EXPERIMENTAL BIOLOGY GROUP : SUMMARIES OF SCIENTIFIC PAPERS

The following are abstracts of papers read at the 38th Scientific Meeting of the Experimental Biology Group (EBG) which was held at the Department of Physiology, Medical School, Observatory, Cape, on 30 October 1970:

ANOMALOUS MIXED LEUCOCYTE REACTIONS IN A FAMILY GENOTYPED FOR HL-A

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Family studies have established that a correlation exists between the major human histocompatibility system (HL-A) and lymphocyte reactivity in the mixed leucocyte reaction. In general, pairs of siblings identical with respect to HL-A fail to stimulate in the mixed leucocyte reaction, while family pairs differing by one or two HL-A alleles stimulate readily. The family reported here consists of both parents and 6 children who were tested for HL-A specificities using 166 antisera in a semimicrocytotoxicity dye exclusion test. Phenotyping for HL-A revealed that in addition to the four HL-A classes expected among the offspring, a fifth class was observed. This child is a probable HL-A recombinant (A c/d) involving maternal chromosomes C and D. Cells from this child, in one way mixed lymphocyte cultures, repeatedly failed to stimulate and were not stimulated by cells from a sibling (AC) with an intact maternal C chromosome, but stimulated and were stimulated by cells from a sibling (AD) with an intact maternal D chromosome. These results are discordant with the widely held view that the HL-A locus itself is responsible for the mixed leucocyte reaction and favour instead the contention that the mixed leucocyte reaction is not a simple function of HL-A but perhaps under the genetic control of a locus closely linked to the HL-A system.

THE EVOLUTION OF MECHANISMS FOR BIOSYNTHESIS OF MACROMOLECULES: A COMPARISON OF FATTY ACID- AND AMINO ACID- POLYMERIZATION SYSTEMS

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Much is now known about the biosynthesis of long-chain fatty acids and proteins. In the first case, polymerization of 8 or 9 identical 2-carbon units (activated as CoA derivatives) is catalysed by a multi-enzyme complex composed entirely of proteins bearing bound prosthetic groups such as 4′-phosphopantetheine and FMN. Proteins, in contrast, are formed by polymerization of 20 different amino acids (activated as tRNA derivatives) on ribonucleoprotein particles (ribosomes), and the sequence is determined by transcription and subsequent translation of the base sequence of informational nucleic acids. Long chains can be produced.

Recently, details of a different polypeptide-synthesizing system have been elucidated. A multi-enzyme complex, free of nucleic acids and composed only of proteins bearing a bound cofactor, 4′-phosphopantetheine, is responsible for the biosynthesis of the cyclic decapeptide antibiotic, gramicidin S. The constituent amino acids are activated to form bound aminoacyladenylates in primary reactions closely resembling those involved in the activation both of fatty acids and of amino acids destined to be incorporated into proteins.[1] The polymerization now proceeds, in strict sequence, from bound thioester intermediates, some linked to the 4′-phosphopantetheine group and others to thial groups of cysteine residues of the enzyme proteins.[2,3] The mechanistic analogy with long-chain fatty acid synthesis is very close, excepting that the antibiotic product is a polymer consisting of heterologous units (amino acids) arranged in a defined sequence, and is thus essentially a small protein. Other antibiotic polypeptides are apparently formed in similar fashion, the chain lengths never exceeding about 20 residues.[4]

One of the chief reasons for exploring this system was the search for 'fossil' peptide-synthesizing processes which would throw light on the evolutionary development of protein synthesis. The multi-enzyme complexes forming peptide antibiotics and long-chain fatty acids may be present-day versions of an ancestral synthetic mechanism, in which polymerization of units was catalysed by relatively simple low-molecular-weight substances with reactive thiol groups favourably aligned to form thioesters and to promote acyl transfers under suitable conditions. Evolutionary pressure for biosynthesis of complex, long-chain proteins, with their extreme versatility and specificity, led to the use of nucleic acids in the vastly more complicated process of gene-dictated ribosomal amino acid polymerization.

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HISTONE—SERUM PROTEIN INTERACTIONS

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In attempting to produce specific anti-histone antibodies it was found that histones interacted with a number of serum proteins. Mixing a solution of acid extracted total histone with serum from immunized and non-immunized rabbits resulted in heavy precipitation. Similar sera run against histone fractions (F1, F2a1, F2a2, F2b and F3) in immunodiffusion plates resulted in a large number of precipitin lines. Insoluble polymerized total histones adsorbed similar amounts of protein from sera of immunized and non-immunized rabbits.

Electrophoresis of the adsorbed proteins showed that they consisted of a mixture of serum albumin and other acidic serum proteins, but contained little gammaglobulin. A technique similar to immunoelectrophoresis was used as well to demonstrate this interaction. Precipitation occurred mainly with serum albumin and other acidic proteins but not with gammaglobulin.

Further investigations were therefore done with ammonium-sulphate-precipitated gammaglobulin preparations. In immunodiffusion tests, however, a somewhat blurred line was seen with fraction F2b and precipitation with fractions F2a1, F2a2 and F3 occurred in or very close to the antigen wells. The latter effect was caused by poor diffusion of fractions F2a1, F2a2 and F3 into agarose gels. This may have been caused by aggregation of histones at basic or neutral pH. In addition the histones which diffused into the gels could not be washed out with water or saline.

Conventional tube precipitation tests with gammaglobulins prepared from immunized and non-immunized rabbits and histone fractions, F1, F2a1, F2a2 and F2b were done. The last three histone fractions precipitated gammaglobulin. The curves were similar in appearance to normal immunoprecipitin curves. Only a portion of the histone was actually precipitated in the tube in which maximal precipitation occurred. The amount of precipitation occurring in the tube showing maximal precipitation increased for periods of more than a week. There was a gradual shift to the right (increased antigen concentration) of the point at which maximal precipitation occurred during this time. The precipitate redissolved in acidic solution.

