GENETICAL SOCIETY OF GREAT BRITAIN

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THE PROBLEMS AND POSSIBLE SOLUTIONS

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The phenomena associated with crossing-over, namely, gene conversion, post-meiotic segregation, polarity, and negative interference, will be briefly described. The reasons why the copy-choice appears untenable will be given. Models based on hybrid DNA will then be discussed. These explain gene conversion as correction of mispairing in DNA. Postmeiotic segregation is attributed to a failure of correction through a lack of excision of a mismatched nucleotide (or possibly to the insertion of the wrong nucleotide, or some other change of nucleotide sequence during the correction process). It appears that the frequency and direction of the repair process in a pair of recombining chromatids may differ. Polarity is explained by postulating that the initial breakage of nucleotide chains occurs at fixed points and that potential mispairing interferes with hybrid DNA formation. The presence of a site of mutation near a primary breakage-point will then reduce the likelihood of hybrid DNA extending to a second site of mutation placed further from the breakage-point. Negative interference is attributed partly to correction of mispairing, but the hybrid DNA models differ in their explanation for parental combinations of genes on either side of a mutant site showing conversion.

MEIOSIS AND MEIOTIC CHROMOSOME REPLICATION IN ASCOMYCETES

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The ascomycete *Neotiella rutilans* (Fr.) Dennis has rather large nuclei. This has made it possible to determine the time of meiotic chromosome replication by means of Feulgen microspectrophotometry. Replication takes place before caryogamy, when the homologous chromsomes are in separate haploid nuclei. Meiosis follows the "*Neurospora* pattern": synapsis takes place immediately after caryogamy between highly contracted (and replicated) homologous chromosomes; during meiotic prophase the paired chromosomes are in an uncoiling phase. Preliminary investigations of the ultrastructure of the chromosomes by means of electron microscopy have shown that a synaptinemal complex is already formed between the contracted and paired chromsomes in the primary ascus nucleus. In interphase, the chromosomes are highly contracted and do not replicate. Partial uncoiling and replication takes place during mitosis. With respect to their nuclear structure and mitotic pattern the fungi resemble the more primitive Protozoa (Euglenoidea and Dinoflagellates) rather than higher organisms with "classical" mitosis, which is characterised by chromosome uncoiling and replication during interphase. The investigations have been done in collaboration with Jane Mink Rossen, M.Sc., and Professor phil. dr. Dieter von Wettstein.

CHIASMA FORMATION AND DNA SYNTHESIS IN " HIGHER " ORGANISMS

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Even if one accepts the association of chiasmata and genetic crossovers there are many problems which require solution before we can say that we understand the processes involved in their formation. Apart from the obvious questions concerning the mechanism—how, exactly, are they brought about at the molecular level—these problems include: (1) Is there always a 1:1 correlation between a genetic crossover and a visible chiasma? (2) What is the physical basis for chromosome pairing? (3) When does chiasma formation take place in relation to the time of DNA synthesis? (4) How does one explain positive and negative interference? (5) How do the controls and interactions which are known to affect chiasma formation or recombination bring about their effects? (6) How far can one generalise from one life form to another?

Although it is premature to say that any one of these points has really been answered in full, recent work, including the study of ^{3}H-labelled chromosomes at meiosis and electron microscopy, is certainly throwing light on some of them and providing a number of provisional answers which may be of guidance in selecting possible hypotheses to explain chiasma formation.

RECOMBINATION IN *CHLAMYDOMONAS*

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Experiments with synchronous populations of meiotic cells have aimed at determining the stage during which recombination, both between and within genes, takes place and the nature of the events involved. The results show that the terminal events for both inter- and intra-genic recombination occur in pachytene. Both kinds of recombination apparently result, however, from a sequence of two steps, the first occurring in preleptotene and the second in pachytene. Each step appears to involve either the synthesis of DNA or the incorporation of new material into DNA. A general requirement for protein synthesis at each step is found but the synthesis of new protein does not seem to be directly involved in the recombination process.

