# Experimental Biology Group: Summaries of Scientific Papers

The following are abstracts of papers read at the 39th Scientific Meeting of the Experimental Biology Group (EBG) held in the Department of Medical Microbiology, Tiervlei Hospital, Bellville, CP, on 18 February 1971:

## BIOCHEMICAL EVIDENCE OF MYOPATHY IN PALE, SOFT EXUDATIVE (PSE) POSTMORTEM DEGENERATION IN PIGS

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The postmortem phenomenon of pale, soft exudative (PSE) degeneration of skeletal muscle in pigs, also known as 'wässeriges Fleisch' or 'Muskeldegeneration' (MD) has been extensively studied and is of both biochemical interest and economic importance.[1] PSE develops in carcasses which have accelerated glycogenolysis in the immediate (1 - 2-hour) postmortem period. The pale appearance, altered texture and decreased water-binding capacity of muscle fibres are thought to be due to denaturation of sarcoplasmic proteins consequent to low muscle pH values while the carcass is still warm.[2] Animals whose carcasses exhibit PSE show no abnormality during life but there appears to be genetic differences in susceptibility between breeds and strains. Pietrain and Landrace pigs have the highest

incidence (up to 40%). Previous studies on anaesthetic-induced glycogenolysis (malignant hyperthermia)[3] indicated a subclinical myopathy which could be detected by serum enzyme markers.[4] We have investigated these parameters in PSE degeneration.

Carcasses of mixed breeds of pig were examined under production conditions at a local abattoir. Blood specimens for enzyme studies were taken during exanguination, within 1 - 2 minutes of electrical stunning, and serum creatine phosphokinase (CPK), aldolase (ALD) and lactate dehydrogenase (LDH) were assayed.[2] Carcasses were evaluated for PSE on a colour score basis (0 - 5, with 2·5 being normal) and on $pH_1$ and $pH_2$ values, which were determined at 1 - 4 hours postmortem in longissimus dorsii. Mean normal values (N = 14) for CPK, ALD and LDH were 571, 19·1 and 279 units respectively, whilst PSE levels (N = 27) were 4 335, 161 and 1 553 units ($P<0{\cdot}01$ for each enzyme). Animals with high CPK levels (800 to 1 000 units) had a 90% incidence of PSE, while 70% of those carcasses with lower CPK levels were normal. A negative correlation between $pH_1$ values (an indication of rate of glycogenolysis) and serum CPK activity was obtained ($\log CPK = 0{\cdot}35\, pH_1 - 4{\cdot}77$, $r = 0{\cdot}42$, $p = <0{\cdot}01$).

These observations suggest that malignant hyperthermia and PSE in pigs may be different expressions of the same underlying subclinical myopathy which can be elicited either by the anaerobic stress of muscular exercise or death, or by anaesthetic agents. Differences in presenting features may be explained by the presence or absence of blood circulation.

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1. Briskey, E. J. (1964): Advanc. Food Res., **13**, 89.
2. Wismer-Pedersen, J. and Briskey, E. J. (1961): Nature (Lond.), **189**, 398.
3. Berman, M. C., Harrison, G. G., Bull, A. B. and Kench, J. E. (1970): *Ibid.*, **225**, 653.
4. Berman, M. C., Du Toit, P. and Kench, J. E. (1971): S. Afr. Med. J., **45**, 580.

## THE EFFECTS OF ALA-DEHYDRATASE INHIBITION WITH LEAD ON HAEM SYNTHESIS BY AVIAN ERYTHROCYTES *IN VITRO*

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Previous experimental results from this laboratory have indicated the possible existence of two functionally distinct pathways for the disposal of 5-aminolaevulinic acid.

Our model clearly requires that haem synthesis should be demonstrable in the absence of ALA'ase activity. To examine this requirement, avian erythrocytes, chosen for their ability to synthesize haem from glycine *in vitro*, were pre-incubated with $10^{-4}$M lead acetate. The cells were then washed and incubated with ($2\text{-}^{14}C$) glycine, and incorporation of radioactivity into haemoglobin haem was measured.

