THE AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF BIOLOGICAL SCIENCES

DEPARTMENT OF GENETICS

ANNUAL REPORT 1971

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RESEARCH WORK

The main interest of the Department is turning towards the interpretation of processes of population change and organic evolution in terms of molecular genetics. This is an integrative approach, considering molecular change in relation to the survival of the organism, rather than a reductionist one characteristic of much of molecular biology. The advantage of such a field of work is that it is relevant to all organisms and thus of general interest, and it combines the concepts and techniques of genetics, evolution and biochemistry.

The Department still retains an interest in the problems of metabolic regulation and the control of genetic recombination. A new activity of this type is the study of regulatory processes in living cells in relation to the structural integrity of membranes.

With the hope of contributing to the solution of some present-day problems, the Department has initiated studies into the genetic basis of the biological degradation of benzene derivatives and is about to commence work on the modification of the amino-acid composition of plant proteins to make them more suited to human nutrition.

Repeated Nucleotide Sequences in *Vicia* Species (Chooi)

Cytological studies have shown that there is a six-fold variation in DNA content per cell between species in the plant genus, *Vicia*. DNA-DNA hybridisation experiments have indicated that the differences in DNA content per cell between six related species could be attributed mainly to highly repeated DNA sequences.

Present experiments are concerned with:-

(a) the mechanism (local; i.e., linear or lateral multiplicity) of evolutionary increase in DNA content per cell in a larger range of *Vicia* species;

(b) a comparison of the repeated nucleotide sequences found in various *Vicia* species;

(c) the significance of the evolutionary increase in DNA.

Both buoyant density and DNA-DNA hybridisation studies of whole DNA and fractionated DNA from *Vicia* species with large differences in DNA content per cell suggest that local multiplicity probably accounts for a large part of the evolutionary increase in DNA.

Assuming that increase in DNA content per cell in *Vicia* species is significant in genetic adaptation to changing environments, it is conceivable that evolutionary increase in DNA content per cell has taken place via selective multiplication of certain parts of the genome. This is consistent with the finding that under different specific environmental conditions the DNAs of *V. atropurpurea* and *V. benghalensis* undergo significant changes in base composition and reassociation characteristics.

Characterisation of Monotreme DNA (Chooi, Baldwin)

Little is known about the degree of relatedness between representatives of the monotremes and about their phylogenetic relationship with other vertebrate groups. Present research is concerned with a study of the relative DNA content per cell and DNA structure of monotremes.

DNA has been purified from Platypus and Echidna. Buoyant density and melting profiles indicate that the base compositions of these two DNAs are about 44% G+C. However, while both Echidna and Platypus DNAs give a single band in cesium chloride equilibrium centrifugation, Platypus DNA has a heavier satellite as well. The presence of the satellite suggests that there is a special set of genes of particular base composition localized in the chromosomes.

Adaptation of Enzymes from Poikilothermic Animals to Temperature (Baldwin)

(a) Control of thermally induced isoenzyme production in Salmonid fishes.

Poikilothermic organisms often possess enzyme systems which are adapted to maintain stable rates of catalysis throughout the normal habitat temperature range. The basis for temperature independent of enzyme reaction rates in Salmonid fishes appears to lie in the temperature-directed production of different isoenzymes displaying adaptive changes in enzyme-substrate affinities. To investigate the thermal switching mechanisms underlying these changes in isoenzyme production, methods have been developed for growing rainbow trout and brook trout liver and muscle cells in culture. Enzyme systems known to show thermally-directed isoenzyme changes in the whole animal have been characterised in the cultured cells, and the cells are being grown at 4° and 18°C to establish if isoenzyme changes will occur

under culture conditions.

(b) Regulation of enzyme activity in the Echidna and Platypus.

The responses of the Echidna and Platypus to fluctuating environmental temperature differ markedly. Active Echidnas maintain body temperature in the range 25° to 33°C, but during prolonged cold periods they become torpid, with body temperature closely paralleling environmental temperatures down to at least 5°C. In contrast, the Platypus appears to maintain a stable body temperature of about 31°C. Because of these differences in response to environmental temperature, these animals were considered ideal for studying the evolutionary adaptation of homologous enzymes to different thermal regimes.

