THE GENETICAL SOCIETY OF GREAT BRITAIN

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GENETIC STUDIES IN DUCHENNE MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy is the commonest X-linked disorder in humans, with an incidence among boys of about 1 per 3000, and a mutation rate of about $1{\cdot}5 \times 10^{-5}$. (Haemophilia is about half as common; we regard colour blindness as a normal variant). However, the counterpart of Duchenne muscular dystrophy is not known by animal geneticists.

54 families with this condition have been studied in the West Midlands. Clinical studies reveal variation in manifestation from case to case. Studies of levels of creatine phosphokinase (a normal enzyme which escapes from damaged muscles) in female relatives indicate that high levels are found in almost all carriers below the age of 20 and in about 70 per cent of older carriers. As mucle is a syncytial tissue, microscopic evidence of Lyonisation is not obvious in spite of normal strength and abnormal leakage.

The estimations of the proportions of carriers among female relatives have been used to assess the relative frequencies of mutation in oogenesis and spermatogenesis. These estimates are difficult due to small numbers and an apparent deficit of affected boys and an excess of carrier girls.

THE FATE OF SATELLITE DNAs I, II and III AND RIBOSOMAL DNA IN A FAMILIAL DICENTRIC TRANSLOCATION CHROMOSOME 13/14

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The distribution of DNA sequences complementary to satellite DNAs I, II and III and ribosomal RNA were studied in a family with a stable dicentric 13 : 14 translocation chromosome. This chromosome showed a partial loss of sequences complementary to all three satellite DNAs, but slightly more of the sequences complementary to satellite I were retained than of the other two satellite DNAs. The partial loss of material from all three satellites indicates that they are not present as single discrete blocks of each sequence, but consist of interspersed blocks with each satellite DNA represented by more than one, and probably several blocks. Ribosomal DNA was totally absent from the translocation chromosome, but an interesting finding was the presence of an increased amount of ribosomal and satellite DNAs in one chromosome 22. This chromosome, distinguished by brilliant satellites (22s+) was found in seven out of nine members of the family with the translocation and in only one of five without it. No living male was found who had only the 22s+ or the t dic (13 : 14). This family may show the presence of compensation for certain sequences lost during chromosome rearrangements.

FIBRILLAR MATERIAL IN CEREAL *MICROSPOROCYTE NUCLEI*

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Treating wheat microsporocytes at different stages of premeiotic interphase with colchicine or low temperature can induce asynapsis at subsequent meiotic prophase (Bayliss and Riley, *Genet. Res. Camb.*, *20*, 193-200; Dover and Riley, *J. Cell Sci.*, *12*, 143-161). Ultrastructural studies of wheat anthers have found bundles of intranuclear microfilaments in microsporocytes but not in other cell types. Such bundles of fibrillar material (F.M.) often appeared to form links between two masses of chromatin or between chromatin and the nuclear membrane. Individual bundles of F.M. were up to 10 μ in length and 0·8 μ in diameter. F.M. may be of widespread taxonomic distribution for it occurs in microsporocyte nuclei of many species in the wheat comparium (including *Triticum*, *Aegilops*, *Hordeum* and *Secale* species, in both diploids and polyploids) and in *Vicia* and *Crepis*. In wheat, rye and Triticale F.M. was most common at, or just before, leptotene (occurring in up to 100 per cent of the nuclei sampled) but was absent at pachytene and later meiotic stages. Thus its formation preceded that of synaptonemal complex. Preliminary serial section reconstructions indicate that F.M. tends to be localised within the nucleus to the telomeric pole, while the number of bundles of F.M. is approximately equal to the chromosome number (2n). Prolonged colchicine treatment during premeiotic mitoses and premeiotic interphase did not prevent the polymerisation of F.M. although it appeared to delay its formation besides inducing the formation of numerous large paracrystals in the tapetum. While the function (if any) of F.M. is unknown, its appearance and distribution suggests that it may play an important role in establishing or maintaining the spatial coorientation of chromosomes prerequisite for pairing at meiosis.

AN INHERITED FAILURE IN DNA REPAIR IN THE HOUSE MOUSE?

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A population in a river valley in Peru, sampled in 1961 and 1968, was proved, by breeding several generations in the laboratory, to carry an incidence of mutants higher than that found in control wild populations studied in the same way: 10 different mutants in eight wild-caught mice. A sample of 300 mice was obtained in 1976, and sibmating from 80 pairs has proceeded to F3 and F4; so far, 11 new mutants have been found, a high incidence considering the limited investigation as yet. Also, unusual mitotic karyotypes have been seen in most wild-caught mice and descendants, and, in a sample of some 20 mice, one wild-caught mouse and one descendant has shown some cells with karyotypes containing univalents, fragments and aberrant chiasmatic configurations. This evidence, findings from a biochemical variant assay made by Prof. R. J. Berry, and any new evidence, will be presented at the meeting.