Pre-incubation with lead irreversibly inhibited ALA'ase to an extent where the enzyme could no longer be detected by spectrophotometric assay. Despite this apparent absence of ALA'ase activity, haem synthesis proceeded at rates between 40% and 80% of those encountered in control erythrocytes.

## POLYSACCHARIDES FROM WATSONIA SPECIES

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We recently[1] described the evidence for crystallinity within fibres obtained from the polysaccharide gum available from *Watsonia pyramidata* corms. This observation has been confirmed, and similar evidence is presented for gums from 4 other Watsonia species.

Corms of *Watsonia pyramidata, W. angusta, W. meriana, W. fourcadei* and *W. ardernei*, collected in March 1970 (by courtesy of the Director and staff of the National Botanic Gardens, Kirstenbosch), were broken open to reveal the gum-containing vesicles. From these fibrous strands of gum were drawn by means of tweezers, and selected on the basis of uniformity of extinction under the polarizing microscope. Composite bundles of 5 - 7 fibres representative of each species were submitted to Cu $K\alpha$ radiation for 10 - 15 hours.

A close match was obtained in the X-ray diffraction patterns for all 5 species examined, though there were considerable differences in the quality of the X-ray diagrams. Gum from the large *W. angusta* corms gave patterns that were most suitable for measurement. As found earlier for *W. pyramidata* gum, the X-ray diffraction patterns were consistent with the assumption of hexagonal unit cells, $a = 14{\cdot}7$, $b = c = 12{\cdot}0$ Å. The presence of meridional reflections on only the third and sixth layer lines (the latter revealed by appropriate tilting of the specimen) indicates a 3-fold screw-axis; this suggests the presence of xylose residues arranged helically.[2] The volume of the above cells is 1830 Å;[3] this, together with the measured density of 1·52, yields for the estimated number of xylose residues per cell circa 2·8 allowing for the presence of galactose and arabinose side-chains as found in *W. pyramidata* gum.

By contrast, no evidence of crystallinity could be found for the gum exudate collected from the bark of *Brabeium stellatifolium*, which, although yielding an elongated strand of polysaccharide material which showed good extinction under the polarizing microscope, nevertheless gave only an amorphous ring pattern on the X-ray diffractogram. This polysaccharide is not a substituted xylan, but is of the substituted arabinogalactan type[3] found extensively among plant gums.

1. Fowle, L. G., Juritz, J. W. F., Klug, A. and Stephen, A. M. (1970): S. Afr. Med. J., **44**, 152.
2. Marchessault, P. H. and Sarko, A. (1967): Advanc. Carbohyd. Chem., **22**, 462.
3. Stephen, A. M. and Van der Bijl, P. (1970): Unpublished data.

## CIRCULAR DICHROISM AS A PROBE FOR CYTOCHROME *c* CONFORMATIONAL CHANGES

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The circular dichroic behaviour of both ferri and ferrocytochrome *c* in alcoholic solutions has been studied with a view to relating the induced structural changes with the resultant property changes of autoxidation[1,2] and ascorbate reduction.[3]

Circular dichroism spectra of ferricytochrome *c* in aqueous buffer and in aqueous alcohol solutions of increasing concentration were recorded in the 625 - 210-nm range. In solutions of methanol, ethanol and propanol at concentrations of 24 mole %, 14 mole % and 6 mole % respectively the spectra changed dramatically, in both the aromatic regions (261 and 251 nm) and the haem regions (550, 530, 493, 419 and 408 nm) when compared to spectra in aqueous solution. Conversely, no changes were observed at 220 nm ($\alpha$ helix) until much higher alcohol concentrations.