Studies of the effect of assay temperature on the kinetics of muscle lactate dehydrogenases indicate that in the Echidna, rate stabilization of catalysis is achieved from 25° to 35°C, and is accompanied by a marked increased in the apparent K_m of the enzyme for the substrates pyruvate and NADH. At temperatures below 25°C, the apparent K_m remains almost constant and reaction rates decrease rapidly over the temperature range encountered in torpid animals. For the Platypus enzymes, changes in apparent K_m with assay temperature are much smaller than observed with the Echidna, and reaction rates display Q10 values of about 2 from 5° to 40°C.

Similar studies on liver supernatant and mitochondrial malate dehydrogenases are being carried out in collaboration with

Dr. Aleksiuk (Honorary Research Fellow in genetics), and in addition, the effects of temperature upon mitochondrial oxidation in tissue homogenates are being investigated.

(c) Structural analysis of the Lactate Dehydrogenase isoenzymes of the monotremes. (Bennett, Baldwin)

The functional adaptation to temperature of the enzyme lactate dehydrogenase (LDH) in Platypus and Echidna appears to provide a very suitable system in which to study molecular changes in relation to adaptation.

LDH exists as a complex isozymic system in vertebrate organisms due to at least two genes which specify different subunits of the active enzyme molecule. The polypeptide products of these genes randomly assemble according to a binomial distribution to make the five different tetrameric molecules H_4, H_3M_1, H_2M_2, H_1M_3 and M_4. The H and M genes are differentially active in different tissues and at different stages of development. Thus, tissues subjected to periods of anaerobiosis are richer in those isozymes composed of M subunits, while heart muscle and brain with a relatively secure oxygen supply tend to be rich in isozymes composed of H subunits. This simple mechanism is found in mammals and most birds. However, other vertebrate classes, particularly fish, frequently exhibit more complex isozyme patterns which are very different from those of the mammals.

Starch gel electrophoresis of heart and muscle extracts of Platypus and Echidna show the typical distribution of five tetramers, though with differing electrophoretic mobilities between the two animals, plus, for each tetramer a maximum of two

additional sub-bands. The apparent temperature adaptation of these LDHs of Platypus and Echidna in terms of their structure and catalytic mechanism is now being studied.

Initially the M_4 tetramer of Platypus LDH has been purified from muscle. The purification is a three-day procedure involving DEAE-cellulose, Sephadex G100 and CM-cellulose chromatographic steps. The M_4 tetramer finally emerges as three quite separate homogeneous peaks of active protein, designated I, II and III, which correspond to the three bands obtained by starch gel electrophoresis of the M_4 isomer. Peaks I and III correspond to two bands of the electrophoretic pattern whilst Peak II gives rise to the Peak I isomer plus the third isomer. Ultracentrifugation of protein from Peak I gives a single monodisperse peak with a sedimentation coefficient of 7.67, which is comparable with LDHs from other vertebrates, whilst Peak II gives a polydisperse pattern. Sucrose density gradient centrifugation of Peak I results in a single coincident band of protein and LDH activity, while Peak II gives the same band of protein and activity plus a single, more slowly sedimenting band of inactive protein, probably the dimer. Peak II protein, therefore, is thought to give rise to two M_4 isozymes in an interacting system with the inactive dimer. Amino acid analyses of Peak I and II proteins repeatably show small but significant differences, especially between the minimally contributing residues, histidine and tyrosine, while their N-terminal analyses both give threonine as the terminal amino-acid.

