EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

The following are summaries of the papers presented at the 16th Scientific Meeting of the Experimental Biology Group (EBG) held on 30 April 1965 in the Lower Lecture Room, Department of Pathology, Medical School, University of Cape Town.

THE EFFECTS OF HISTAMINE AND 5-HT LIBERATORS ON EXPERIMENTAL NON-DIETARY CIRRHOSIS IN RATS

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The experimental cirrhosis produced in rats by intravenous injections of egg-yolk or other foreign protein, suffers from being non-progressive, even when intensified by adrenalectomy. If the animals are depleted of histamine before egg-yolk injections are commenced, and a histamine liberator given with all subsequent egg-yolk injections, a more sustained lesion is produced, though its onset may be delayed. This apparently progressive lesion is accompanied by unusual degrees of hepatomegaly, focal hepatic necrosis and, exceptionally, bile ductular proliferation.

Depleting the animals of 5-HT with reserpine during the course of egg-yolk injections gives variable results, but large and toxic doses intravenously appear to prevent egg-yolk fibrosis in about one-half the animals tested.

SOME EFFECTS OF PEPTIDES ON MICROSOMAL PROTEIN SYNTHESIS

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The exact role of peptides in cellular metabolism or in protein synthesis has not been clearly defined. Currently accepted mechanisms do not provide for the participation of preformed peptides in protein synthesis. There is, however, evidence, mainly from plants[1] and from bacteria,[2] that peptides are able to influence protein synthesis or function as protein precursors. Studies on peptide excretion in children during protein depletion and repletion have suggested that peptides may be intermediates during protein synthesis.[3]

In order to obtain more direct evidence on the role of peptides, their effect has been studied on *in vivo* protein synthesis using a system from rat liver. Homogenized liver from fasting rats was separated by differential centrifugation into nuclear, mitochondrial microsomal and a supernatant fraction, from which a 'pH 5' enzyme precipitate was prepared.[4] On incubating ^{14}C-L-leucine in a suitable salt mixture, with an energy generating system and either supernatant or 'pH 5' fractions, microsomal protein was labelled and its specific ^{14}C activity measured. With this system gly-l-leu (6×10^{-4}M) caused 51% inhibition of ^{14}C-leucine uptake. The degree of inhibition was proportional to log (peptide concentration). Other peptides at a concentration of 6×10^{-4}M had the following effects on incorporation: l-leu. gly. + 11%, l-ala. leu. + 5%, gly. gly. gly. — 11%, gly. l-tyr. — 17%, gly. gly. — 39%.

The possibility that hydrolysis of leucyl peptides, with consequent dilution of the ^{14}C amino acid with unlabelled leucine, could contribute to the effects, was also investigated. The activity of glycyl-l-leucine peptidase in the various fractions was (in units/mg. protein): whole homogenate 0·67, nuclei 0·014, microsomes 0·11, supernatant 1·95 and pH 5 fraction 0·080. Equimolar (6×10^{-4}M) l-leucine produced less inhibition (35%) than gly-leu. Dilution of the ^{14}C label was, therefore, not the only mechanism of inhibition nor could it account for slight activation by some peptides and the inhibition by peptides containing no leucine.

Apart from these unexplained phenomena it appears that peptides can compete with free amino acids for incorporation into protein, and thus may exert an important influence on the entry of amino acids into the protein molecule.

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SEROLOGICAL STUDIES OF BROME-GRASS MOSAIC VIRUS AND ITS PROTEIN FRAGMENTS

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Brome-grass mosaic virus (BMV) has been found to be widespread in pasture grasses and wheat in South Africa.[1] This virus has some unique properties such as an unusual RNA composition (MW = 1×10^6), an isoelectric point of pH 7·9, and a smaller size at pH 6 than at pH 7·0.[2] This structural transition is indicated by higher values of the intrinsic viscosity, sedimentation and diffusion coefficients of the virus at pH 6·0.[3] Another characteristic property of BMV is its instability above pH 6·5, the virus being degraded into a number of serologically related antigens with different electrophoretic mobilities.[4] This phenomenon warranted a detailed investigation of the serological behaviour of BMV and its protein fragments.

A number of antisera were prepared against BMV and its breakdown products. When virus purified in the presence of pH 7 phosphate buffer was used to inject rabbits intramuscularly, an antiserum (titre 1/64) was obtained that reacted in gel double-diffusion tests with both complete virus particles and with various virus degradation products. Up to 4 precipitin lines were obtained in some tests when the antigen was kept above pH 7·0 at room temperature. BMV protein with a sedimentation coefficient of 2·8 S (a dimer of M = 40,000, prepared by dialysis of the virus against 0·1 M $CaCl_2$ pH 6·0) also reacted with this antiserum, forming a pattern of interference (spur) with adjacent precipitin lines caused by complete virus particles or reaggregated protein. The position and curvature of the bands helped in their identification and were used to determine under which conditions of pH, temperature and ionic environment the protein failed to reaggregate and the virus to break up. Comparative diffusion measurements were made in an analytical Tiselius cell. The virus was stabilized in 0·1 M acetate buffer pH 4·0 at 4° and 37°C, and when injected into rabbits it produced an antiserum that failed to react with unaggregated BMV protein, although it reacted with reaggregated protein. This indicates a change in the pattern of antigenic determinants of the protein when it is assembled into the capsid.

It seems well established that all the protein sub-units of a virus are structurally and chemically identical and the results imply a change in the folding of the polypeptide chain of the sub-units upon aggregation into virus particles. A similar conclusion has recently been reached by Rappaport *et al.*[5] in a serological study of tobacco mosaic virus and turnip yellow mosaic virus.

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THE INCORPORATION OF δ-AMINOLAEVULINIC ACID 5-^{14}C INTO PLASMA BILIRUBIN IN NORMAL AND PORPHYRIC HUMAN SUBJECTS

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Approximately 80 μC (4·2 m.moles) of ALA-5-^{14}C was administered by mouth to 2 normal subjects, one patient with South African genetic porphyria, one patient with symptomatic porphyria and one patient with acute intermittent (Swedish) porphyria, and the specific activity of plasma bilirubin was estimated at varying times thereafter for one week. In both normal and porphyric subjects, plasma bilirubin showed a peak in radioactivity within the first 24 hours after administration of the radioactive ALA, without a noticeable peak at 4 days. In the porphyric subjects, where more frequent plasma samples were taken during the first 24 hours, this early labelled fraction consistently showed two distinct peaks, the one preceding the other by approximately 3 hours. There were no significant differences in the time of appearance of radioactivity in bilirubin or the specific activity of bilirubin at the peak when normal subjects were compared with porphyrics. Since there is good reason to believe that this early labelled fraction represents bilirubin derived from liver haem, these experiments indicate that there is no defect in liver haem synthesis in porphyria.

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