Ferrocytochrome *c* spectra were determined anaerobically but otherwise under identical conditions. Alcohols produced no changes in the CD spectra except at high concentrations (60% v/v) where the spectra resembled that of ferricytochrome *c*. Similar changes were noted in the absorbance spectrum at 550 nm. This cytochrome *c* still reacted with carbon monoxide however, a property peculiar to the reduced form of the protein.

The concentrations of alcohol which produced changes in the CD spectra of ferricytochrome *c* correspond to those at which ascorbate was no longer able to reduce cytochrome *c*.

This correlation implicates changes in the haem and aromatic environments with the failure of cytochrome *c* to be reduced. Conversely, the failure of the 220-nm band to undergo change at the same alcohol concentration suggests that no major change in the protein structure has occurred, and that the small changes that have taken place are localized in the haem crevice. This view is strengthened by the strong dependence of the effects of the alcohol on their hydrophobicity—the haem crevice is known to be very hydrophobic in nature.[4]

In contrast the failure of CD to exhibit any changes in the structure of ferrocytochrome *c* even at alcohol concentrations exceeding those which initiate rapid autoxidation of cytochrome *c* suggests that changes in structure leading to this change of property are very small.

It is thus apparent that only slight changes in conformation, possibly an opening of the haem crevice, will allow ferrocytochrome *c* to become autoxidizable while more drastic changes, possibly the loosening of a ligand to the iron of the haem, are required to prevent reduction. These results highlight the importance of structural changes for the function of cytochrome *c* in electron transfer and confirm our previous suggestions that the paths of oxidation and reduction are different in cytochrome *c*.

1. Kaminsky, L. S. and Davison, A. J. (1971): Biochemistry (in the press).
2. *Idem* (1970): Paper presented at the 2nd International Congress of the Porphyrias, Cape Town, December 1970.
3. *Idem* (1971): Biochemistry (in the press).
4. Dickerson, R. E., Takano, T., Kallai, O. B. and Samson, L. (1971): J. Biol. Chem., (in the press).

## EVIDENCE FOR A CYCLIC AMP-DEPENDENT PROTEIN KINASE IN THE THYROID GLAND

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Much evidence has accumulated indicating that the action of thyroid-stimulating hormone (TSH) is mediated via the adenyl cyclase—cyclic AMP mechanism. Wilson[1] showed that dibutyryl cyclic AMP (DBC) duplicated the stimulatory action of TSH on iodide transport, thyroxine synthesis and protein synthesis. Furthermore, puromycin and actinomycin D were found to inhibit the stimulatory actions of both TSH and DBC on iodide transport. More recently, Wilson and Wright[2] have shown that DBC enhanced DNA-dependent RNA synthesis in a similar manner to TSH, thereby implying that cyclic AMP also acts at the genetic level by controlling in some way the transcription of DNA.

Histones and possibly other nuclear proteins have been implicated as gene repressors inasmuch as evidence has been obtained that they inhibit the activity of DNA as primer for RNA synthesis. Histones may be chemically modified, either by acetylation, methylation or phosphorylation and recent investigations have suggested that enzymatic chemical modification may be among the possible mechanisms for decreasing their repressor activity and account for their specific role in controlling DNA transcription in different cells.

A cyclic AMP-dependent protein kinase which phosphorylates histones has been found in liver and brain.[3,4]

This observation suggests a mechanism for the induction of RNA synthesis by those hormones which cause increases in intracellular cyclic AMP levels and whose actions are inhibited by actinomycin D. We therefore undertook to demonstrate the existence of such a cyclic AMP-dependent protein kinase in the thyroid gland.

This paper presents evidence for the existence, in homogenates of thyroid tissue and isolated thyroid cells, of a protein kinase which phosphorylates calf thymus histone using $\gamma$-$^{32}$P-ATP. Furthermore, $10^{-6}$M cyclic AMP increased the phosphorylating activity of the enzyme by twofold and the enzyme was sensitive to cyclic AMP concentrations of less than $10^{-9}$M. The stimulatory effect of cyclic AMP on the crude enzyme was observed using lysine-rich F1 histone and lysine and arginine-rich F2b histone. The normal twofold stimulation was found with F1 histone whereas a 3-fold stimulation was observed with F2b histone.