A model which is consistent with all the observed facts suggests that the M_4 tetramer exists in three forms M_4 (Peak I), M_2M_2' (Peak II) and M_4' (Peak III) with the M_2M_2' tetramer capable of dissociation into dimers and reassociation to give M_4 but not M_4' under the experimental conditions. This model implies a duplication of the M gene to give rise to an M' gene so that random association of their dimeric polypeptide products yields the binomial distribution of three types of M_4 tetramers. The model would also explain the presence of up to three sub-bands for each of the primary LDH isoenzymes by superimposition of the binomial distribution of M and M' polypeptides on the primary M and H polypeptide distribution. Peptide mapping of the three M_4 isozymes, which is currently in progress, should unequivocally test this conclusion and also provide information on the nature of their amino acid differences.

It is possible that an extension of this model may be applicable in Echidna and may explain the complex isozyme systems in the lower vertebrates.

The study will be continued by purification of the Platypus H_4 and the Echidna M_4 and H_4 enzymes in an attempt to elucidate the functional significance of their LDH isozyme systems by detailed kinetic and structural analyses.

Evolution and Adaptation of histidinol dehydrogenases

(Creaser, Loper[+], Dhawale and Lindsay[Ⱡ])

This investigation is a comparative study of the structure and enzymology of the essential biosynthetic enzyme, histidinol

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+ Visiting Research Worker
Ⱡ Member of the Department of Botany

dehydrogenase, which occurs in a variety of different modes of organisation. The two groups which have been most clearly defined are the operon systems in *Salmonella, Escherichia, Micrococcus* and *Bacillus* and the multifunctional histidinol dehydrogenase - cyclohydrolase enzyme found in several Ascomycetes. A survey of the dehydrogenase-cyclohydrolase enzyme from several fungi and yeasts has shown that there are marked differences in the stability and ease of reconstitution of these complex enzymes.

Good progress has been made in the purification and elucidation of the mode of organisation of the histidinol dehydrogenase from *Pseudomonas*. It had previously been thought, on genetical and preliminary biochemical evidence, that *Pseudomonas* had a dehydrogenase-cyclohydrolase enzyme similar to that of Neurospora but it has proved possible to separate these two enzyme activities on purification. Experiments on derepression with analogs and with new mutants could be interpreted on the basis of *Pseudomonas* having a 'mini-operon' consisting of histidinol dehydrogenase, cyclohydrolase and one other enzyme.

It is known that an *Arthrobacter* from soil can grow on histidinol as a sole source of carbon and nitrogen and produces an inducible histidinol dehydrogenase. The majority of inducible enzymes are not essential for growth of the cell in all environments and it became of interest to investigate the mechanism of the *Arthrobacter* adaptation. When grown on minimal salts-glucose medium, a single histidinol dehydrogenase is produced which is the normal biosynthetic one. When histidinol is added to the

medium, a second enzyme is induced which attains a high level of activity in the cell. It is suggested that this second enzyme is produced by a duplicated histidinol dehydrogenase gene which has evolved into part of an inducible degradative pathway for histidinol utilisation. Comparative protein chemistry could confirm the postulated duplication and permit assessment of the degree of divergence since duplication. Efforts to select a *Pseudomonas* mutant with such a degradative enzyme have not been successful, but it was possible to select a mutant with a greatly enhanced level of the normal enzyme. In this case, evolutionary adaptation may result from a changed control system.

Comparative studies on histidinol dehydrogenases from organisms adapted to extreme environments have commenced with a range of *Bacillus* species which grow naturally at temperatures up to 70°C. Efforts are being made to extract and purify the enzyme from moderate and extreme halophiles.

Genetic Determination of the Quaternary Structure of β-Galactosidase. (Langridge)

The active form of β-galactosidase in *Escherichia coli* is a tetramer composed of four identical sub-units held together by hydrophobic interactions and hydrogen bonds (the quaternary structure). To obtain information on the position of regions of the protein responsible for maintaining these associations, the enzymes from about 200 point mutations in the gene for β-galactosidase have been examined. Mutations affecting quaternary structure were identified by the increased sensitivity of the mutant enzymes to dissociation by urea and by density-gradient centrifugation.

About five per cent of point mutations appear to unstabilize the quaternary structure. Mapping by three-point linkage tests indicates that there are about four sequences of amino-acids involved in sub-unit association. These sequences overlap, or are close to, the sequences responsible for substrate binding. The proposition is being tested that the hydrophobic environments for substrate binding are provided by the four clefts formed between sub-units when they associate.

