

EXPERIMENTAL BIOLOGY GROUP: SUMMARIES OF SCIENTIFIC PAPERS

Abstracts of papers read at the 21st Scientific Meeting of the Experimental Biology Group (EBG) held at the Karl Bremer Hospital on 16 September 1966:

UNUSUAL INTRACYTOPLASMIC INCLUSIONS FOUND IN FOETAL LAMB KIDNEY CELL CULTURES INFECTED WITH WESSELSBRON VIRUS

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An electron-microscopic study of ultra-thin sections of foetal lamb kidney cells infected with Wesselsbron virus (an arbovirus) revealed unusual intracytoplasmic inclusions. Infected cells showed distinct changes 40-48 hours after infection, at which stage many orderly-patterned inclusions in 'crystalline-like' array were seen in the cytoplasm of a very high proportion of the cells. They were intimately associated with the endoplasmic reticulum which showed gross vesiculation in the vicinity of the arrays. The 'crystalline-like' inclusions consist of electron-translucent sub-units which, by their hexagonal and rhombic shapes, suggest an arrangement of short hexagonal cylinders.

Four days after infection the orderly-patterned inclusions were no longer apparent in the infected cells but characteristic

areas of dense aggregates were visible in the cytoplasm in comparable numbers and situations similar to those of the prior arrays from which they are presumed to have originated. It is presumed that these intracytoplasmic inclusions are associated with viral multiplication and represent structures involved in or required for the assembly of virus particles. Virus particles were occasionally observed in the cisterns of the endoplasmic reticulum.

Thin sections of foetal lamb kidney cells infected with an attenuated strain of Wesselsbron virus showed intracytoplasmic inclusions with irregular array. Numerous virus particles, measuring 34 μ in diameter, were found in clusters in the cytoplasm.

THE IODINE AND PROTEINS IN CONGENITAL GOITRE OF CATTLE

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A congenital goitre occurring in an inbred herd of Afrikaner cattle¹ has been shown to contain an excess of deiodinase² and an abnormal thyroglobulin which comprises most of the goitre iodine.³ The abnormal thyroglobulin contains thyroxine.³

After the administration of radioactive iodine (¹²⁵I), 48% of the radioactivity of the goitre was located in the insoluble fraction whereas only 18% of the normal thyroid radio-iodine was insoluble.

Goitre and normal thyroid tissue were homogenized in 0.25 M sucrose from which nuclear, mitochondrial, microsomal, and soluble fractions were prepared by differential ultracentrifugation. These fractions were analysed for their enzyme (deiodinase and protease) activities and for their iodine:nitrogen ratios (μ g. I/mg. N).

Iodotyrosine deiodinase activities were only observed in the mitochondrial and microsomal fractions and were higher in the fractions obtained from the goitre than in those prepared from normal bovine thyroids. Incubation of all four fractions with ¹³¹I-labelled thyroglobulin caused release of ¹³¹I-labelled iodo-

tyrosines whereas none of the fractions obtained from normal calf thyroid hydrolysed the ¹³¹I-labelled thyroglobulin to any appreciable extent during the same period of incubation.

I/N ratios in the mitochondrial and microsomal fractions obtained from normal thyroid homogenates were 5.24 and 6.22 whereas those of the goitre were only 0.53 and 0.52 respectively. A similar difference was found in the I/N ratios of the nuclear and soluble fractions of the normal thyroid which were both approximately 2, whereas in the goitre they were both 0.093.

In view of the heterogeneity of the abnormal thyroglobulin in the goitre,³ attempts were made to separate its components by gel-filtration through columns of Sephadex G-100, granulated 7% agar gel and 3% agarose beads. With none of these columns was resolution of the thyroglobulin-like iodoprotein achieved, although the main iodoprotein could partly be separated from the serum proteins.

1. Schulz, K. C. A. (1962): *Proceedings of the Second Congress of the Genetic Society*, p. 90.
2. Van Zyl, A., Schulz, K., Wilson, B. and Pansegrouw, D. (1965): *Endocrinology*, **76**, 353.
3. Robbins, J., Van Zyl, A. and Van der Walt, K. (1966): *Ibid.*, **78**, 1213.

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EXPERIMENTAL INFECTION OF COMMON TERNS WITH INFLUENZA A/TERN/SA/1961 VIRUS (TERN VIRUS)

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In 1961 an epizootic occurred among Common Terns along our Cape coastline and we isolated and identified the causative virus as influenza A/Tern/SA/1961.

Eventually, earlier this year, a method of capturing live healthy terns was devised and 15 Common and 2 Swift Terns were obtained for experimental purposes. We were able to reproduce experimentally in Common Terns the acute illness with a high mortality rate which affected these birds in the 1961 epizootic. We also confirmed that large doses of tern virus administered intramuscularly had no apparent harmful effect on the Swift Tern but did stimulate antibody formation. At death the Common Terns had a viraemia and consequently virus was present throughout the tissue; however, fluorescent antibody studies showed that virus multiplication occurred in

muscle, heart, brain, lung and spleen. Virus was isolated from palatal and cloacal swabs of most of the birds and viral antigen was demonstrated in the glands of the palate and cloacal mucous membrane in 2 of the 6 birds in which they were examined.

Cloacal and palatal secretions or excretions seem the likely source of virus for spread of the infection. In addition, viral antigen was demonstrated in the palatal and cloacal glands in the tern which was recovering from infection 14 days after inoculation with tern virus; this suggests that infection may persist at these sites after recovery from the systemic infection.

The attempt to infect one bird by means of lice transferred to it from sick birds was unsuccessful; the role of arthropods in the spread of this infection is unknown.