EFFECTS OF THE Y-CHROMOSOME IN MICE: A STUDY OF TESTIS WEIGHT, PLASMA TESTOSTERONE AND BEHAVIOUR

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Different observers have reported Y-linked effects on several characters, including testis weight, sexual behaviour and aggressive behaviour (Hayward and Shire, *Nature*, *250*, 499, 1974; Weir and Hogle, *Genetics*, *74*, 294, 1973; Selmanoff, Jumonville, Maxson and Ginsburg, *Nature*, *253*, 529, 1975). It is not known whether these effects are related developmentally, or merely by linkage; nor is it clear whether any of these quantitative effects are mediated by variation in androgen metabolism.

Reciprocal F1 males between CBA/FaCam and C57Bl/6Fa mice were backcrossed to the two parental strains, providing young adult male offspring from each backcross which differ (on average) only in the origin of the Y-chromosome. These animals were put through a series of behavioural tests, first for sexual behaviour with standard oestrous females, and then for aggressive behaviour towards a defeated male in the home cage. The mice were then killed; body weight, testis weight, kidney weight and seminal vesicle weight were measured, and plasma testosterone levels were determined by radioimmunoassay.

Compared with mice carrying the Y-chromosome derived from C57Bl/6Fa, mice carrying the Y-chromosome from CBA/FaCam showed a significant increase in the proportion of animals fighting, and a significant reduction in testis weight (whether or not corrected for body weight). There were no significant differences between the groups in sexual behaviour, plasma testosterone values, or the weights of the androgen target organs (kidneys and seminal vesicles).

These results suggest that the Y-effect on sexual behaviour described in the literature is separate from the Y-effects on aggressive behaviour and testis weight, and fail to provide any evidence that androgens mediate the Y-effects on aggressive behaviour and testis weight. On behavioural grounds, it is difficult to compare measures of sexual and aggressive behaviour; but on the evidence of the present study, it appears that there are major genetic components controlling the development of aggressive and sexual behaviour which are distinct.

GENETIC CONTROL OF ANTIBODY RESPONSE TO ΦX174 PHAGE IN INBRED MICE

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Immunogenetic or IR-gene systems have been described whereby some strains produce high and other strains low antibody responses when immunised with the same dose of antigen.

The control of immune response in 12 inbred strains of mice, consisting of 234 animals including 60 controls was investigated following immunisation with bacteriophage ΦX174 (titre 1×10^{13} pfu/ml, Miles Laboratories) in complete Freund's adjuvant and then boosted with the antigen in saline.

Antibody levels were measured using ^{125}I-labelled phage in an antigen excess assay.

A continuous distribution of antibody responses was found, with strains BALB/C, ASW and B10.BR being high responders, B10D2, B10A, SWR/J, A/JAX and C57Bl/6 being intermediate responders, whilst strains NZW, Theta, DBA/2 and C3H were found to be low responders.

ΦX174 phage antigens stimulate in different strains of mice antibody response levels which appear to be dependent on the genome of each mouse strain, possibly controlled by a large number of IR-genes.

Alternatively, these responses could be explained by a cross-tolerance or molecular mimicry mechanism.

MANIPULATION OF THE STERILITY SYSTEM IN SIBLING SPECIES CROSSES WITHIN THE *ANOPHELES GAMBIAE* COMPLEX

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Mosquitoes of the *Anopheles gambiae* complex are the main vectors of malaria and filariasis in Africa and are therefore responsible for a vast amount of morbidity and over a million deaths annually.

The male hybrids from crosses between the sibling species within the complex are sterile. Using a sex linked marker it has been shown that the X chromosome is one of the interacting factors causing this sterility. By a four generation crossing scheme a stock was produced with its X and Y chromosomes from species A and its autosomes partly from species B.

This stock was fertile, but crossing the females to species B yielded sterile males. Such males have autosomes very largely of species B origin and there seems a good chance that their mate recognition system in the field would be that of species B and that they would therefore be competitive sterile males for use against wild species B populations. By the use of a translocation linking dieldrin resistance to the Y chromosome and dieldrin treatment of first instar larvae it can be arranged that batches of mosquitoes consisting only of such sterile males are reared.

DEVELOPMENTAL AND GENETIC ANALYSIS OF THE TUMOROUS HEAD MUTATION IN *DROSOPHILA MELANOGASTER*

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Tumorous head (*tuh*) is a recessive homeotic mutation affecting the eye-antennal disc of *D. melanogaster*. It has two gene action, *tuh-1* located on the X chromosome at approximately 68·5 (*Pyati, Molec. gen. Genet., 146*, 189-190), and *tuh-3* which we mapped as being between 58·54 and 58·56 on the 3rd chromosome. We have also found a new enhancer of the *tuh-3* gene.

The adult phenotype includes duplications, deficiencies and transformations of the head. Homeotic transformations seen are those of antenna to leg and rostralhaut to genitalia. The eye is usually reduced with head tissue and vibrissae bristles replacing the missing facets.