It is suggested that the first step in recombination is the production of structural changes in DNA molecules during the premeiotic synthetic period. These are potential sites for both types of recombination. Such structural changes are then resolved and corrected during pachytene, during which process inter- and/or intragenic recombination may take place at any given site.

POLARIZED GENE CONVERSION

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Polarised intragenic recombination has been explained in terms of " fixed regions of pairing ". On hybrid DNA models of recombination, this is envisaged as the restriction of primary nucleotide breakage to fixed points. Some data show that the direction of polarity within a cistron changes such that conversion is more frequent towards the ends of, and less frequent towards the centre of, a cistron. This is consistent with a hybrid DNA model in which hybrid DNA formation is more frequent at the ends of a cistron. Do such fixed points of pairing, on breakage, coincide with ends of cistrons, possibly with the ends of *each* cistron? Analyses of

recombination involving adjacent genes could perhaps answer this question. An absence of recombinants having flanking markers of the minority non-parental combination would be predicted in the progeny of crosses between two mutants that map in adjacent polarons. A second prediction, on at least one model, is that non-crossover conversion events in one cistron (polaron) provide an opportunity for coincident conversion in an adjacent cistron. These aspects of intragenic recombination will be discussed with reference to recent data.

THE EFFECT OF MUTATIONS TO RADIATION SENSITIVITY ON CROSSING-OVER AND GENE CONVERSION IN USTILAGO

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Some current models of genetic recombination predict that one or more of the enzymic mechanisms responsible for the repair of damaged DNA would also play some part in the process of gene conversion (non-reciprocal recombination) and possibly also in that of crossing-over. This prediction can be tested by examining the effect of radiation sensitive mutations on these processes. Such mutants can be readily isolated in the smut fungus *Ustilago maydis*; three of these, *uvs-1*, *uvs-2* and *uvs-3*, which are recessive, non-allelic and phenotypically distinct, have been used in recombination studies. In Ustilago it is possible to synthesise vegetative diploid strains which are heterozygous and heteroallelic for biochemical markers, and with these to measure the spontaneous or UV-induced frequency of reciprocal mitotic crossing over proximal to the heterozygous loci, as well as that of gene conversion within heteroallelic loci. The frequencies of these events in diploids which are, in addition, heterozygous for *uvs-1*, *-2* or *-3*, have been compared with those in diploids in which the *uvs* mutant is homozygous. Experiments are also in progress to determine the effects of the mutants on crossing-over and conversion at meiosis. Mutants *uvs-1* and *uvs-2* have a strong influence on recombination, particularly in almost abolishing UV-induced mitotic gene conversion, which indicates that a repair mechanism is involved in this process. The possible relationship of the results to models of genetic recombination will be discussed.

INTEGRATION OF TRANSFORMING DNA IN BACTERIA

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A unique feature of transformation is that the fate of transforming DNA can be analysed biologically, and by chemical and physical methods, at various times after fixation by competent cells. Such methods permit an analysis of the kinetics of integration and its resolution into discrete steps. Employment of these techniques in a number of laboratories has provided results bearing on current models for recombination and these will be reviewed.

TWO CLASSES OF RECOMBINATION DEFICIENT MUTANTS OF *E. COLI* K-12

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The recombination deficient (Rec^-) mutant JC1569 of Clark and Margulies (1965) and Rec^- mutants such as AB2463 *rec-13* isolated by ourselves, show an abnormally high spontaneous and radiation-induced release of nucleotides containing thymine from their DNA. Exogenous H^3-thymidine is not incorporated during this extensive post-irradiation degradation of the DNA. These mutants appear to

be partially defective in their ability to repair DNA containing single strand cuts, as the one hit (37 per cent. survival) dose for AB2463 is 3 ergs./mm.^{2} of 2537 A UV light, while that for the double mutant AB2480 *rec*-13 *uvr*A (pyrimidine dimer excision$^-$) is only 0·2 ergs./mm.^{2} The fifteen-fold greater resistance of strain AB2463 as compared to AB2480, suggests that over 90 per cent. of single strand cuts left by enzymatic removal of photoproducts are successfully repaired in AB2463. The repair defect is evidently not complete. Recombination deficient mutants of a second class have been obtained. AB2470 is recombination deficient when mated with Hfr H, permits normal zygotic induction of λ prophage, and is of intermediate radiation sensitivity between the original strain and AB2463. It shows less radiation induced degradation of its DNA than occurs in wild type, and it recovers the ability to incorporate exogenous thymidine after UV.