In addition to the known poor antigenicity of histones, interaction with acidic serum proteins, gammaglobulin from non-immunized rabbits and agarose has complicated immunological studies on histones.

HEREDITARY METABOLIC MYOPATHY IN PIGS AND ITS ASSOCIATION WITH MALIGNANT HYPERPYREXIA

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Malignant hyperpyrexia, induced by halothane ($CF_3CHBrCl$) in pigs who appear otherwise normal, has been shown to be associated with acute changes in glycogen, mineral and water metabolism in muscle.[1,2] In the analogous condition in humans there is biochemical evidence of underlying muscle disease in susceptible individuals and their relations.[3,4] Pigs who develop hyperpyrexia have raised resting serum levels of creatine phosphokinase (CPK) as well as exaggerated leakage after exposure to halothane.[5]

The three muscle 'marker' enzymes CPK, lactate dehydrogenase (LDH) and fructose-1,6-diphosphate aldolase (ALD) have been measured in the serum of normal and hyperpyrexia-susceptible pigs. At least two populations of pig could be identified by means of their resting serum enzyme levels; the higher level group corresponded with abnormally reacting pigs. CPK levels were the most sensitive index of myopathy. There was some overlap between normal and susceptible groups with regard to ALD and LDH levels but the correlation was still significant. Serum enzyme levels can thus be used to select and predict development of hyperpyrexia.

The ATP-supported Ca++ accumulating ability and Ca++-stimulated ATP-ase activity of the sarcoplasmic reticulum was studied in normal and myopathic animals in view of associated muscle rigor and release of Ca++ from affected muscle. Normal sarcoplasmic reticulum preparations accumulated Ca++ at a rate which was not affected by halothane in concentrations up to 5% (v/v) in the gaseous phase. The uptake of Ca++ by myopathic sarcoplasmic reticulum was greater than controls, both in the presence and absence of halothane. These and results of ATP-ase assays may be another expression of increased membrane permeability. They do not support a primary role of the sarcoplasmic reticulum in the genesis of malignant hyperpyrexia.

This newly described subclinical myopathy may explain some stock losses and undesirable properties of meat in the pork industry, which are secondary to accelerated postmortem glycogenolysis in muscles.

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FLAVONOIDS OF THE PROTEACEAE: A CHEMICAL CONTRIBUTION TO STUDIES ON EVOLUTIONARY RELATIONSHIPS AMONG THE SOUTH AFRICAN PROTEOIDEAE

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The fresh leaves of over 100 species of indigenous Proteaceae were subjected to acid hydrolysis according to the procedure of Bate-Smith.[1] Paper chromatography, in conjunction with ultraviolet spectroscopy when necessary, was used to detect in the hydrolysates the possible presence of cyanidin (Cy) and delphinidin (Del) (as their chlorides), and of kaempferol (K), quercetin (Q) and myricetin (Myr), these flavonoids being considered significant in determining phylogenetic relationships.[1] Where both cyanidin and delphinidin appeared in the same hydrolysate, indicating the presence of both of the leucoanthocyanidins in the original leaf sample, the relative proportions were visually estimated. The results are reported in Table I. To these have been added the results for 31 species which have been reported in the literature,[1,2] many of which have been re-examined by ourselves, since these results have a direct bearing upon the objective of this survey. Based upon the presence or absence of the aforementioned flavonoids, 4 groups are recognized. The figures in each column are the number of species of each genus which fall into that group.

TABLE I. RESULTS

	Group I	*Group II*	*Group III*	*Group IV*
Myr	+	−	−	−
Del	+	+	−	−
Cy	low + or −	+	+	−
Q	+	+	+	±
K	±	±	±	±
Genus				
Aulax	0	3	0	0
Faurea	0	0	0	1
Leucadendron	17	8	9	1
Leucospermum	15	2	0	0
Mimetes	0	0	3	0
Paranomus	0	3	0	0
Protea	0	1	10	35
Serruria	4	1	0	0
Spatalla	0	1	0	0

On the basis that the presence of trihydroxy phenolic compounds such as delphinidin and myricetin is a primitive character, and their absence an advanced character[1] and further that the loss of the ability to synthesize leucoanthocyanidins from an evolutionary line cannot be regained,[1] the sequence in the evolution of these groups as determined from a chemical basis, may be represented as follows:

Group I → Group II → Group III → Group IV.

In the light of these results, a suggestion on evolutionary relationships in the Proteoideae as based on chromosome studies[3] may require re-examination. There may also be grounds for remodelling sections in the genus *Protea* as delimited at present.[4]

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AN ANALOGUE COMPUTER FOR SYSTEMS CONTROL AND SIMULATION IN THE INVESTIGATION OF SODIUM TRANSPORT ACROSS FROG SKIN

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Short-circuit current (SCC) and potential difference (PD) are measured in the study of sodium transport across frog skin. The repetitive measurement of these parameters over long periods is extremely tedious, and we have automated the procedure. An inexpensive computer, utilizing only 8 operational amplifiers, has been constructed and programmed to function as a control system for the automatic continuous measurement of SCC and PD.

From electrophysiological and isotope data, we have calculated the various fluxes and transfer coefficients of Na across normal frog skin, treating this as a 3-compartment system. This can be readily simulated on the computer. The instrument has consequently proved invaluable for both the acquisition and interpretation of data obtained from a variety of experimental procedures on frog skin.