The degree of penetrance of tumorous head is temperature-sensitive both during oogenesis and embryogenesis. Experiments indicate that the *tuh-3* gene is active before the 8th hour of embryogenesis.

MUTAGENESIS BY INSERTION OF TRANSPOSON 7

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Transposons are specific DNA sequences, carrying recognisable genes, that transpose between replicons in the absence of normal recombination. We have been using transposon 7 (Tn7, which carries trimethoprim and streptomycin resistance determinants, Barth *et al., J. Bact., 125*, 800, 1976) to mutagenise the wide host-range plasmid RP4. The advantages of this method are (i) each isolated plasmid is mutant by insertion of Tn7 at a single site, (ii) this site can be physically mapped by the use of restriction endonucleases or heteroduplex analysis, (iii) the newly introduced restriction sites on the transposon can be used to excise or clone specific portions of the target molecule. Interpretation difficulties arise from the variable-size deletions frequently produced at the site of Tn7 insertion, and from its probable polar effects on operons. Using this method, we have mapped genes on RP4 responsible for tetracycline and kanamycin resistance, surface exclusion and conjugal transfer. The latter involves at least five genes clustered into two widely separated regions. From the location of regions devoid of Tn7 insertions and from excision experiments, we also have evidence on the location of essential RP4 genes.

THE *TIF*-1 CONDITIONAL MUTATOR OF *E. COLI*: STIMULATION BY NITROFURAZONE AND EFFECTS OF ANTIMUTAGENS

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The *tif*-1 mutation (Kirby *et al., J. Bacteriol., 111*, 447, 1972) is a temperature-sensitive allele at the *rec* A locus with pleiotropic effects. At 41° a whole series of *Rec*- and *Lex*-dependent functions is induced, including filamentation, prophage induction in λ lysogens,

Weigle reactivation of UV-irradiated phage, enhanced UV and gamma radiation mutability (Bridges, *Molec. Gen. Genet.*, *151*, 115, 1977) and a mutator effect (Witkin, *Proc. Nat. Acad. Sci.*, *71*, 1930, 1974; George *et al.*, *Molec. Gen. Genet.*, *140*, 309, 1975).

The present results, in a *tif sfi uvr* A *trp* strain of *E. coli*, show that the mutator effect of *tif* (scored by *trp*$^-$ ochre $\rightarrow$ Trp$^+$ reversions and D-fucose^r (*ara* C$_c$) forward mutations) is greatly stimulated, at 30° or 37°, by nitrofurazone at 1 μg per ml. This stimulatory effect of nitrofurazone is over 10-fold greater than that of adenine, 75 μg per ml. The spontaneous, adenine- and nitrofurazone-enhanced mutator effects of *tif* are greatly reduced by guanosine plus cytidine addition. Other antimutagens, such as caffeine (1000 μg per ml), spermine (250 μg per ml), quinacrine (10 μg per ml), acriflavine (5 μg per ml), and adenosine (200 μg per ml) are without similar effect in this *tif* mutator system. Spot-tests with T_4 ochre mutant C428 show that the adenine- or nitrofurazone-enhancement of *tif* mutator action applies mainly to these phenotypically Trp$^+$ revertants due to ochre suppressor mutations.

DO CHEMICAL CARCINOGENS ACT BY ALTERING EPIGENETIC CONTROLS THROUGH DNA REPAIR RATHER THAN BY MUTATIONS?

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It has been debated whether carcinogenesis has a mutational or epigenetic basis (*e.g.* J. H. Coggin and N. G. Anderson, *Adv. Cancer Res.*, *19*, 106, 1974). Recent work shows a correlation between cancer and mutation production by a wide range of chemicals. (J. McCann and B. N. Ames, *Proc. Nat. Acad. Sci. USA*, *73*, 950, 1976). A proponent of a strict epigenetic mechanism of carcinogenesis has to explain how chemical damage to DNA can alter an epigenetically regulated state of gene expression without requiring mutation.

Holliday and Pugh (*Science*, *187*, 226, 1975) proposed that the methylated state of particular DNA sequences could stably control gene expression. During development, specific bases are methylated on both strands of these sequences by transiently-active switch methylases. Half-methylated sequences arise after DNA replication and, to maintain the methylated state, the newly replicated strand is methylated by constitutive enzymes specific for half-methylated sequences.

If a DNA lesion occurs in or near one of the methylated sequences and is repaired, by excision or recombination mechanisms, just before or after DNA replication, a sequence unmethylated on both strands may be generated; this would no longer be a substrate for a maintenance methylase and would result in a stable change in genetic control. The change is not mutational since the sequence of base-pairing specificities is unaltered. The weak mutagen ethionine, which is a potent liver carcinogen, could act by inhibiting DNA methylation. Further aspects of these ideas will be discussed.

