THE GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers presented at the HUNDRED AND SIXTY-SEVENTH MEETING of the Society held on 12th and 13th November 1971 at the INSTITUTE OF CHILD HEALTH and the UNIVERSITY COLLEGE LONDON.

PRENATAL DIAGNOSIS: PRESENT STATUS AND FUTURE PROSPECTS

A. E. H. EMERY

University Department of Human Genetics, Western General Hospital, Edinburgh

Prenatal diagnosis with the selective abortion of abnormal fetuses is becoming an established procedure in the management of families at risk of producing a child with a serious genetic disorder. Techniques are available either to study the fetus directly, or indirectly by investigating changes in maternal blood or urine. However, apart from the study of amniotic fluid and its contained cells, the remaining techniques are either only applicable in late pregnancy or are still at the experimental stage.

Sex determination is useful in X-linked recessive disorders with abortion of male fetuses of known carriers or of female fetuses of hemizygous affected males. The main application of cytogenetic studies has so far been in the prenatal diagnosis of Down's syndrome in cases where the mother has an increased risk of having an affected child. By the biochemical study of cultured amniotic fluid cells about 15 different inherited metabolic disorders have so far been diagnosed *in utero*. The biochemical study of amniotic fluid itself, apart from rhesus incompatibility, has received comparatively little attention. Yet this approach has obvious advantages over methods which depend upon the protracted culture of amniotic fluid cells.

The future of prenatal diagnosis is likely to see an increase in the number of metabolic disorders which can be diagnosed *in utero*, the applicaion of genetic linkage studies and the addition of such techniques as fetoscopy and fetal biopsy.

AMNIOCENTESIS FOR ANTENATAL DIAGNOSIS

A. C. TURNBULL

Department of Obstetrics and Gynaecology, The Welsh National School of Medicine, Cardiff

Amniocentesis is being utilised with increasing frequency for intra-uterine diagnosis. In the vast majority of patients this procedure has been performed in the second half of pregnancy, for the management of rhesus isoimmunisation, to assess the maturity or well being of the fetus *in utero*. In recent years, however, amniocentesis has been performed early in the second trimester of pregnancy to detect a variety of genetic defects in the fetus.

Transabdominal is preferable to transvaginal amniocentesis. The procedure is performed under local anaesthesia, using a disposable spinal needle with stylet. After puncture, the stylet is removed and 10 ml. amniotic fluid aspirated using a sterile plastic syringe. Strict aseptic precautions are used throughout the procedure. Amniocentesis should if at all possible be performed in a unit close to the laboratory, as investigations of the amniotic fluid should be started at once. Patients need not stay in hospital overnight; in some centres they remain in the out-patient department for only 1 hour.

On the evidence available at present, amniocentesis in early pregnancy appears to be a reasonable safe procedure for mother and fetus when performed by clinicians experienced in the technique. Probably the main risks are related to puncturing placental vessels. Placental localisation by ultrasonic means may help to avoid this risk. From the clinical viewpoint, it is important that the safety of transabdominal amniocentesis performed early in pregnancy should be fully established, but relatively

few are performed in any one centre at present. Collaborative studies are being arranged under the aegis of the M.R.C. and will include careful observation of the mother and fetus during pregnancy after amniocentesis and follow-up of the infant after birth.

FETOSCOPY

J. B. SCRIMGEOUR

Department of Obstetrics and Gynaecology, University of Edinburgh

This technique must still be regarded as unproven in the clinical context although initial clinical work has been promising. While its potential for the antenatal diagnosis of some genetic defects is well recognised, the chance of precipitating abortion by the procedure itself has yet to be determined. This fact alone severely limits its indications for use at the moment, and it is therefore felt that only patients who have a very high risk of recurrence of a defect may be offered this. A full explanation of the technique and the possibility of abortion thereafter must be made to both parents and a signed consent form completed.

The position at present is that amniotic fluid, if uncontaminated by bilirubin or meconium, can transmit light sufficiently well to allow inspection of the fetus within the amniotic sac. If the volume of liquor is sufficient the fetus may be moved around and this allows a complete examination of its outer surface. As embryologists and opthalmologists cannot be certain of the effect of infrared and ultraviolet light on the developing eye a special filter has been incorporated into the light source. Photography has proved possible but if the fetus looks normal and continuation of the pregnancy is planned, for the same reason *no* photographs should be taken.