Genetics of Alcohol Dehydrogenase in Yeast (Langridge, Bennett, Stewart[P])

Yeast alcohol dehydrogenase is probably the only enzyme available at present which can be subjected to selection pressure and whose primary structure and tertiary conformation are known. Therefore, it may provide a suitable system in which a nucleotide substitution giving a selective advantage may be studied in terms of amino-acid replacement, alteration in kinetic rate and change in conformation.

The dehydrogenase oxidizes alcohols to aldehydes or ketones which are in turn oxidized by other enzymes to organic acids utilizable by the cell. At present, haploid yeast cells are being selected for utilization of the organic acids, aldehydes and ketones that would be derived from two- to eight-carbon alcohols. When the genotype of the yeast has been adjusted in this manner, mutations of the alcohol dehydrogenase to use different alcohols can be obtained.

P Member of the Department of Developmental Biology

Bacterial Degradation of Benzene Compounds (Langridge, Lindsay[P])

Most of the herbicides, pesticides, insecticides and detergents have substituted benzenes as major components of the compounds. These benzene rings are quite resistant to microbial breakdown and form an important source of environmental pollution. The evolution of the ability to degrade primarily and secondarily substituted benzenes is being studied, as well as the spread of the responsible gene systems in natural populations of bacteria. Bacteria have been obtained from the local sewage works which will metabolize all primarily substituted benzenes, except nitrobenzene. They are now being examined for their gene systems enabling secondarily substituted benzenes to be utilized as carbon sources.

Aspects of the Genetic and Phenotypic Control of Aromatic Biosynthesis in *Neurospora crassa* (Doy, Hoffmann*)

(a) DAHP Synthase

Work has been continued on this allosteric isoenzymic system but at a reduced level. A method was developed for the purification of DAHP synthase (Trp). Purification methods are now available for all the allosteric isoenzymes. With the genetic knowledge available, these isoenzymes provide a system for the study of the interaction between elements of control and for the evolution of allosteric control within one organism.

P Member of the Department of Botany

* Former member. Based on work done while a member of the Department.

(b) Two sets of gene clusters and their products

Work on the enzymes and gene clusters that constitute the common aromatic pathway and an inducible related catabolic pathway has continued in association with work at Yale University. The most important finding is that *qa-1*, a regulatory gene contiguous with three structural genes of quinate catabolism (*qa-2*, *qa-3* and *qa-4*), controls their expression by a positive mechanism resembling the arabinose system in the prokaryote, *Escherichia coli*. *Qa-1* also determined whether or not *arom-1* auxotrophs grow when the common path intermediate, shikimate, is supplied as the sole source of aromatic path end products. This is an important result since it implies either that the proteins coded by the *qa* cluster interact with those of the *arom* cluster, or else that *qa-1* controls a product (such as a permease) concerned with the accessibility of shikimate to the functional *in vivo* organisation of enzymes. Methods have been developed for the purification in good yield of the *arom* cluster multifunctional protein and this can be dissociated into functional sub-units of relatively small molecular weight. Results with wild-type material now correlate much better with results using extracts of *arom* cluster mutants.

(c) Pleiotropic effect of *arom* cluster mutations on DAHP synthase

Certain mutations within the *arom* cluster alter the molecular weight of DAHP synthase which suggests an *in vivo* functional organisation between components of the common pathway.

Plant Tissue Culture and Development, particularly of Haploid Lines (Gresshoff, Doy)

Work has commenced on a project in which it is hoped to develop methods for the biochemical-genetic analysis of higher plants, particularly from haploid tissue culture. The media used are all of defined composition.