CHARACTERISATION OF A PLASMID OF *ESCHERICHIA COLI* CONTROLLING ADHERENCE TO HUMAN INTESTINAL MUSCOSA

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A human enteropathogenic *Escherichia coli* strain of serotype 026 K60 H11 produces colicin, is resistant to some antibiotics, and adheres specifically to the mucosa of human foetal small intestine *in vitro*. About 30 per cent of the total cellular DNA of this strain can be isolated as covalently closed circular molecules comprising three plasmid species with approximate molecular weights 56×10^6, 7×10^6 and $4{\cdot}5 \times 10^6$. The largest, designated pLG101, is self-transmissible to *E. coli* K12, and recipients carrying solely this plasmid show the phenotypic characters mentioned above. We conclude therefore that the capacity of strain 026 K60 H11 to adhere specifically to human foetal intestinal mucosa is mediated by the colicinogenic multiple drug resistance plasmid pLG101.

PROTOPLAST FUSION LEADING TO HIGH FREQUENCY RECOMBINATION IN STREPTOMYCES

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Protoplasts of *Streptomyces* spp. can be prepared and regenerated with comparative ease (M. Okanishi, K. Suzuki and H. Umezawa, *J. gen. Microbiol, 80*, 389-400, 1974). We have used treatment with polyethylene glycol, under conditions found to be optimal for animal cells (G. Pontecorvo, P. N. Riddle and A. Hales, *Nature, 265*, 275-258, 1977), to fuse protoplasts of genetically marked derivatives of several streptomycetes. The fused protoplasts were regenerated under non-selective conditions and the spores derived from the confluent regenerated cultures were characterised for parental and recombinant genotypes (D. A. Hopwood *et al., Nature, 268*, 171-174, 1977). In intra-specific combinations we find very high frequencies of recombination. For example, in *S. coelicolor* A3(2), recombinants in a six-factor protoplast " cross " represented up to 20 per cent of total progeny. This was the case even in a combination of two strains each lacking both known plasmid sex factors, SCP1 and SCP2; in such a combination, normal matings give recombinants at a maximum frequency of 10^{-7}. Recombinants arising after protoplast fusion include an unusually high proportion of multiple crossover classes; this may reflect the occurrence of " rounds of mating " involving numbers of parental and recombinant genomes during protoplast regeneration. Certain interspecific protoplast fusions have also yielded recombinants. The implications of this work, particularly in relation to industrial strain improvement programmes, will be discussed.

MITOCHONDRIAL INSTABILITY IN A STRAIN OF *SACCHAROMYCES CEREVISIAE*

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A haploid strain of *S. cerevisiae* (A285) gives rise to heterogeneous, sectored colonies on glucose-agar medium. Cells sampled from these colonies segregate on plating into normal and respiratory deficient colonies. The latter show distinct categories of stable respiratory deficiency from the totally incompetent *petite* mutant to partial respiratory competence, measured in the first instance as ability to utilise glycerol as a carbon source.

The respiratory mutants also vary in (1) ability to utilise the sugars galactose, α-methyl-D-glucoside and maltose, petites being unable to use any of these sugars, (2) resistance to the drug chlorimipramine (Anafranil).

FINE STRUCTURE AND BIFUNCTIONALITY OF THE *hisB* LOCUS OF *ASPERGILLUS NIDULANS*

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Mutants which nutritional, complementation, and mapping criteria identify as *hisB* are of two types: those that have lost the ability to produce ascospores on selfing (self-infertile), and those which have retained this wild type property. Reversion to *hisB*$^+$ by second-site (intragenic) mutation, often simultaneously restores to self-infertile mutants their wild type self-fertile status.

Similarly, self-fertile *hisB* mutants may become self-infertile on reversion to histidine independence. The ability to prduce ascospores by different combinations of *hisB* mutants is directly related to the degree of allelic complementation. Non-complementing combinations produce no ascospores; weakly complementing combinations produce few ascospores; and strongly complementing combinations appear fully fertile.

These results indicate that at the *hisB* locus one bifunctional or two contiguous genes are present. Other evidence shows that recombination between *hisB* and flanking markers often leads to changes at the *hisB* site.

THE *P*, *K* AND *91* GENETIC CONTROLS OF GENE CONVERSION IN *ASCOBOLUS IMMERSUS*

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The frequencies and patterns of gene conversion at four closely linked white ascospore mutation sites, *w*-10, *w*-78, 3C1 and NG1, are largely controlled by three genetic factors, *P*, *K* and *91*. These factors are closely linked to the sites they control but generally do not co-convert with them, although the white mutants often co-convert with each other. Two complete genetic cycles of crosses for the control factors have been made: *P*, $+ \times P$, *m*; *K*, $+ \times K$, *m*; $P+ \times K$, *m*; *K*, $+ \times P$, *m*; *91*, $+ \times 91$, *m*; *P*, $+ \times 91$, *m*; *91*, $+ \times P$, *m*; *K*, $+ \times 91$, *m*; *91*, $+ \times K$, *m*. In one cycle the mutant (*m*) was *w*-78, probably a base-substitution mutation, and in the other it was 3C1, probably a frame-shift mutation.

