, Bellville, CP, on 25 October 1968:

MOLECULAR SIEVE CHROMATOGRAPHY OF *ACACIA* STEM EXUDATES

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Molecular weight distributions of constituents of the gum exudates from stems of *Acacia podalyriaefolia* and *A. melanoxylon* have been determined by molecular sieve chromatography (gel filtration) on polyacrylamide gels, which were found to fractionate carbohydrates as follows:

Gel	*Fractionation range*, $\overline{M}_w$*
Biogel P-300	5,000 - 100,000
Biogel P-10	250 - 15,000
Biogel P-4	$<$4,000

*Weight-average molecular weight.

With perspex or polythene tubes as columns, samples (2 - 5 mg. in 1 ml. M NaCl) were eluted with M NaCl,[1,2] 1 ml. fractions being collected and assayed for carbohydrate.[3] Linear calibration curves related log molecular weight to elution volume over the appropriate range of $\overline{M}_w$.

Acacia podalyriaefolia exudate. This polysaccharide gum, essentially a highly branched arabinogalactan,[4] was isolated from its relatively non-viscous aqueous solution by ethanol precipitation. Chromatography on Biogel P-300 (50 × 1·25 cm.) gave a single sharp peak on plotting carbohydrate content of

fractions *vs.* elution volume, indicating $\overline{M}_w$ 31,500 (33,500 from sedimentation and diffusion measurements).[5] Re-chromatography of selected fractions confirmed that the bulk of the polysaccharide had molecular weight 31,500, the remainder ranging down to 25,500.

The product (1 G) of mild acid hydrolysis (0·01N H_2SO_4, 96°, 50 hr) of the polysaccharide (1·6 G), collected by precipitation from aqueous ethanol to remove mono- and oligosaccharides, gave on chromatography an elution pattern showing three broad overlapping peaks at molecular weights 26,500, 18,000 and 8,200; it contained approx. 96% galactose residues. Re-chromatography indicated a distribution over the range 31,000 - 8,000, $\overline{M}_w$ being 18,000 (18,650 by sedimentation and diffusion).[5]

Acacia melanoxylon exudate. Only about 50% of this unusual Acacia bark exudate is carbohydrate, including glucose, rhamnose and arabinose residues. Methylation analysis[5,6] shows the presence (on average) of short sugar chains. Acid hydrolysis liberates sugars and leaves a water-insoluble residue, showing steroid reactions* and strong absorption at 315 mμ. The acetylated exudate contains two components at least (thin-layer chromatography),[6] while NMR spectroscopy of the acetates further indicates carbohydrate and steroidal constituents.

Chromatography on Biogel P-300 (60 × 0·9 cm.) of that part (A1) of the exudate precipitated from aqueous solution by ethanol showed two small regular peaks due to polysaccharide $\overline{M}_w$ 93,000 and 51,000 (UV absorbance zero), and a broad peak (approx. 95% of total material) at $\overline{M}_w$ 5,000 or below, containing carbohydrate and UV-absorbing material. The same mixture A1 on Biogel P-4 (30 × 0·9 cm.) showed a sharp high peak at the void volume ($\overline{M}_w$ 4,000 or above) and one very broad peak at $\overline{M}_w$ 870. Resolution of the low-molecular-weight components was then achieved on Biogel P-10 (55 × 1·25 cm.), elution of A1 giving, in addition to a small peak at the void volume (high-molecular-weight polysaccharide), three distinct peaks enclosing almost equal areas at $\overline{M}_w$ 5,000, 1,200 and 670, all of which exhibited marked UV absorbance at 315 mμ (peaks in absorbance in the elution curve coinciding with peaks in carbohydrate content). The aqueous ethanol-soluble fraction (A2) of the *A. melanoxylon* exudate, chromatographed on Biogel P-300, was found to contain no high-molecular-weight material (>5,000).

Preparative-scale fractionation is being investigated with a view to isolating polysaccharide (from A1), and to determining the sugar sequences in the low-molecular-weight constituents of differing size (in A1 and A2).

The support of the CSIR and of the University of Cape Town Staff Research Fund is acknowledged, as well as the contributions to this work of Prof. J. K. N. Jones, Dr A. Polson and Mr G. R. Woolard.

*The exudate itself is reported by Prof. N. Sapeika to show pharmacological properties typical of a saponin.

