EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

The following are abstracts of papers read at the 30th Scientific Meeting of the Experimental Biology Group (EBG) which was held at the University College of the Western Cape, Bellville, on 28 February 1969:

REGIONAL DIFFERENCES IN UPTAKE OF PLASMA ALBUMIN AND CHOLESTEROL BY THE NORMAL AORTA

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Atheromatous lesions show a characteristic distribution throughout the arterial tree. It is therefore apparent that there must be processes peculiar to those regions at which lesions do form that determine atheroma formation, and that an understanding of these processes is a prerequisite for establishing the mechanism of their formation. Neither the encrustation theory nor the 'filtration' theory, as currently expressed, can account for atheroma formation with a characteristic distribution of typical lesions.

In studies on the uptake of plasma constituents by the aorta, it was previously shown that both plasma cholesterol[1] and plasma albumin[2] gain access at the luminal surface of established atheromatous lesions at a much greater rate than at adjacent normal areas. It was concluded that the continued growth of the lesion is dependent, at least in part, upon this increased rate of access. It was therefore postulated that the localization of lesions at particular sites may also be dependent upon such a process; the rate of access at those sites at which lesions do form being greater than at areas which tend to be spared.

Normal rabbits with normal aortas were given ^{131}I-labelled rabbit albumin intravenously and killed 6 hours later. Uptake by the aorta was studied by autoradiography on sections cut from different regions of the vessel wall. Similar studies were done in normal rabbits with normal aortas killed 30 hours after administration of tritiated cholesterol by nasogastric tube. These studies show that there is indeed a much greater rate of access of both these plasma constituents at those sites where atheromatous lesions do form than at adjacent areas where lesions tend not to form.

It is concluded that the predilection of certain areas of the aorta to atheroma is compatible with an increased rate of access of plasma constituents to these areas. It is suggested that where plasma lipid levels are elevated the cells at these areas are provided with lipid in quantities which exceed their metabolic capacity, leading to the accumulation of lipid at these sites. The reason for the increased rate of access at these sites is not elucidated by these studies.

1. Krut, L. H. (1968): S. Afr. Med. J., **42**, 792.
2. *Idem* (1969): *Ibid.*, **43**, 126.

CYLINDRICAL GRADIENT ROTOR FOR PREPARATIVE PURPOSES

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A zonal rotor is described in which the principle of the reograd (reorienting gradient) rotor of Andersen, Price, Fisher, Canning and Burger (1964) is utilized. It differs from the reograd rotor in its height:diameter ratio; the manner in which the septa form independent compartments when in operation; the presence of a central core from which the septa radiate; and the manner by which the gradient is introduced.

The rotor may be run in the Spinco preparative rotor and may be used for isopycnic and velocity separation. A maximum of 20 ml. of sample may be fractionated. Its applicability to biological systems is demonstrated in the separation of the components from an artificial mixture of two haemocyanins.

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β-D-GLUCURONIDASE ACTIVITY OF ABALONE EXTRACTS

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β-D-Glucuronidase preparations from humps of perlemoen (abalone, a marine mollusc) have been used to effect the cleavage of aldobiouronic acids normally highly resistant to hydrolysis by chemical means. Such compounds are conveniently prepared from naturally occurring polysaccharides (gums and hemicelluloses) by removing other glycosidically bound sugar residues with hot dilute mineral acids, this treatment leaving the bond between glucuronic acid and the adjacent sugar unit intact.

Grade I β-D-glucuronidase (Seravac Laboratories; *ca.* 100,000 Fishman units per vial containing 50 mg. protein), prepared by selective ammonium sulphate precipitation from perlemoen visceral extracts, was incubated at pH 4 and 37°C with 6-O-(β-D-glucopyranosyluronic acid)-D-galactose (I), 4-O-(α-D-glucopyranosyluronic acid)-D-galactose (II), and the 4-O-methyl-D-glucuronic acid analogue (III) of compound I in separate experiments, progress of hydrolysis of the substrates being followed by sampling at intervals between 10 min. and 24 hr and measuring reducing power towards copper by Nelson's arsenomolybdate colorimetric procedure. Increase in reducing power with time was conveniently plotted in terms of glucose, the colour responses of equivalent amounts of glucose, galactose, glucuronic acid and glucurone being comparable. Suitable controls were set up to make sure that the hydrolyses observed were due to enzyme participation.