Gene conversion patterns for the two types of mutation were very different but responded in very similar ways to the control factors, which affected both the total observed conversion frequencies and the relative frequencies of different segregation classes. Two outstanding features of the action of these controls of conversion were their incomplete dominance and their *cistrans* position effects.

MODIFICATION OF DOMINANCE OF A CYCLOHEXIMIDE RESISTANCE MUTATION IN *CORPRINUS CINEREUS*

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One of a series of mutants selected for resistance to 1 mg/litre cycloheximide (actidione) was partially dominant in a dikaryon with a sensitive strain. The mutant was shown to carry two mutations: a recessive resistance mutation belonging to the complementation group *cx*^{r}*-2*, and a linked modifier mutation which does not confer resistance but causes dominance when combined with the recessive resistance gene. The modifier also affects other resistance alleles at the *cs-2* locus, but not *cx*^{r}*-3* or *cx*^{r}*-4* mutations.

The properties of diploid strains carrying various combinations of resistance and modifier genes will be described, and the results compared with those reported by Senathi Rajah and Lewis (*Genetical Research*, *25*, 95-107, 1975).

THE REACTIVATION OF TRANSCRIPTION IN *XENOPUS* NUCLEI BY CYTOPLASMIC EXTRACTS

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The genome of the mature erythrocyte of *Xenopus* is very highly repressed. The template activity of nuclei isolated from these cells is similarly very low, even in the presence of excess RNA polymerase. The rate of transcription is greatly enhanced after exposure of the nuclei to certain cytoplasmic extracts.

The livers of immature rats are one source of such extracts and the activity in these preparations is unusually stable. This has permitted its identification with protein(s) and its partial purification.

The transcripts on both treated and control nuclei are predominantly of a rather low molecular weight, but comparison of the newly synthesized RNAs by competitive hybridisation to *Xenopus* DNA shows that certain portions of the genome are expressed in treated

nuclei which are not represented in control nuclear RNA. The cytoplasmic extracts are, therefore, genuinely " reactivating " normally repressed sequences. A proportion of the novel transcripts possess amino-acid accepting activity.

The potential of this system for studies on eukaryotic gene regulation is outlined.

DETECTION OF DEFECTIVE OR UNINDUCIBLE HSV GENOMES LATENT IN HUMAN TRIGEMINAL GANGLION EXPLANTS

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From 20 human cases of death from trauma a total of 39 trigeminal ganglia have been isolated and explants cultured. In 16 explant cultures from 12 individuals latent herpes simplex virus type 1 was spontaneously obtained upon cultivation. The remaining 23 were superinfected with *ts* mutants of HSV-1 strain 17 at the non-permissive temperature of 38·5° or at 31°. In six the *ts* mutant virus was complemented by information residing in the ganglion explant material, while in three wild type virus was regained. Whether this wild type virus was produced by (a) recombination or (b) reversion of the input *ts* mutant, or (c) induction of a latent HSV genome triggered by superinfection is not known, but under test. Two further cultured explants from one individual were superinfected with mutant HSV virus which lacked the structural gene for thymidine kinase and in both of these the induction of HSV specific thymidine kinase activity could be demonstrated.

GENETIC VARIATION OF HERPES SIMPLEX VIRUS ISOLATES FROM HUMAN TRIGEMINAL GANGLIA

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Herpes simplex virus type 1 is known to form latent infections in human trigeminal ganglia. Explantation or co-cultivation of ganglia tissue with animal and human cell lines has yielded 26 virus isolates from 12 individuals. The virus isolates have been typed as HSV type 1 by antibody neutralisation, infected cell polypeptide analysis and DNA restriction endo-nuclease profiles. Isolates from different individuals yielded distinct restriction endonuclease profiles, which indicates that a high degree of genetic variability exists. Isolates from the right and left ganglia of the same individual or independent isolates from the same ganglion exhibit only minor differences in the recognized variable regions of the HSV type 1 genome, and are clearly derived from the same virus strain. The observed genetic variation in the HSV type 1 isolates will be discussed.

TEMPORAL CONTROL IN HERPES SIMPLEX VIRUS TYPE 1 INFECTION STUDIED BY SUPERINFECTION WITH VIRUS MUTANTS

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The availability of several different *ts* mutants of HSV-1 all of which are unable to synthesise DNA or induce deoxypyrimidine kinase (dPyK) at the non-permissive temperature (38°), allows investigation of possible temporal controls which may be involved in the replicative cycle of HSV-1.