DIE AKUTE GEVOLGE VAN INTRA-VENEUSE INFUSIE VAN 'N BETA-ADRENERGIESE BLOKKEERDER OP MIOKARDIALE METABOLISME EN ENERGETIKA IN PASIËNTE MET ISGEMIESE HARTSIEKTE

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Miokardiale metabolisme en energetika is bestudeer by 'n reeks manlike en vroulike pasiënte tussen die ouderdom van 34 en 71 jaar, wat met isgemiese hartsiekte voorgedoen het. Aangesien daar in 'n hele aantal van die pasiënte kliniese bewyse van akute koronêre gebrek was, is die hemodinamika en metabolisme slegs onder rustende toestande bepaal. Die verskeie parameters is weer eens bepaal nadat propranolol intraveneus toegedien is.

In die meeste pasiënte was die kardiaale indeks, linker ventrikulêre werkverrigting en DTM (druk-tyd per minuut) onder rustende toestande verhoog. Koronêre bloedvloei was in die meerderheid van gevalle verlaag, miokardiale suurstof ekstraksie was normaal, en die miokardiale verbruik van suurstof was gevolglik verminder. Die bewyse vir anerobiese metabolisme met rus is voorsien deur die verhoogde laktataat vlakke in arteriële bloed, deur negatiewe of lae positiewe redoks-potensiaal gradiënte, deur die verbruik by voorkeur van koolhidraatbronne vir energievoorsiening, en deur lae vryvetsuur suurstof ekstraksie verhoudings in feitlik elke geval. Miokardiale doeltreffendheidsindekse was in alle pasiënte baie hoër as normaal, en die suurstof verbruikskoeffisiënt ($\text{ml.O}_2/100 \text{ G./sistoliese sek./kg-m.}$) was veel laer as die normale waarde van 0.075. Beide dié bevindings dui op die buitensporige werkverrigting in terme van suurstof verbruik.

Twintig minute na die aanvang van die propranolol infusie is alle parameters weer bepaal. Met enkele uitsonderinge is 'n treffende vermindering in die kardiaale omset waargeneem ($\pm 250\%$), en linker ventrikulêre werkverrigting is in verhouding verminder. Daar was 'n meegaande daling in sistemiese arteriële drukke, maar daar was geen gemiddelde druklesing laer as 75 mm.Hg nie. In die meerderheid van gevalle was die DTM laer as die ooreenkomstige waarde vóór die infusie bepaal, alhoewel die afname van 'n kleiner graad was as die verandering in drukwerkverrigting. Koronêre bloedvloei en die bepalende faktor daarvan, nl. miokardiale suurstof verbruik, het geen betekenisvolle verandering aangetoon nie. Miokardiale doeltreffendheidsindekse het in die rigting van normaal verander. Die eksterne (meganiese) doeltreffendheid het amper-normale waardes bereik terwyl die $\text{DTM}/\text{Q}_{\text{O}_2}$ nog heelwat bo-kan die normale 250 gebly het. Met enkele uitsonderinge het die arteriële laktataat-vlakke gedaal, en glukose verbruik en suurstof ekstraksieverhoudinge het toegeneem. Die redoks-potensiaal gradiënt het in elke geval 'n treffende ommaswaai na erobiese aangedui. Daar was egter geen meegaande toename in vryvetsuur ekstraksie of verbruik nie: die onderdrukte metabolisme van hierdie substraat is voortgesit selfs waar miokardiale suurstof verbruik die kontrole waardes oorskry het.

OBSERVATIONS ON THE HYDROLYSIS OF PROTEINS IN MINERAL ACIDS

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The accurate determination of the amino acid composition of a protein depends upon the purity of the protein, complete hydrolysis of this protein and the accurate determination of the amino acids in the protein hydrolysate. While the first and last of these objectives can today be achieved, optimum conditions for hydrolysis have not been established. This is reflected in the discrepancies between the results reported by different workers. As certain amino acids such as serine, threonine, tyrosine and cystine are progressively destroyed during hydrolysis while others such as valine and isoleucine are resistant to hydrolysis, it is customary to hydrolyse for different periods of time and extrapolate the values obtained to zero time. This report describes preliminary results of a systematic investigation into various factors which may affect hydrolysis in an attempt to define the optimum conditions.

Human gammaglobulin G homogeneous on ultracentrifugation and immuno-electrophoresis was prepared by the method of Baumstark *et al.*¹ The dried protein was hydrolysed at 110°C in 6N HCl in evacuated tubes sealed under nitrogen. After hydrolysis the acid was removed by lyophilization. The residue was taken up in buffer and analysed on a Beckman Model 120 B amino acid analyser. Each experiment was per-

formed in duplicate, and the following variables were examined: (i) duration of hydrolysis, (ii) purity of reagents, (iii) presence of oxygen in hydrolysis mixture, (iv) protein to acid ratio (w/w), (v) the effect of hydrolysis on a synthetic amino acid mixture simulating gammaglobulin in composition.

Results. Redistillation of HCl is not required provided A.R. reagent is used; the presence of atmospheric oxygen does not affect the results; increasing the acid to protein ratio did not improve the results; strict precautions should be taken during sealing to avoid products from the burner entering the tube, since significantly lower results for serine and cystine will be obtained.

Most amino acids are completely liberated after 16 hours with the exception of glutamic acid, valine and isoleucine. Threonine, serine, cystine and tyrosine are progressively deaminated with a parallel increase in free ammonia. The reproducibility of serine recoveries from 22 hours onwards is poor, producing serious errors in extrapolated values.

On hydrolysis a synthetic amino acid mixture behaves in a similar way as the corresponding protein with the exception of tyrosine. Cystine values are unreliable at all times.

1. Baumstark, J. S. *et al.* (1964): Arch. Biochem., 108, 515.