The procedure should be preceded by placental localisation, and ultrasonography appears most reliable at this stage. Eighteen weeks gestation has so far proved to be the optimal time for attempting fetoscopy but reliance should be placed on the actual size of the uterus rather than the calculated gestational age.

GENETIC ASPECTS OF DIAGNOSTIC ULTRASOUND

S. CAMPBELL

Institute of Obstetrics and Gynaecology, Queen Charlotte's Hospital, W.6

Diagnostic ultrasound has been used in obstetrics since 1958. Certain aspects of this new discipline of interest to geneticists will be discussed. 1. The safety of ultrasound. Reference will be made to *in vivo* experiments which have recently been carried out in Queen Charlotte's Hospital. 2. The localisation of the placenta prior to amniocentesis. The risks of this important cytogenetic diagnostic procedure are reduced if the placenta can be accurately located. The place of ultrasound is evaluated. 3. The diagnosis of fetal abnormality. At the moment this is confined to the detection of cephalic abnormalities. Anencephaly in particular can be diagnosed before 20 weeks maturity. Babies of low growth potential can also be diagnosed by serial ultrasonic cephalometry. These babies have an increased incidence of fetal abnormality.

DIAGNOSIS OF GENETIC DEFECTS BY DEREPRESSSION OF GENES *IN VITRO*

P. F. BENSON

Paediatric Research Unit, Guy's Hospital Medical School, London, S.E.1

The use of cultured human cells for diagnosis of genetic defects is restricted by failure of phenotypic expression at the great majority of loci. The activities of some enzymes in cultured cells are very low or undetectable but may be induced by

addition of inducers, or other effectors, to the culture media, or by selecting cells in phases of growth when the rates of synthesis of relevant enzymes or other proteins are maximal.

Studies will be presented which suggest that in certain cases derepression of genes could allow detection of homozygous or heterozygous mutants in cultured fibroblasts, amniotic cells or lymphocytes which would not be possible in uninduced cells.

PRENATAL DIAGNOSIS OF TAY-SACHS DISEASE AND RELATED DISORDERS

R. B. ELLIS

Institute of Child Health, Guildford Street, London, WC1N 1EH

In view of the recent upsurge of interest in biochemical methods for the prenatal diagnosis of genetic disorders, the present state of the art will be critically reviewed. Particular attention will be given to the limitations of the methods and to the dangers and difficulties involved. The relative merits and disadvantages of cultured and fresh amniotic fluid cells and of the cell-free fluid as a source of enzymes will be considered. Comment on the ways that the subject could develop and be organised will be included.

The general approach to, and problems of, biochemical methods for prenatal diagnosis will be illustrated by reference to studies on Tay-Sachs disease: the disease for which most prenatal work has been reported.

USES OF LINKAGE FOR PRENATAL DIAGNOSIS

J. H. RENWICK

Landon School of Hygiene

Abnormal phenotypes due to aneuploidy or certain homozygous genotypes may now be diagnosed directly on amniotic cells in culture. There are no corresponding means of direct diagnosis of heterozygous genotypes as yet but indirect predictions can be made from the phenotype controlled by a closely linked locus.

Three or four such linkages can now be used in this way. These will be discussed.

DETECTION OF THE SECRETOR STATUS OF AMNIOTIC FLUID

P. S. HARPER

Department of Medicine, University Hospital of Wales, Cardiff

A study of 68 samples of amniotic fluid taken early in pregnancy showed that 54 (79·4 per cent.) contained soluble ABH blood group substances, agreeing closely with the expected proportion of " secretors ". Presence or absence of the substances was not related to maternal secretor status, and no close correlationwas found between the type of blood group substance present and maternal blood group. Out of 19 cases in which fetal blood group could be determined and where blood group substances were present in amniotic fluid, only one disagreement was found. The results suggest that the secretor status of the fetus can be determined early in pregnancy from amniotic fluid. Taken in conjunction with the recently confirmed linkage of the secretor locus with that for myotonic dystrophy this may allow in certain cases a prediction to be made as to whether the individual will later be affected.