Experiments at the permissive temperature (31°) have shown that superinfection can occur at least up to 8 hours post infection. When *ts* virus infected cells are superinfected with ts^+ virus at 38° both rescue of the *ts* genome and recombination can only be detected until approximately 6 hours post primary infection. The fate of the *ts* and ts^+ genomes in these experiments has been monitored by additionally employing different plaque morphology markers. It is found that the ability of a ts^+ dPyK$^-$ superinfecting genome to induce the *ts* D mutant's dPyK activity occurs on the same time scale as the rescue of the *ts* D function. At later times when rescue of the primary infectant can no longer be achieved, DNA synthesis nevertheless takes place indicating replication of the superinfecting virus. Orthogonal experiments indicate that defects in the superinfecting virus can be rectified by the already replicating ts^+ genome on apparently the same time scale.

A MODEL OF TRANSCRIPTION AND ITS USE IN MEASURING RATES OF INITIATION AND PROPAGATION

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A mathematical function has been devised which describes the change with time in the amount of RNA made for a single scripton. The amount of RNA made per scripton (s) at some time (t) is expressed as a function of the rate of initiation (V_i), the rate of propagation (V_p), and the length of the scripton (D). The value of (s) when plotted against (t) is seen to be most sensitive to changes in V_i, this is consistent with the observation that most known mechanisms for the control of transcription operated by changing the initiation rate.

The shape of the curve of (s) versus (t) is similar to that obtained experimentally when T7 DNA is transcribed *in vitro* by *E. coli* RNA polymerase. A computer program has been used to fit curves to the experimental data by a regression technique, and so estimate V_i and V_p. The values thus obtained by analysing the time course of RNA synthesis, agree well with those reported in the literature. The model provides the basis for a simple, rapid and accurate method for estimating V_i and V_p. It will be useful for investigating materials and conditions which alter the rate of transcription. Time course analysis is especially suited to the transcription of DNA molecules with a single scripton.

THE IMPORTANCE OF A DETAILED KNOWLEDGE OF THE NATURE OF DNA DAMAGE BY CHEMICAL MUTAGENS AND CARCINOGENS

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Leaving aside phenomena such as non-disjunction it is axiomatic that induction of mutation by chemicals should involve interactions with DNA. In this Symposium which includes the Mendel Lecture by Professor Charlotte Auerbach, it is appropriate to note that di-(2-chloroethyl) sulphide (mustard gas), the first reported chemical mutagen (Auerbach and Robson, *Nature*, *157*, 302, 1946), was the first compound whose mechanism of action was clearly identified as being a consequence of its ability to cross-link two guanine moieties in cellular DNA. Subsequent studies with a series of electrophilic reagents, some direct acting and others generated within cells by metabolic enzymes, has indicated that not all DNA reactions are equally relevant for the induction of mutations. This is true not only when considering the lesions induced by different chemicals but also for the different sites of reaction within the DNA by the same mutagen. Part of this difference in response is a direct consequence of the lesion modifying DNA replication, but the role of cellular DNA repair mechanisms is particularly relevant. These effects have been clearly illustrated in a study of the mutagenic response to alkylating agents of a series of *E. coli* WP2 strains having different repair capacities.

In the case of the large molecular size mutagens and carcinogens such as the polycyclic hydrocarbons it seemed unlikely that subtle differences in the nature of the DNA reaction might be significant to the biological response. However recent studies on the induction

of 8-azaguanine resistant mutants in mammalian cells has shown this not to be true. In particular it has been demonstrated that the mutagenic efficiency of the metabolically generated diolepoxide ultimate carcinogen of benzo (*a*) pyrene, is significantly greater than that of the closely related K-region epoxide. The relevance of this and similar observations of mutation induction in mammalian cells in culture to the somatic mutation theory of cancer will be discussed. A complete knowledge of the chemistry of mutagen-DNA reaction is therefore a prerequisite for an understanding of the biological response.

DNA INTERACTION AND CHROMOSOMAL ABERRATIONS

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In recent years the use of mammalian cells with an elevated sensitivity to induced chromosome structural changes has added very considerably to our knowledge of the lesions and mechanisms involved in aberration formation. In many cases the hypersensitivity has been related to DNA repair defects, *e.g.*

(1) Xeroderma pigmentosum cells and some Chinese hamster cells defective in excision or post-replication repair (PRR) sustain more chromosome damage and sometimes more sister chromatid exchanges than normal wild-type cells when exposed to UV or certain chemical clastogens. That cyclobutane dimers are a major lesion leading to chromosomal aberrations is demonstrable in photoreactivation experiments with amphibian cells.
(2) Ataxia telangiectasia cells are sensitive to aberration induction by ionising radiation and may have a defect in rejoining of DNA strand breaks. For other X-ray sensitive cells (*e.g.* mouse lymphomas, trisomic cells) no repair defect has been identified.
(3) Fanconi's anaemia cells have a specific sensitivity to DNA inter-strand cross-linking agents and appear unable to " unhook " the cross-links.
(4) Yoshida lymphosarcoma cells of the rat which are sensitive to bifunctional alkylating agents are defective in PRR. Resistant lymphosarcoma cells can be sensitised by inhibition of their PRR with caffeine.