Expecting that varieties may differ genetically in their ability to initiate anther growth on defined media, many varieties were tested with the result that haploid tissue cultures of *Arabidopsis thaliana*, *Lycopersicum esculentum* (tomato) and *Hordeum vulgare* (barley) were established. The method applies only to some varieties and requires the removal of anthers during late prophase of meiosis and transfer to a high auxin and low kinetin medium to initiate callus growth. Differentiation is then achieved by transfer to low auxin and high kinetin medium with a light-dark cycle. Flowering plants then develop but are, of course, sterile. Chromosome counts of callus material and root tips confirmed that the cells were haploid.

Similar studies with tomato have resulted in differentiation to produce a plantlet. Haploid callus derived from anther culture may be converted to a fruit which is seedless but with the form, colour and taste of normal fruits.

Methods are being developed for the culture of single cells in low plate dilutions as a preliminary to mutant selection and for tests of the transfer of genetic information between cells of related and diverse species.

A diploid callus of *Zea mays* (maize) has been differentiated into a system of roots and shoots.

An Organelle in *Escherichia coli* concerned with envelope Synthesis (Cho*, Doy)

Work has been completed on the investigation of aspects of the internal organisation of *Escherichia coli* by electron microscope studies of auxotrophs defective in envelope synthesis. Spermine treatment of a diaminopimelic acid-requiring strain under conditions of diaminopimelic acid starvation (thereby blocking cell envelope synthesis) resulted in the detection of a polar organelle. This organelle is connected to the plasma membrane and consists of ribosomes in association with a structure in interlocking electron-dense and -light components. We have proposed that this structure is an apparatus for the synthesis and assembly of protein and phospholipid components of the cell envelope including the organisation of cell division. If some of the electron-light components represent nucleic acid, then this organelle may also be involved in the replication and transfer of DNA. This is the first organelle other than membrane invaginations that has been discovered in *Escherichia coli* or any other prokaryote.

Repression of Aromatic Amino Acid Biosynthesis in *Escherichia coli* K-12 (Brown*, Somerville℣)

Work continued on regulatory mutants of *Escherichia coli* K-12 in which the synthesis of several enzymes of aromatic biosynthesis

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* Former member. Based on work done whilst a member of the Department.

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was constitutive. These enzymes were: 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) synthetases (Phe) and (Tyr), chorismate mutase T-prephenate dehydrogenase and transaminase A. In wild-type strains these enzymes were repressed by different amino-acids. DAHP synthetase (Phe) was multivalently repressed by phenylalanine *plus* tryptophan while DAHP synthetase (Tyr), chorismate mutase T-prephenate dehydrogenase and transaminase A were repressed by tyrosine in low concentration (10^{-4}M). DAHP synthetase (Tyr) and chorismate mutase T-prephenate dehydrogenase but not transaminase A, were also repressed by phenylalanine in high concentration (10^{-3}M).

Apart from the constitutive synthesis of DAHP synthetase (Phe), these regulatory mutants had the same phenotype as strains mutated in the tyrosine regulatory gene (TyrR). The mutations causing this phenotype mapped in the same region as TyrR at approximately 26 min. on the chromosome, being cotransducible with TrpA, TrpE, CysB and PyrF. It is thought that these mutations may be alleles of the TyrR gene and that synthesis of the enzymes listed above is controlled by this gene. The alternative possibility that these mutations occur in a separate regulatory gene close to TyrR is also under examination.

Chorismate mutase P and prephenate dehydratase activities which are carried on a single protein were repressed by phenylalanine alone and were not controlled by TyrR. Formation of this protein is presumed to be controlled by a separate, unknown regulator gene

or genes. Regulatory mutations conferring constitutive synthesis of chorismate mutase P-prephenate dehydratase and presumed to affect this unknown gene(s) have been induced and are being mapped.

Phenylalanine transaminase was not repressible under the conditions studied and was not affected in any of the regulatory mutants examined to date. Two enzymes of the common aromatic pathway, 5-dehydroquinate synthetase and 5-dehydroquinase, behaved similarly. DAHP synthetase (Trp) and tryptophan synthetase were repressed by tryptophan and were solely under the control of the TrpR regulatory gene.