CULTURE OF CELLS FROM AMNIOTIC FLUID FOR CHROMOSOME ANALYSIS

N. M. GREGSON

Department of Pathology, Salisbury General Hospital, Salisbury, Wilts.

The techniques for obtaining chromosome preparations from cultured amniotic fluid cells are described. Small aliquots of amniotic fluid specimens are routinely

used for direct determination of sex by quinacrine fluorescence and sex chromatin study.

The rate of success and the time required between amniocentesis and chromosome analysis are given, with discussion of the various difficulties involved.

PRENATAL CHROMOSOMAL ANALYSIS: APPLICATION AND GENETIC ASPECTS

P. E. POLANI

Paediatric Research Unit, Guy's Hospital Medical School, London, S.E.1

The indications for which prenatal chromosomal analysis has been used will be discussed and some of the findings that have accrued from the work of a number of laboratories will be presented. Undoubtedly, serious risk of Down's syndrome has been the commonest indication for chromosome studies on amniotic cells. The results from Downs's syndrome translocation heterozygotes suggest that the frequency of segregation leading to an unbalanced chromosome complement with translocation trisomy 21 is more common than estimates from family studies have hitherto suggested.

ETHICAL ASPECTS

C. O. CARTER

M.R.C. Clinical Genetics Unit, Institute of Child Health, W.C.1

There are three aspects of the ethics of prenatal diagnosis to be considered: 1. The ethics of abortion itself—this is essentially a religious issue; 2. The ethics of balancing the safety of the procedure against the potential value of the information gained; 3. The ethics of abortion relative to minor degrees of abnormality perhaps discovered incidentally.

NON-COMPLEMENTATION BETWEEN RECESSIVE SUPPRESSOR GENE MUTATIONS IN *COPRINUS LAGOPUS*

N. K. TODD and L. A. CASSELTON

Department of Plant Biology and Microbiology, Queen Mary College, London

The phenomenon of non-complementation between recessive, non-allelic suppressor gene mutations in *Coprinus lagopus* has been known for some years (D. Lewis *Genetical Research*, *2*, 141-155, 1961; D. H. Morgan, *Genetical Research*, *7*, 195-206, 1966). Based on current knowledge of su^+ gene action, we could predict that non-complementation and recessiveness are two properties to be expected of missense su^+ genes specifying modified tRNA species with altered codon specificities.

Missense su^+ tRNA does not compete for expression with its corresponding su^- tRNA, but with the tRNAs, specified by one or more other genes, which normally translate the mutant codon. Compared with a haploid, the relative amount of su^+ tRNA in a su^+/su^- diploid cell is halved. If the su^+ tRNA has a low efficiency, its activity in the diploid may no longer be expressed—hence the su^+ gene mutation will appear recessive. In a diploid carrying two different su^+ genes, the relative amount of su^+ tRNA is the same as in the haploid. The suppressor activity will thus be expressed, leading to apparent non-complementation. Our prediction requires that such su^+ genes are allele specific, since this is a fundamental property of missense su^+ genes.

Two groups of su^+ genes will be described which combine all these properties.

MUTATION FROM BIFUNCTIONALLY TO MONOFUNCTIONALLY DEFECTIVE *PYRIMIDINE-3* MUTANTS OF *NEUROSPORA*

A. RADFORD

Department of Genetics, Leeds University, Leeds, LS2 9JT

Pyrimidine-3 mutants are known which have lost both the pyrimidine-specific carbamyl phosphate synthetase (CPS) and aspartate transcarbamylase functions of that locus. Most such alleles are capable of complementation, some in polar and some in non-polar fashion. On the basis of complementation polarity, it appears that the CPS function is translated proximally and the ATC function distally, with both functions probably carried on a single polypeptide. Previous work has shown that many doubly defective polar mutants can have the distal function restored by a mutationally induced relief of polarity. Non-polar doubly defective alleles can also have the ATC activity restored by a mutational event, and six alleles of this type have been studied. Two appear to be simple base pair substitutions, but the other four alleles are not so simple to classify. Possible interpretations of their complementation behaviour and chemically-induced revertibility will be discussed.