These examples will be used to discuss the current " state of the art " on mechanisms of induced structural damage. Cells which are sensitive to induced numerical changes via non-disjunction are required for understanding the mechanisms involved in this phenomenon of which, at present, we know very little.

INDUCED MUTATION IN BACTERIA—A NEW LOOK AT AN OLD PROBLEM

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The formation of base-pair substitution mutations after irradiation of *Escherichia coli* with ultraviolet light or ionising radiation and after treatment with many chemical mutagens is believed to occur during the operation of an error-prone repair system, probably operating on single strand gaps in DNA. This system requires the activity of a number of genes, most characteristically the *lexA*$^+$ and *rec*$^+$ genes. Studies in which bacteriophages were used as the target for error-prone repair suggested that the system might be inducible in a similar way to prophage induction for which protein synthesis was required and a repressor needed to be inactivated. Induction of prophage λ is inhibited by the *recA*$^-$ allele and delayed and restricted by the *lexA*$^-$ allele. It is known that the product of the *recA*$^+$ gene is a protein which binds to single-stranded DNA and at least one form of which can specifically inactivate λ repressor by proteolytic cleavage.

At present the repressor for error-prone repair remains hypothetical. Indeed the concept of inducible error-prone repair is an oversimplification since recent evidence suggests that error-prone repair may be basically constitutive but with a requirement for an

additional inducible component when it acts on newly-synthesised, as distinct from " old ", DNA.

The demonstration that DNA polymerase III is required for error-prone repair is currently prompting an examination of its role. (For all other types of repair requiring polymerase action, polymerases I and/or II can substitute for polymerase III). " Inducible " error-prone repair may turn out to reflect the suppression of activities associated with polymerase III in the region beyond the replication fork which normally function to prevent the occurrence or fixation of errors during the replication of undamaged DNA.

MUTATION IN EUKARYOTIC MICROBES

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Numerous models of mutation in eukaryotic cells have taken as their basic assumption common mechanistic features between prokaryotes and eukaryotes. In view of the evolutionary divergence between the prokaryotes and the eukaryotes, which is marked by the presence or absence of true nuclei, mitosis, meiosis and certain cell organelles such as mitochondria, such models may be naïve in their assumptions of common mechanisms. The simple eukaryotic microbes such as the yeasts provide us with convenient organisms for the study of the differences and similarities of the mechanisms of mutation at the different levels of cellular organisation.

Studies of DNA repair and mutation in eukaryotic microbes provide convincing evidence of the increased complexity of these processes compared with the prokaryotes. For example, yeast error-prone repair, leading to mutagenic change, involves the action of the products of at least seven genes after UV exposure alone. Similar complexities of repair and mutagenic change have been revealed in studies of spontaneous and chemically induced mutation in eukaryotes.

The position of a mutagen treated eukaryotic culture during the mitotic and meiotic division cycles has an important influence upon the mutational process and provides us with some insight into the interactions between chromosome replication, repair and recombination, for both the nuclear and mitochondrial genome. Experiments will also be described which illustrate the importance of the metabolic state and cytoplasmic constitution of cells upon mutation induction.

At the molecular level the specificity of mutation has been characterised in eukaryotic cells particularly by the study of the structural gene for iso-l-cytochrome c, which illustrates the interactions which take place between mutagens, repair enzymes and particular nucleotide sequences.

A NEW MODE OF CONTROLLING GENE EXPRESSION BY MINI IS-ELEMENTS

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The mini IS-elements IS6 and IS7 have been isolated as constitutive revertants from gal OP-308::IS2 in which the expression of the gal operon is turned off by IS2 in orientation I. Both IS6 and IS7 map within IS2 proximal to the *gal* structural genes, and both turn on the expression of these genes. IS6 is 115 bp long and causes 50 per cent constitutive synthesis of the gal enzymes, while IS7 is only 65 pb long and is 20 per cent constitutive compared to the gal^+ wild type operon. Both mini IS-elements are rather unstable, *i.e.* they are excised with frequencies between 10 per cent and 90 per cent. This efficient loss of the integrated element allowed the isolation of more stable derivatives. In one instance increased stability was due to an internal deletion within IS6 reducing its size from 115 bp to 65 bp, while retaining its turn-on properties. The importance of the findings on the evolution of new genetic systems will be discussed.

THE CONTRIBUTION OF MUTATION AND ADAPTATION TO THE ORIGIN OF DRUG RESISTANT PHENOTYPES IN MAMMALIAN CELLS

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Three classes of purine analogue resistant V79 cells have been characterised with respect to their biological and biochemical phenotypes. Clones isolated after exposure to single high doses show high level resistance, low HGPRT levels and are HST^s. Resistance is stable on prolonged growth (>2 years) in the absence of selective pressure and such lines do not revert spontaneously. Their HGPRT has altered electrophoretic mobility and enhanced heat sensitivity relative to wild type enzyme.