Our studied indicate that the genes specifying the enzymes of aromatic biosynthesis are functionally grouped into three distinct regulatory units (regulons). One regulon is controlled by TyrR and specifies the enzymes: DAHP synthetase (Tyr), DAHP synthetase (Phe), chorismate mutase T-prephenate dehydrogenase, and transaminase A. The second is controlled by TrpR and specifies DAHP synthetase (Trp) and the enzymes of the tryptophan pathway. The third is controlled by an unknown regulatory gene(s) and specifies chorismate mutase P-prephenate dehydratase.

Utilization of shikimic acid by *Escherichia coli* (Doy, Brown)

It was found earlier that mutants of *Escherichia coli* W and K-12 blocked in the common aromatic pathway before shikimic acid generally grow poorly on shikimic acid as sole aromatic supplement. This limited growth response has been correlated with a relatively

poor ability of these mutants as well as wild-type *E. coli* W and K-12, to concentrate ^{14}C-shikimate inside the cell.

E. coli K-12 mutants but not *E. coli* W mutants showed an even poorer growth response to shikimate when the medium contained citrate. The site of action of citrate on shikimate utilization is unknown.

Derivatives of pre-shikimate auxotrophs of *E. coli* W and K-12 selected for their ability to grow rapidly on shikimate were able to concentrate approximately 10-times more free ^{14}C-shikimate inside the cell than their respective parents. The mutations conferring this enhanced growth response on *E. coli* K-12 strains blocked in 3- deoxy-D-*arabino*heptulosonic acid-7-phosphate synthetase, 5-dehydroquinase or 5-dehydroshikimate reductase all mapped at the *shiA* locus. ShiA specifies the low capacity shikimate transport system of wild-type *E. coli* K-12 and is 25% cotransduced with the *his* region. Mutations which conferred enhanced growth response to shikimate on an *E. coli* K-12 strain blocked in 5-dehydroquinate synthetase were not cotransduced with *his*. Their location is at present unknown.

Control of genetic recombination in *Neurospora crassa*

(Catcheside, Barbara Austin*, Corcoran and Teresa Angel*)

Genetic recombination by crossing over or gene conversion was long thought to be a largely mechanical process. However, many genes are now known to participate at various stages and it is becoming clear that a series of biochemical reactions, mediated by

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* Former member. Based on work done while a member of the Department.

enzymes, are concerned. The reactions subsequent to pairing of homologous chromosomes appear to involve successively the formation of cuts in one of the two chains of the chromosomal DNA molecule, the formation of hybrid DNA between pairs of homologous chromosomes, some erosion of parts of DNA chains followed by repair through resynthesis and, finally, the closure of the gaps. In some organisms, especially bacteria and some fungi (including Neurospora), genes are known which determine the enzymes participating in the later stages, particularly of repair. Mutants of these genes cause a general lack of recombination, affecting all parts of the chromosomes in a nucleus.

For several years another group of genes has been studied in Neurospora. These have effects upon recombination in specific local regions. They are of two categories (i) *rec* genes, the dominant alleles of which appear to reduce recombination locally, and (ii) *cog* genes, the dominant alleles of which increase recombination locally. The *cog* genes are situated within the local region in which recombination is altered and each is believed to be a sequence in the DNA which is recognised and cut by a specific endonuclease. The *rec* genes are usually remote from the region in which recombination is controlled; each is believed to specify a regulator controlling the activity of a gene responsible for a specific endonuclease.

Recent work has shown *rec-2* to be between the *spray* and *amination-1* loci in linkage group V. This gene affects non

allelic recombination between the *pyrimidine-3* and *histidine-5* loci in linkage group IV but has no effect upon these loci themselves. Evidence is strengthening that *rec-2* and *rec-w* are the same gene and a more searching test is under way. The *rec-w* gene affects allelic recombination in the *histidine-3* locus (linkage group I) and non allelic recombination between this locus and *adenine-3*, a *cog* locus being identified in this region by a variant. Previously it has been shown that *rec-3*, which controls allelic recombination in the *amination-1* locus (linkage group V), is the same as *rec-x*, which controls allelic recombination in the *histidine-2* locus (linkage group I). It has now been shown that *rec-3* also controls non-allelic recombination in the *histidine-2 arginine-3* region and that *rec-2* may affect a different part of this same region. The number of *rec* genes having a controlling function, and therefore the number of genes responsible for specific endonucleases, appears to be fairly considerable, perhaps between ten and fifty. However, data upon which a statistical estimate could be based are still incomplete.