REGULATION OF *DE NOVO* PURINE NUCLEOTIDE SYNTHESIS BY ENZYME REPRESSION IN *SACCHAROMYCES CEREVISIAE*

T. S. GROSS and R. A. WOODS

Department of Genetics, Sheffield University

Accumulation of intermediates of *de novo* purine nucleotide synthesis in *ade 2* and *ade 3* mutants of yeast is dependent on the level of purine in the growth medium. Amino-imidazole ribotide (AIR) accumulation in an *ade 2* mutant is negligible at >150 μM adenine and 4-amino-5-imidazole carboxamide ribotide (AICAR) accumulation in an *ade 3* mutant is similarly affected. Comparisons of AICAR levels in *ade 3*(*his*$^-$) and *ade 3*(*his*$^+$) mutants in media differentially supplemented with adenine and histidine has enabled us to determine the contributions of the adenine and histidine pathways to AICAR accumulation. In *ade 3*(*his*$^-$) strains AICAR is formed solely via histidine biosynthesis whereas in *ade 3*(*his*$^+$) both pathways contribute.

When repressed cultures of *ade 2* and *ade 3* are transferred from high adenine to low adenine media they rapidly synthesise AIR and AICAR respectively. This recovery from repression does not occur in the presence of cycloheximide and is thus dependent on protein synthesis.

We have assayed phosphoribosyl-pyrophosphate amidotransferase (PRPP-ATase, the first enzyme of purine biosynthesis) and found that activity is dependent on the level of purine supplementation. Cultures recovering from repression show a 6-fold increase in specific activity which is completely inhibited by cycloheximide. We infer that the enzymes of purine biosynthesis in yeast are subject to control by repression.

GENETIC AND BIOCHEMICAL STUDIES ON RESISTANCE TO THE PURINE ANALOGUE 4-AMINO PYRAZOLO (3, 4-d) PYRIMIDINE (4-APP) IN *SACCHAROMYCES CEREVISIAE*

W. R. PICKERING and R. A. WOODS

Department of Genetics, Sheffield University

Wild type *Saccharomyces cerevisiae* is sensitive to 4-APP (Lomax & Woods, *J. Bacteriol.*, *100*, 817, 1969) and shows negligible growth at 10 μg./ml. We have isolated 36 4-APP resistant mutants, following mutagenisis with E.M.S., all of which grow

at 150 μg./ml. These mutants have been allocated to 7 genes, *app 1* to *app 7*, three of which, *app 1*, *app 3* and *app 4* have both dominant and recessive alleles. Four of the genes, *app 3*, *app 5*, *app 6* and *app 7* are loosely linked. Cross-resistance to other purine analogues shows gene specificity; all 7 confer resistance to 8-azadenine and 6-methyl-purine; only *app 1*, *4* and *5* cause resistance to 8-azaguanine. None of the mutants excretes purine.

Activity of the purine phosphoribosyltransferases was studied in representative mutants of each gene since loss of activity of these enzymes is a common cause of analogue resistance. Activity with adenine, hypoxanthine and guanine as substrates was found in all of them. The level for *app 2*, *app 3*, *app 6* and *app 7* were the same as in wild-type whilst those for *app 1*, *app 4* and *app 5* were significantly higher for all three substrates. The implications of these findings will be discussed.

GENETIC REGULATION OF THE PENTOSE PHOSPHATE PATHWAY OF *ASPERGILLUS NIDULANS*

O. HANKINSON and D. J. COVE

Department of Genetics, University of Cambridge

During the reduction of nitrate to ammonium by *Aspergillus*, NADPH is oxidised to $NADP^+$. Action of the pentose phosphate pathway (P.P.P.) affords the major means whereby the organism reduces $NADP^+$ to NADPH.

When *Aspergillus nidulans* is grown with a mixture of urea and nitrate as nitrogen source, the levels of four enzymes of the P.P.P. are two-fold greater than when it is grown with urea. This increase is not dependent on the metabolism of nitrate and is probably mediated by the same process (D. J. Cove, *Proc. Roy. Soc., Lond. B., 176*, 269-275; 1970) which is responsible for the induction of nitrate reductase and nitrite reductase.

Two classes of mutants defective in the P.P.P. were isolated. All grow poorly on nitrate and nitrite. One class has reduced activities of the above four P.P.P. enzymes, but retains inducibility by nitrate for these enzymes, but retains inducibility by nitrate for these enzymes. Members of the other class lack transaldolase and accumulate sedoheptulose-7-phosphate, which accumulation is increased twenty-fold in the presence of nitrate. This increase is dependent upon the metabolism of nitrate. It therefore appears that the flow through the P.P.P. is regulated normally both by enzyme levels and by the availability of a product of nitrate metabolism.