Resistant clones isolated after chromic (3-4 weeks) exposure to sub-toxic analogue concentrations, have similar phenotypes to those of the first class, *i.e.* AZ^r HAT^s. However, they do revert spontaneously. After 6 weeks growth in the absence of selective pressure, HAT^r colonies were detected at a frequency of 1×10^{-4}: on continued growth in MEM the frequency of HAT^r colonies increased exponentially, and by 16 weeks approached 100 per cent. Loss of purine analogue resistance was detectable only after 14 weeks growth in the absence of selection. The rise in HAT^r clones was paralleled by a rise in ^{11}C-hypoxanthine uptake and HGPRT activity.

In contrast, clones isolated after 1-2 weeks exposure to sub-toxic analogue concentrations show lower levels of purine analogue resistance, are fully HAT^r and have near wild-type HGPRT levels. Such lines are even more unstable, and resistance is lost in 6-8 weeks. These, and other data which will be presented, suggest that resistance in these two latter classes may be of adaptive origin.

To further characterise clones of the first class, reversion studies were undertaken. No spontaneous revertants were detected in studies on three induced AZ^r clones and although HAT^r clones were detected on EMS mutagenesis they proved to be amethopterin and AZ resistant. No spontaneous revertants have been detected in a detailed screening of a spontaneous TG^r line. However, on EMS mutagenesis, HAT^r clones are detectable early (6 hours), they fall in frequency 6-48 hours and subsequently rise to approximately plateau levels by 96 hours. In contrast to the observed expression curve for $TG^s \rightarrow TG^r$, for $TG^r \rightarrow TG^s$, there is no obvious relationship between cell division and induced frequency. Biochemical and biological characterisation of HAT^r clones has revealed considerable phenotypic diversity and although many are indistinguishable from Wt, others, particularly those expressing early, show varying levels of TG^r associated with HAT^r again suggesting an adaptive origin for some clones.

MUTATION IN MOUSE SPERMATOGONIAL STEM CELLS

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Mutation studies in the whole animal must contend with a number of factors that play no part in cell mutation studies *in vitro*. Germ cell production in the male progresses through a series of spermatogonial types into meiosis and their cell numbers are maintained by a spermatogonial stem cell population. In the mouse, a heterogeneity in radiation sensitivity within the stem cell population has been considered the basis for the " humped " dose-response curves for genetic damage and this may reflect the wide range of cell cycle times exhibited by the stem cell. Following depletion of the germinal epithelium by radiation or other noxious agents repopulation is rapidly achieved by the surviving stem cells and a shortening of their cell cycles may be largely responsible for the reduced yields of genetic damage observed with most fractionated and some chronic radiation exposures.

GENETIC INSTABILITIES AND TRANSPOSABLE ELEMENTS IN HIGHER ORGANISMS

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The classical work linking genetic instabilities to transposable elements was carried out by Barbara McClintock on *Zea mays*. Such elements are widespread in maize and there is evidence that phenomena probably similar in nature, though less completely analysed, are not infrequent in other flowering plants, for example *Antirrhinum majus*. During the last 5 years also, transposable elements have also been much studied in *Drosophila*, firstly as causes of genetic instability (particularly through the work of M. M. Green), and more recently as determinants of male recombination. All of these transposable elements in higher organisms appear closely analogous in their properties to the IS sequences of bacteria. They may be connected with the inverted-repeat (" foldback ") DNA sequences which are plentiful in the genomes of higher eukaryotes, and which, according to at least one report, may be rather freely transposable. It is tempting to ascribe, as McClintock has done, a role in normal cell differentiation to controlled transpositions. Consistent with such an interpretation is the rather closely controlled timing of transposition events seen in some of the maize examples. However, the near-randomness as regards site of reinsertion is much more difficult to reconcile with a normal role.

THE ROLE OF GENETIC CHANGE IN THE INITIATION AND PROGRESSION OF NEOPLASIA

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Neoplastic growth requires an initial step which alters one or more normal cells into cells with malignant potential. The agents which are capable of effecting this change are, with only one or two exceptions, mutagens. Evidence is accumulating that this initiation step involves an alteration in the genome akin to, if not identical to, mutation. It is an important reservation, however, that evidence exists that in some forms of neoplasia regulatory rather than mutational events are of primary importance. Cells which have undergone this initiation event do not progress inevitably to frank neoplasia. Altered cells may be constrained by normal regulatory mechanisms. If they do progress towards malignancy there is often clear evidence of cell selection and clonal development. A continuing instability of the genome may be necessary to generate the diversity upon which selective forces can operate to produce this cellular evolution, but secondary independent mutational events may also play an important part.

It will be important in the future not only to study the nature of the initiation event, but also to study how changes induced by mutagenic carcinogens relate to the genetic changes actually observed in cancer cells.