Further preliminary investigations indicate that the thyroid protein kinase has (*i*) a higher cyclic AMP-dependent phosphorylating activity towards histones than other proteins such as protamine, casein, albumin and thyroglobulin; (*ii*) a $Mg^{2+}$ dependency with maximum activity occurring at 3 mM; (*iii*) a smooth pH dependence with optimum pH at 6·8; and (*iv*) a higher activity in Tris buffer than in phosphate buffer.

1. Wilson, B. (1968): Endocrinology, **83**, 877.
2. Wilson, B. and Wright, R. (1970): Biochem., Biophys. Res. Comm., **41**, 217.
3. Langan, T. A. (1968): Science, **162**, 579.
4. Mayomoto, E., Kuo, J. F. and Greengard, P. (1969): Science, **165**, 63.

## A NEW METHOD FOR HISTONE ISOLATION

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The standard method for the preparation of histones, the basic proteins associated with the DNA of chromatin, involves acid extraction of histones with 0·25N hydrochloric or sulphuric acid.[1] The method presented here, in which histone and DNA are separated by precipitation of the DNA with protamine, avoids this harsh extraction step and may therefore yield a protein preparation more closely resembling that found *in vivo*.

Deoxyribonucleoprotein (DNP) or chromatin from calf thymus was dissolved (4 mg/ml) in a solution of 2·0M NaCl, 0·05M sodium bisulphite, 0·05M sodium acetate, pH 5·0. At 2·0M NaCl the histones and DNA are completely dissociated.[2] An equal volume of histone-free protamine was added (20 mg per ml 2·0M NaCl, 0·05M sodium bisulphite, 0·05M sodium acetate, pH 5·0) and the solution dialysed against 1·0M NaCl, 0·05M sodium bisulphite, 0·05M sodium acetate, pH 5·0. At this ionic strength the DNA was completely precipitated as a DNA-protamine complex, and the histone and excess protamine which remained in the supernatant were then separated by gel filtration on Sephadex G-50, yielding a histone preparation free of DNA and protamine.

The yield of histone on an analytical scale was 90%. It was estimated electrophoretically[3,4] that some histone was still acid extractable from the DNA-protamine precipitate but represented only a few percent of histones F3 and F2a1, and was virtually free of the fractions F2a2, F2b and F1.

The histone preparation appeared to be almost completely free of any degradation or proteolytic products, as well as any non-histones.

This isolation procedure has been carried out on a scale by which gram quantities of histone can be prepared in a relatively short time. A 75 × 15-cm column, packed with Sephadex G-50, was used to separate up to three grams of histone per run with 0·005M sodium bisulphite, 0·005M sodium acetate, pH 5·0 as eluant. It was found impossible to de-salt the histones simultaneously during the separation from protamine on the Sephadex bed, as a considerable retardation of the histone peak occurred when distilled water was used as the eluant.

The whole histone could be resolved into two fractions on a Sephadex G-100 column at pH 5·0. The first peak could be further fractionated by ammonium sulphate at 70% saturation into a precipitate of F3 and F2a1 and a supernatant containing F1. Histones F2a2 and R2b, which comprise the second peak, could be partially resolved on a Sephadex G-100 column at pH 4·0.

1. Butler, J. A. V., Johns, E. W. and Phillips, D. M. P. (1968): Progr. Biophys. Molec. Biol., **18**, 211.
2. Ohlenbusch, H. H., Olivera, B. M., Tuan, D. and Davidson, N. (1967): J. Molec. Biol., **25**, 299.
3. Panyim, S. and Chalkley, R. (1969): Arch. Biochem., **130**, 337.
4. Panyim, S., Jensen, R. H. and Chalkley, R. (1968): Biochim. biophys. Acta (Amst.), **160**, 255.