SOME SIDE EFFECTS OF BACTERIAL MUTATIONS AFFECTING RECOMBINATION

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In different laboratories a number of mutants have recently been isolated from female strains of *E. coli* K12 which do not permit the integration of genes derived from male donors during conjugation. These recombination-negative (*rec*$^-$) mutants are interesting from two points of view. Firstly, a combined genetic and chemical study of their properties opens up the way to the understanding of the enzymatic processes involved in recombination. Secondly, they enable direct tests to be made of the relation between recombination and other biological phenomena.

In this communication it is the second of these aspects of the *rec*$^-$ mutants which will be discussed. In particular the influence of the *rec*$^-$ mutations on the following phenomena will be considered:

1. Radiation survival of the *rec*$^-$ bacteria.
2. Radiation survival of coli phages when assayed on *rec*$^-$ strains.
3. Phage recombination during growth in the *rec*$^-$ strains.
4. Generalised and specialised transduction.
5. Lysogenisation and induction.
6. Mutation.

GENES AND ENZYMES INVOLVED IN THE REPAIR FROM LETHAL UV DAMAGE AND RECOMBINATION IN BACTERIA

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By conjugation between Hfr $\times$ F$^-$ strains of *Escherichia coli* the location on the bacterial chromosome of various genes was determined that control: (*a*) the sensitivity of the strains to ultraviolet irradiation (*uvr*); (*b*) the ability to reactivate UV irradiated bacteriophages (*hcr*); (*c*) the sensitivity of the strains to X irradiation (*exr*) or (*d*) the ability to recombine (*rec*).

The influence of any of these genes on various phenotypic properties was studied.

From extracts of *Micrococcus lysodeikticus* an enzyme preparation was obtained which is able to repair lethal UV damage in bacteriophage DNA *in vitro*. The enzymes involved might be endonucleases with a very restricted action, specific for UV irradiated DNA. The possible role of these enzymes in the recombination process will be discussed.

EVIDENCE OF RECOMBINATION BETWEEN IMMUNOLOGICALLY DISTINCT STRAINS OF FOOT-AND-MOUTH DISEASE VIRUS

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A series of experiments will be described which have provided qualitative evidence of the occurrence of recombination between immunologically distinct strains of Foot-and-Mouth Disease Virus (FMDV)—a small RNA-containing virus.

The two strains of FMDV used in these experiments were distinguishable by their heat resistance, response to a specific inhibitor (guanidine HCl), plaque morphology, maximum growth temperature, pathogenicity and immunological properties. Various methods of selection from mixed infections of cell cultures consistently yielded a small amount of progeny virus, recombinant in terms of unselected markers.

The nature of the experiments and the low frequency of the recombinant virus necessitate cautious interpretation of the results, but it is evident that a process similar to recombination can be detected. Furthermore, analysis of serum neutralisation data for parental and mixed infection progeny virus suggests that recombination may be more frequent than ascertainable by screening for marker exchange.

Some of the problems associated with attempts at formal genetic analysis in this and other animal virus systems will be discussed briefly.

THE INDUCTION OF CROSSING-OVER IN THE ABSENCE OF MUTATION

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Using a chemically defined and axenic treatment medium, it has previously been established that formaldehyde exhibits no mutagenic activity towards *Drosophila melanogaster* larvae unless adenylic acid (or adenosine) is present in the treatment medium.

Data will be presented to show that formaldehyde is capable of inducing crossing-over in the *Drosophila* male under conditions both where it does and does not induce sex-linked recessive lethal mutations.

Since crossing-over may involve chromosome breakage, the observations have been extended to include the induction of translocations by formaldehyde. Formaldehyde is found to induce translocations under conditions both where it does and does not induce sex-linked recessive lethal mutations.