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PLASMA AMINO ACID CONCENTRATION AND THE REGULATION OF ALBUMIN SYNTHESIS

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The existence of separate, independent control mechanisms for albumin synthesis and catabolism under conditions of protein deprivation has been demonstrated in this laboratory.[1] In these experiments, reduction or withdrawal of dietary protein was followed by a fall in synthesis rate of albumin, and it was postulated that this was a consequence of a reduction in the availability of certain amino acids. Conversely, refeeding of protein resulted in a significant rise in albumin synthesis on the first day of the new diet to above normal levels, and this was thought to be due to renewed availability of amino acids. This work led us to postulate that synthesis of albumin was governed by the availability of certain amino acids in the plasma. The present experiments were designed to test this hypothesis.

Male albino rats of the Wistar strain, housed in conditions of controlled temperature and humidity, were fed one of two diets: a normal protein diet consisting of 20% mixed proteins, or a protein-free diet. Appropriate vitamin and mineral supplements were added to the protein-free diet. Under light ether anaesthesia, 50 ml. of blood was drawn into heparinized syringes by cardiac puncture from two groups of animals—well-nourished animals and animals fed a protein-free diet for 2 days. The amino-acid concentration of the plasma was measured in blood obtained from each group of rats. The concentration of the branch-chain amino acids leucine, isoleucine and valine was reduced in the rats on the protein-free diets, while the albumin concentrations were not significantly altered.

Accordingly, to test the role of amino acids in controlling albumin synthesis in rat liver, undiluted blood from rats fed the 20% protein diet and from rats starved of protein for 48 hours was used to perfuse liver of well-nourished animals according to the method of Miller *et al.*[2] Albumin synthesis rate was measured by the method of McFarlane.[3,4]

The two perfusates produced no change in the synthesis rate of urea, but livers perfused with blood from animals on a protein-free diet synthesized significantly less albumin than livers perfused with blood from well-nourished rats. Livers from well-nourished rats were next perfused with blood from rats starved of protein for 2 days to which valine, isoleucine and leucine supplements had been added. The synthesis rate of albumin was significantly increased when the branch-chain amino acids were added to the perfusate.

These results would appear to support our hypothesis that the diminution in synthesis rate of albumin during protein depletion may be caused by a reduction in the availability of certain amino acids and is not due to any change in plasma albumin concentration.

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DOES mRNA MEDIATE THE ACTION OF THYROID-STIMULATING HORMONE ON I^- TRANSPORT?

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The action of thyroid-stimulating hormone (TSH) on I^- transport by isolated cells has been shown to be biphasic in nature. I^- accumulation expressed in terms of ^{131}I-C/M ratios (intracellular $^{131}I^-$ concentration divided by the extracellular $^{131}I^-$ concentration) is depressed within ½-hour of TSH addition and is slowly stimulated with continuous TSH treatment, reaching almost maximum stimulation after 6 hr. A kinetic analysis of this phenomenon indicated that the early depression in C/M

was due to an increase in I⁻efflux from the cells, whereas the later stimulation of C/M was associated with a slowly developing augmentation of I⁻influx.

In the present study we examined the possibility that during the latent period for the stimulatory action on I⁻influx, the activation of essential intermediate processes is slowly achieved. Since several investigators have demonstrated an early stimulation of glucose oxidation by TSH, we first investigated the possible role of glucose metabolism in I⁻transport. Pre-incubation of the isolated thyroid cells for 10 hr. in the absence of glucose, followed by incubation in the presence of the antagonist 2-deoxyglucose did not significantly alter the I⁻trapping of control cells. Furthermore, both ½-hr. and 6-hr. effects of TSH were still fully evident.

Since protein synthesis was found to be stimulated by TSH, we considered the possibility that one of the intermediate mechanisms of TSH action might involve protein induction. We observed that the inhibitor of DNA-dependent RNA synthesis, actinomycin D (4 μg./ml.), and the inhibitors of protein synthesis, puromycin (0·5 mM) and cyclohexamide (0·1 mM), had no effect on basal C/M but essentially abolished the 6-hr. enhancement of C/M. Washing the inhibitor-TSH-treated cells several times with incubation medium reversed the inhibitory effects of actinomycin, puromycin and cyclohexamide on the 6-hr. TSH stimulation of I⁻trapping. Re-incubation of the washed inhibitor-TSH-treated cells for 1 hr. induced a full TSH stimulation similar to that observed when the cells are incubated for 6 hr. with TSH alone.