Studies on Membrane Function and Biosynthesis (Rolfe+)

A wide variety of environmental agents appear to interact with cells by exerting some direct effect on the cell membrane. These include some hormones (insulin and epinephrine), acetylcholine, and certain factors which appear to regulate the rate of cell division and possibly some of the pathways of cellular

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+ Visiting Research Fellow

differentiation in higher organisms. Although the cell membrane plays an important role in these biological processes, very little is currently known about its structure and function. We have studied these relationships by investigating the genetic control and biosynthesis of particular proteins and lipids of the membrane of *Escherichia coli*.

The interaction of colicins (protein antibodies liberated by certain bacterial strains) with *Escherichia coli* cells provides a model system for studying the processes by which environmental factors can interact with the cell membrane and rapidly cause intracellular metabolic changes. Bacterial mutants (colicin-tolerant) which still adsorb colicins but are no longer killed by them, have been isolated, genetically mapped and characterized in some detail. Mutations mapping within these loci were shown to have pleiotropic effects on a number of properties such as multi-drug sensitivity, dye and detergent sensitivities and altered growth characteristics.

One of these colicin tolerant loci, the *tol A,B* locus, controls multiple colicin tolerance in *E. coli*. It consists of at least three cistrons which appear to affect the glycolipid composition of the bacterial cell envelope. Mutants at the *tol C* locus are tolerant only to colicin E1. This locus has recently been shown to code for membrane components which are involved in the biosynthesis of the phospholipid cardiolipin. The *tol L* locus mutants (tolerant to colicins E2 and E3 at 40°C), may turn

out to be the most interesting group as they appear to be involved in the control and synthesis of a protein known to bind the intracellular messenger, cyclic AMP.

These studies will be pursued further with the aim of setting up a model system to investigate environmental interactions involved in the regulation of growth and differentiation of mammalian cells.

Structural Studies of Histidinol Dehydrogenases (Bennett, Creaser)

Work has continued on the comparative amino acid sequences of histidinol dehydrogenase from *Neurospora crassa* and *Euglena gracilis*. An automated method of column peptide fractionation has been used initially to compare the pattern of tryptic peptides of the enzyme from the two organisms and then as a preparative technique for the provision of peptides for sequence analysis. *Euglena gracilis* histidinol dehydrogenase yields a tryptic peptide pattern very different from that of *Neurospora crassa*. Analysis of tryptic peptides of Neurospora and Euglena histidinol dehydrogenase has reached the stage of determination of their amino acid composition and sequences.

Amino acid substitutions in Electrophoretic Variants of Human Transferrin (Bennett, Kirk[P])

Normal human transferrin and that of an electrophoretic variant "Beryl" has been purified to homogeneity by rivanol precipitation, DEAE-Sephadex chromatography and starch-block electrophoresis. Treatment with neuraminidase to remove carbohydrate showed that differences in electrophoretic mobility must be due

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[P] Member of the Department of Human Biology

to changes in the primary structure. An automated method of column peptide fractionation was utilised to fractionate the tryptic peptides of the two transferrins and forty-three peaks were repeatably recognised. A difference in one peptide peak was detected between the two patterns and the nature of the difference in terms of its amino acid composition is being determined.

Improved Protein Sequencing Method (Bentley, Creaser)

It has been known for some time that a chelated cobalt compound can cleave terminal peptide bonds and a sequencing method, based upon this observation, is under investigation. To date, it has been possible to show that the procedure can be used for extremely rapid qualitative N-terminal amino acid determination and can also be used for the quantitative measurement of the N-terminal amino acid in a variety of peptides and proteins. Extension of the procedure to enable complete sequences to be determined is under way.