Rates of hydrolysis of substrates I - III were all far lower than that of the standard substrate used for β-D-glucuronidase activity, phenolphthalein β-D-glucuronide (IV). Hydrolysis nevertheless did occur, this being confirmed by paper chromatography. In a typical experiment I (1·42 mg., as Ba salt) was completely hydrolysed after 24 hr incubation with enzyme (4·6 mg.), this amount having been shown to be sufficient to accomplish hydrolysis of the same molar quantity of IV in less than 10 min. The time of half-hydrolysis of I was of the order of 1 hr, as estimated from the smooth curve relating increase in reducing power with time. The expected inhibiting effect of liberated glucuronic acid was noted. II (Ba salt) was only partially hydrolysed, using the equivalent of 2·8 mg. enzyme, after 24 hr. The fact that hydrolysis occurred at all suggests the presence of an α-D-glucuronidase in the enzyme preparation. Under comparable conditions III (Ba salt) was hydrolysed, but even more slowly than II. After 24 hr considerable proportions of unchanged II and III were revealed on paper chromatograms.

Even if it were possible to bring about hydrolysis of the glycosidic bond in aldobiouronic acid residues in oligosaccharides of higher molecular weight by β-D-glucuronidase,[1] it would be necessary to separate contaminating carbohydrases from the enzyme for successful removal of D-glucuronic acid, leaving the remainder of the structure intact. Preliminary investigation has shown that considerable β-D-glucopyranosidase activity (salicin used as substrate) is manifested by the β-D-glucuronidase used in the current series of experiments. Work is being continued to determine the levels of α- and β-D-galactopyranosidase, α-D-mannopyranosidase and α-D-glucopyranosidase activity.

The project was financially supported by the CSIR. We acknowledge gifts of chemicals from Dr J. Largier (Seravac) and Prof. J. R. Nunn (Rhodes University). Mr M. D. Malan participated in the work, and Prof. C. von Holt made certain facilities available.

1. Pridham, J. B. (1954): Biochem. J., 57, xxviii. (Polysaccharide gums resist enzymic hydrolysis.)

STUDIES ON THE VENOM OF THE BOOMSLANG, *DISPHOLIDUS TYPUS* A. SMITH

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Venom was collected from 48 boomslang by dissection of the venom glands. The average yield was 2·5 mg. dried venom per specimen.

This venom was shown to hydrolyse casein and the synthetic amino acid esters L - p - toluenesulphonyl-arginine methyl ester (TAME) and L-benzoyl-arginine ethyl ester (BAEE), with pH optima of 9·5, 8·5 and 8·5 respectively. The activity towards casein was approximately ¼ of that shown by puffadder venom and more than 10 times that shown by several South African elapids.

The proteolytic nature of the venom suggests that this activity may be responsible for some of the characteristic haemotoxic effects following envenomation by *D. typus*.

HALOTHANE-INDUCED HYPERPYREXIA IN LANDRACE PIGS

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Malignant hyperpyrexia is a rare but often fatal complication of general anaesthesia in man.[1] A common factor in many of these cases appears to be the halogenated hydrocarbon $CF_3.CHCl.Br$ (halothane), and a familial distribution suggests a genetic defect. A strain of Landrace pig which is available locally has been found to go 'hot' after halothane inhalation anaesthesia.[2]

The clinical picture in humans and pigs appears to be similar, i.e. pyrexia with core temperature reaching 43 - 45°C, stiffness of muscles and often a fatal outcome.

We have investigated the biochemical changes triggered by halothane in susceptible (hot) pigs and have shown that these are dramatic and primary and are not secondary to raised body temperature.

Susceptible pigs, kept anaesthetized for up to 150 min. with the barbiturate, thiopentone, showed no untoward features. Within 2-3 minutes of inhalation of 3% halothane in oxygen, muscle twitchings proceeding to a picture identical with rigor mortis developed. The temperature of various organs monitored by thermistor probes started to rise within 5 - 8 minutes, most dramatically in the liver, and reached 43 - 44°C in the rectum and the oesophagus. Skin temperature fell. Changes in blood chemistry were noted within 2 - 4 min. These included a rise in serum Na^+, K^+, glucose, total protein, Ca^{++}, Mg^{++}, lactate, pyruvate and excess lactate. Blood pH fell from 7·35 to 6·60. Severe lactate acidosis was reflected by a base excess of less than −22 mEq./litre. The arterial PCO_2 rose above 150 mm.Hg.

These findings indicate: (*i*) a net shift of water into cells; (*ii*) leakage of K^+, Ca^{++}, and Mg^{++} out of cells; (*iii*) massive glycolysis. The glycolysis was confirmed by a fall in muscle glycogen from 0·6 to 0·4 G/100 ml. Muscle ATP levels were only slightly reduced. Arterial pO_2 levels were maintained above 200 mm.Hg and blood pressure was increased. No obvious tissue anoxia could account for glycolysis.