THE REGULATION OF A WHEAT MALATE DEHYDROGENASE ISOZYME BY RYE CHROMOSOMES

R. B. FLAVELL and F. J. McPHERSON

Plant Breeding Institute, Trumpington Cambridge, CB2 2LQ

A quantitative analysis of malate dehyrogenase isozymes has been carried out in a hexaploid wheat *Triticum aestivum* variety Holdfast, a diploid rye, *Secale cereale* variety King II, and a series of seven addition lines each having the Holdfast wheat chromosome complement and, in addition, a different homologous pair of King II rye chromosomes. In young shoots of three of these addition lines, grown in a defined salts medium lacking sucrose, the level of at least one isozyme was elevated. This did not occur in shoots grown in a medium containing 0·5 per cent. sucrose or in the *Triticale* possessing the full wheat and rye chromosomal complements grown in the absence of exogenous sucrose. On the basis of cellular localisation and substrate inhibition studies the isozyme activity enhanced by the rye chromosomes is indistinguishable from an isozyme activity in Holdfast wheat and dissimilar from all the malate dehydrogenase isozyme activities observed in King II rye. It is concluded that three different rye chromosomes produce gene products which can interact with the wheat malate dehydrogenase regulatory system.

GENETIC STUDIES ON MALARIA PARASITES

D. WALLIKER, R. CARTER and S. MORGAN
Protozoan Genetics Unit, Institute of Animal Genetics, Edinburgh

During the life-cycle of malaria parasites, micro- and macro-gametocytes, which develop in the blood of the vertebrate host, undergo fertilisation in the mosquito vector. By allowing mosquitoes to feed on blood infected with two strains of the rodent malaria parasite *Plasmodium berghei*, we have been able to produce recombinant parasites.

Two lines, A and C, of the subspecies *P.b. yoelii* were first hybridised. Line A is characterised by an electrophoretic form of glucose isomerase termed GPI-1 and is resistant to the anti-malarial drug pyrimethamine. Line C has an enzyme-form GPI-2 and is pyrimethamine-sensitive. After mosquitoes had fed on a mixture of lines A and C, infections were established in mice. By cloning the resulting blood parasites, we were able to identify GPI-1 sensitive and GPI-2 resistant lines, as well as lines showing parental characters. Control lines confirmed that the recombinants were produced by cross-fertilisation of gametes, and not by mutation.

Line A has also been crossed with a *P. berghei* line from Nigeria, characterised by GPI-2 and pyrimethamine-sensitivity. These lines appear to hydridise less readily than lines A and C, there being a lower frequency of recombination between the markers.

THE GENETIC FUNCTION OF MITOCHONDRIAL DNA

P. BORST
Department of Medical Enzymology, Laboratory of Biochemistry University of Amsterdam, Holland

NO ABSTRACT RECEIVED

MITOCHONDRIAL GENETICS OF PARAMECIUM

G. H. BEALE, A. TAIT and J. K. C. KNOWLES
Institute of Animal Genetics, University of Edinburgh

Erythromycin-resistant and chloramphenicol-resistant mutants have been obtained in *Paramecium aurelia* Syngens 1 and 4, both spontaneously and following mutagen treatment. All these mutants are inherited via the cytoplasm at conjugation.

Using a micro-injection technique, it has been shown that mitochondria from erythromycin-resistant cells, when injected into sensitive paramecia, cause transformation of the latter to resistance.

By polyacrylamide gel electrophoresis it has been shown that the erythromycin-resistant paramecia contain an altered mitochondrial ribosomal protein.

THE INFORMATION IN MITOCHONDRIAL DNA

D. Y. THOMAS
National Institute for Medical Research, Mill Hill, London, N.W.7

Mitochondrial genes conferring resistance to seven inhibitors have been so far identified. Replication mapping using the mutagen nitrosoguanidine has confirmed the order of five of these genes previously established by conventional genetic methods. The map is circular.