OTHER ACTIVITIES

The Department has received a grant of $14,270 from the Rockefeller Foundation of New York to support the first year's work on the modification of the amino-acid composition of plants by mutation and selection. Dr. D. M. Halsall has been appointed a Visiting Research Fellow under this grant and will join the staff in January 1972.

Dr. J. C. Loper of the University of Cincinnatti spent twelve months with the Department studying the genetics of the histidine biosynthetic pathway in *Pseudomonas*.

Dr. D.E.A. Catcheside left in February to join the School of Biological Sciences in Flinders University, Dr. K.K. Jha left in May to take up a fellowship in the University of North Carolina and Dr. K. D. Brown left in September to join the Department of Microbiology in Sydney University

Dr. C. H. Doy has been appointed to the Advisory Board of the journal, Biochemical Genetics.

The following Research Scholars worked in the Department: K. Bentley, A. Bowling, M. Dhawale, P. Gresshoff and R. Varela Torres.

PUBLICATIONS

BROWN, K.D.* Maintenance and exchange of the aromatic amino acid pool in *Escherichia coli* K-12. Journal of Bacteriology, 106: 70-81 (1971).

BROWN, K.D.* Regulation of phenylalanine and tyrosine biosynthesis in *Escherichia coli*. Proceedings of the Australian Biochemical Society, 4 (1971).

BROWN, K.D.* and SOMERVILLE, R.L.Ꝑ Repression of aromatic amino acid biosynthesis in *Escherichia coli*. Journal of Bacteriology, 108: 386-399 (1971).

CATCHESIDE, D.G. Regulation of crossing-over in *Neurospora crassa*. Genetics Lectures 2: 7-18. Genetics Institute of Oregon State University (1971).

CATCHESIDE, D.G. and AUSTIN, Barbara*. Common regulation of recombination at the *amination-1* and *histidine-2* loci in *Neurospora crassa*. Australian Journal of Biological Sciences, 24: 107-115 (1971).

CHOOI, Wai Yean. Comparison of the DNA of six *Vicia* species by the method of DNA-DNA hybridization. Genetics 68: 213-230 (1971).

CREASER, E. H. and RUSSELL, Leslie M. Further characterization of a protein promoting aggregation of retina cells. Biochemical Journal, 123: 127-128 (1971).

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* Former member. Based on work done while a member of the Department.

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CREASER, E.H. and VARELA-TORRES R.* Immunological comparisons of histidinol dehydrogenases. Journal of General Microbiology, 67: 85-90 (1971).

HALSALL, Dorothy, M., CATCHESIDE, D.E.A.* and DOY, C.H. Some properties of the 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase isoenzymes from mutant strains of *Neurospora crassa*. Biochim et Biophys Acta, 227: 464-472 (1971).

HALSALL, Dorothy, M., and CATCHESIDE, D.E.A.* Structural genes for DAHP synthase isoenzymes in *Neurospora crassa*. Genetics, 67: 183-188 (1971).

PUBLICATIONS IN PRESS

BROCK, R.D.Ꝑ, FRIEDERICH, Elizabeth, A.Ꝑ and LANGRIDGE, J. The modification of amino acid composition of higher plants by mutation and selection. International Atomic Energy Agency Publications, Vienna.

CHO, K.Y.* and DOY, C.H. Ultrastructure of spermine treated *Escherichia coli* including a polar organelle concerned with envelope synthesis. Australian Journal of Biological Sciences.

CREASER, E.H. Repressors and derepressors of gene activity. In Metabolic Inhibitors. J. H. Quastel and R. Hochster (Eds.)., Academic Press.

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GRESSHOFF, P.M. and DOY, C.H. Haploid *Arabidopsis thaliana* callus and plants from anther culture. Australian Journal of Biological Sciences.

ROLFE, B.[+], ONODERA, K.[‡] and BERNSTEIN, A.[‡] Genetic analysis of components of the membrane of *Escherichia coli I*. Interaction of genes involved in the functional organisation of the nitrate respiration system. Journal of Membrane Biology.

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