In order to delimit the actinomycin, puromycin and cyclohexamide-sensitive periods, these inhibitors were added at various times after the addition of TSH. It was found that the actinomycin-sensitive period (1½ - 2 hr.) was of much shorter duration than either the puromycin or cyclohexamide sensitive periods (4 - 5 hr.).

These findings suggest that:

1. TSH stimulation of the I⁻trap is not secondary to increased glucose metabolism but is dependent on increased protein synthesis.

2. There exists a distinct possibility that TSH stimulates the synthesis of mRNA, a process that is completed within about 2 hr. The enhanced mRNA synthesis accelerates the synthesis of a specific 'regulator' protein which, in turn, accounts for the slowly developing stimulation of the I⁻trap.

3. TSH does not seem to act directly at the transcriptional level, but on some mechanism which is able to control the synthesis of mRNA.

4. The early enhancement of I⁻efflux (i.e. decreased C/M ratio) is not secondary to changes in either glucose metabolism or protein synthesis, and may possibly reflect a direct action of TSH in altering the permeability of the plasma-cell membrane.

URINARY PORPHYRINS IN SYMPTOMATIC PORPHYRIA

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Patients with symptomatic porphyria excrete urine containing uroporphyrin (8-COOH) and coproporphyrin (4-COOH) and porphyrins believed to have 7-, 6-, and 5-COOH groups. Since haem synthesis is believed to proceed by stepwise decarboxylation of uroporphyrin, it was felt that administration of a ^{14}C-labelled precursor of uroporphyrin to symptomatic porphyrics and observation of the time relationships of the specific activities of the excreted porphyrins would provide a convenient means of studying the kinetics of the decarboxylation *in vivo*. Before this could be done, however, it was necessary to identify definitively the nature of the porphyrins other than uro- or coproporphyrin.

Urinary porphyrins were absorbed on talc, methylated and separated by thin-layer chromatography yielding 5 distinct methyl ester bands. The individual methyl esters were eluted, crystallized and further studied by chromatography and electrophoresis of the free acids, examination of the products of decarboxylation with 0·3 N HCl at 180°C, infrared spectroscopy and mass spectrometry.

The results of these studies indicate the presence of uroporphyrin, coprophyrin, heptacarboxylic porphyrin and hexacarboxylic porphyrin. The methyl ester presumed by other workers to be a pentacarboxylic methyl ester proved to be a mixture of two porphyrins—one a pentacarboxylic porphyrin and the other a hitherto undescribed and unidentified porphyrin.

Mass spectrometry of uroporphyrin methyl esters gave a fragmentation pattern indicating a unique splitting of neutral fragments of m/e58 ($CH_2.CO_2$) from the propionate and acetate side-chains.

UPTAKE OF PLASMA ALBUMIN BY THE ATHEROMATOUS AORTA

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Previous studies[1] on atheromatous aortas of rabbits exposed *in vivo* to tritiated cholesterol have shown, *inter alia*, that plasma cholesterol enters the aorta from the luminal surface and that the rate of uptake by the atheromatous lesion is much greater than occurs in immediately adjacent areas of the aorta that are free of atheroma. This increased uptake by the lesion has been attributed, at least in part, to an increased permeability of the lesion to plasma cholesterol. If this interpretation is correct, one may expect that the lesion is also more permeable to other plasma constituents. This hypothesis was tested in atheromatous rabbits exposed *in vivo* to ^{131}I-labelled albumin.

It is shown in autoradiographic studies that plasma albumin gains access to and is transported across the lesion at a much greater rate than occurs in adjacent normal areas. It is clear from these studies that the media peripheral to a lesion can allow passage of plasma albumin at a substantially greater rate than is normally required of it. The same can be said for plasma cholesterol.[1] It would therefore appear that the rate at which these plasma constituents gain access to the vessel wall from the luminal surface is normally limited by processes at this surface. The nature of the alteration at this surface in atheroma to increase its permeability is not elucidated by these studies.

There are distinct differences in the rate of access of plasma albumin to different regions of the aorta, as has also been observed in the normal aorta of the dog.[2] Where atheroma is present, however, uptake is very much greater at all aortic areas.

The fact that the atheromatous lesion allows an increased rate of access of plasma constituents as dissimilar as albumin and cholesterol suggests that other plasma constituents may also gain entry to the affected region at increased rates. The possible implications of this development in the atherogenic process are manifold.

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