The major products of mitochondrial protein synthesis are six polypeptides in the molecular weight range 11,000 to 34,000. There is evidence that the structural genes for these polypeptides is in mitochondrial DNA.

INHERITANCE OF MITOCHONDRIAL DNA IN YEAST

D. H. WILLIAMSON and D. Y. THOMAS

National Institute for Medical Research, Mill Hill, London, N.W.7

The way in which mitochondrial DNA molecules are transmitted to daughter cells during vegetative growth in yeast is poorly understood. In a diploid cell there are perhaps 100 mitDNA molecules contained in 20-50 mitochondria, and it is possible that all these molecules are equally capable of replication and that the mitochondria are randomly distributed between parent and daughter cells by passive cytoplasmic transfer. However, genetic studies on mitochondrial inheritance, and theoretical considerations, suggest an alternative mechanism in which one copy serves as template for all the mitDNA molecules acquired by the mature daughter individual. This hypothesis is amenable to experimental test at the molecular level with both randomly dividing and synchronous populations, and the results obtained so far will be discussed.

ZYGOTE CELL LINEAGES IN THE STUDY OF MITOCHONDRIAL RECOMBINATION

D. WILKIE and D. Y. THOMAS

Department of Botany and Microbiology, University College, and National Institute for Medical Research, Mill Hill, London

A total of 72 zygotes from crosses between strains of *Saccharomyces cerevisiae* involving three or four mitochondrial markers were micromanipulated and successive daughter cells (buds) were isolated. Clones derived from each of the first three or four daughter cells were sampled and results generally showed the clones to be mixed, although the number of phenotypes was small. Daughter cells from the same zygote tended to segregate the same few phenotypes and in the same proportions. The actual phenotypes seen differed from zygote to zygote. In some cases second generation buds, *i.e.* cells budded off daughter cells, were cloned. In many cases these clones were mixed and the segregants seen frequently differed from those in the clone from the first generation bud. It appears that recombination of mitochondrial markers occurs in zygote buds as well as in the zygote itself.

If isolation of buds from zygotes is continued beyond the fourth or fifth bud, clones derived from these buds tend to be of one phenotype. Also the clone finally derived from the zygote itself is usually homogeneous with constituent cells showing a parental phenotype. Electron microscope studies (Dr D. G. Smith) indicate that mitochondria undergo degeneration in zygotes from these crosses. A model of mitochondrial inheritance will be presented.

NUCLEIC ACID AND PROTEIN SYNTHESIS IN CHLOROPLASTS

J. INGLE

Department of Botany, University of Edinburgh

Nucleic acids unique to the chloroplast will be compared and contrasted with those of the nucleus/cytoplasm and mitochondria. The genetic potential of the chloroplast DNA will be discussed, and considered in relation to the synthesis of the chloroplast RNA and chloroplast proteins.

KILLER PARTICLES OF PARAMECIUM AND THEIR DEGREE OF INDEPENDENCE

I. GIBSON

School of Biological Sciences, University of East Anglia, Norwich

The well known killer particles of Paramecium, kappa, lambda, mu, alpha, etc., depend on a gene of the host Paramecium for their maintenance. Can they be

grown outside the cell and what could be the biochemical basis of the relationship with the Paramecium? Do these studies tell us anything about symbiosis?

PLANT MYCOPLASMAS: ARE THEY RELEVANT TO ORGANELLE EVOLUTION?

M. J. DANIELS

Department of Genetics, John Innes Institue, Norwich, NOR 70F

Recently a number of plant diseases of the " yellows " type, hitherto assumed to be caused by viruses, have been shown to be the result of infection of the phloem by mycoplasma-like organisms transmitted by leafhoppers.

The evidence for this is based on electron microscopic examination of diseased plants, on successful treatment with tetracyclines (which are ineffective against viruses), and in a few cases on culturing mycoplasmas from pathological specimens. Little is yet known about the biology of the mycoplasmas.

Current progress in plant cell biology, notably in the fields of protoplast and tissue culture technique, gives grounds for believing that plant mycoplasmas may provide favourable material for studying the molecular biology of interactions between microbes and higher cells. Mycoplasmas are believed to be the smallest, and genetically the simplest cells capable of independent growth although it is possible that some have already evolved to the point of obligate parasitism. The results of this research may therefore be of interest to students of organelle evolution.