Oxygen consumption, measured before and after the halothane 'trigger', was doubled by the time the core temperature reached 42 or 43°C. In the early stages (1 - 2°C rise) it was increased greater than expected following a passive temperature rise (15°C). However, increased O_2 consumption did not account for excess heat produced, even assuming zero skin losses, thus indicating that non-oxidative pathways are major sources of heat. In one experiment, out of a total of 150 kcal. excess heat produced, glycolysis, neutralization of lactic acid

and hydrolysis of high energy phosphate esters could account for ±25% of this excess. The origin of the remaining 75% may only be speculated upon. Whatever its origin, these studies indicate that non-oxidative pathways can be a potential source of considerable heat production which may be of physiological importance.

It is possible, by the extrapolation to the syndrome of anaesthetic-induced malignant hyperpyrexia in man, to make concrete suggestions for its diagnosis and management.

1. Leading Article (1968): Brit. Med. J., **3**, 69.
2. Harrison, G. G., Biebuyck, J. F., Terblanche, J., Dent, D. M., Hickman, R. and Saunders, S. J. (1968): *Ibid.*, **3**, 594.

LISOSOME IN DIE SELLE VAN DIE PANKREAS-EILANDWEEFSEL NÁ SENUWEEPRIKKELING

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Lisosome kan in die sitoplasma van α- en β-selle van die pankreas-eilandweefsel in metabolies-normale diere onderskei word.

Volgens Orci en sy medewerkers[1] is daar baie meer lisosome in die α-selle tydens twee tipes van diabetes in proefdiere, nl. spontane diabetes in die muissoort *Acomys cahirinus*, en eksperimentele diabetes wat in rotte opgewek is deur toediening van streptozotosien. Hulle postuleer dat die lisosome basies van belang is in die vernietiging van die sekresie-produk, glukagon, wat 'onnodig' sou wees in die diabetes-toestand.

'n Toename in die getal lisosome (en verwante strukture) is waargeneem in die α-selle van die eilandweefsel ná simpatiese prikkeling én in die β-selle na vagus-prikkeling. Hierdie struktuurtjies kan onderverdeel word in sekondêre lisosome, telolisosome (met membraanagtige insluitsels), fagosoom-vakuoles en oorblywende liggaampies. 'n Paar voorbeelde van 'n interessante assosiasie tussen sekondêre isosome en mitochondria is gevind.

Aangesien simpatiese prikkeling 'n verhoogde vrystelling van glukagon bewerkstellig[2] en vagus-prikkeling gepaard gaan met 'n verhoogde insulien-vrystelling[3,4] kan die hipotese van Orci *et al.* dus nie onderskryf word nie. Dit wil eerder voorkom asof die lisosome op een of ander manier meehelp in die meganisme van hormoon-vrystelling deur die selle van die eilandweefsel.

1. Orci, L., Junod, A., Pictet, R., Renold, A. E. en Rouiller, C. (1968): J. Cell Biol., **38**, 462.
2. Esterhuizen, A. C. (1968): Ongepubliseerde gegewens.
3. Kaneto, A., Kosaka, K. en Nakao, K. (1967): Endocrinology, **80**, 530.
4. Daniel, P. M. en Henderson, J. R. (1967): J. Physiol., **192**, 317.

THE POSSIBLE ROLE OF CYCLIC AMP AS AN INTRACELLULAR MEDIATOR OF THYROID-STIMULATING HORMONE ACTION

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Isolated thyroid cells prepared by tryptic digestion of bovine thyroid glands were incubated with thyroid-stimulating hormone (TSH) or with dibutyrylcyclic - 3′,5′ -AMP (DBC). Both these agents altered I^- trapping in a biphasic manner; i.e. I^- accumulation was depresssed during the first hour, and then gradually stimulated to above normal levels during the succeeding 5 hours.

As in the case of TSH, the slowly developed stimulation of I^- accumulation elicited by DBC was abolished by actinomycin D, puromycin or cyclohexamide. These inhibitors did not impair basal I^- transport nor the acute depression following shortly after TSH or DBCa addition.

$^{131}I^-$-incorporation into thyroglobulin and into thyroxine was stimulated in an essentially identical fashion by TSH and DBC. Furthermore, stimulation of the incorporation of leucine-^{14}C into protein by DBC was also demonstrated.

Other nucleotides such as AMP and ATP did not elicit TSH-like actions. Similarly, butyrate was found to be inactive.

The present findings suggest that TSH action may be mediated by an adenyl cyclase—cyclic AMP mechanism.