THE INFLUENCE OF THE CYTOPLASM ON RADIATION SENSITIVITY AND INDUCED MUTATION RATE IN *ASPERGILLUS NIDULANS*

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An ultra-violet-induced cytoplasmic variant in a line 29Y of *Aspergillus nidulans* produced two classes of conidia, variant and non-variant. Variant conidia were more resistant to UV than non-variant; there were no differences between the two classes after gamma irradiation.

No mutations to 8-azaguanine resistance were detected following UV or gamma radiation in the cytoplasmic variant although high mutation rates were recorded for the non-variant. 8-azaguanine resistance could, however, be combined with the variant cytoplasm by heterokaryosis. The occurrence of UV-induced 6-methyl-purine resistant mutants in the cytoplasmic variant, albeit at a reduced rate when compared with the non-variant, showed that an undetectable mutation rate was not necessarily a concomitant of this cytoplasmic alteration. The differences in induced 6-methyl-purine resistance rates, like the survival differences, disappeared after gamma irradiation.

Formal proof of the cytoplasmic basis of these differences was shown in a heterokaryon test. When UV sensitivity and induced mutation rate to 8-azaguanine resistance were used as phenotypic characters they were seen to behave in the same way with a new nuclear background (line 29) as they did with the original nucleus (29Y).

FINE STRUCTURE MAPPING OF THE *AM* GENE OF *NEUROSPORA CRASSA*

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Crosses have been made between nine *am* mutants in all possible combinations, and an attempt has been made to arrange the mutant sites in a linear order using (*a*) frequencies of am^+ recombinants and (*b*) the distributions of two flanking markers (*sp* and *inos*) among the recombinants. The map constructed on the basis of the recombination frequencies is uncertain in some parts and shows some gross departures from additivity. As regards the flanking markers, the most frequent genotype among the am^+ recombinants tends to be, not one of the recombinant types, but one or other of the two parental combinations, depending on the orientation of the *am* alleles with respect to the markers in the parent strains. The most usual result can be formally described by saying that, in most cases, am^+ recombinants arise by conversion without associated crossing-over, and that, in a cross between two given mutants, one mutant site gets converted more often than the other. The mutants can be arranged in a linear order such that, in a cross between any two of them, the one to the left is converted preferentially. This order agrees with the tentative map based on recombination frequencies.

The genetic map shows some correlation with the complementation map. Mutants which show apparent absence of the gene product (glutamate dehydrogenase), as opposed to an altered product, appear to be scattered throughout the map.

REGULATION OF ARGININE BIOSYNTHESIS IN *NEUROSPORA CRASSA*

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The regulation of arginine biosynthesis in *E. coli* by repression was demonstrated by Vogel and others in the 1950's. The differential rate of synthesis of several of the enzymes was commonly decreased ten- or twenty-fold by repression and repressions of several hundred fold were observed in some cases. The best data for *Neurospora* (Vogel, 1965) are for ornithine-glutamate transacetylase, for which a differential rate of synthesis under conditions of derepression exceeded the repressed rate by 4·7 times. The work to be described concerns another of the early enzymes in the pathway, N-acetyl-ornithine-δ-transaminase. Derepression has been demonstrated in *arg* mutants grown to a state of arginine exhaustion but, again, the effect is not very large. Repression of the enzyme in wild type and in *arg* mutants has been looked for, using very high exogenous levels of arginine, citrulline and ornithine, with negative results.

METABOLIC CO-OPERATION BY CELL TO CELL TRANSFER BETWEEN GENETICALLY DIFFERENT MAMMALIAN CELLS IN TISSUE CULTURE

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A stable genetic variant of BHK21 cells lacks inosonic pyrophosphorylase activity. In autoradiographic studies with (^{3}H) hypoxanthine or (^{3}H) inosine, incorporation into nucleic acids was found to be less than 0·4 per cent. of that of the normal clone.

When normal and variant cells are grown together in mixed culture (1:300) in the presence of (^{3}H) hypoxanthine the heavily labelled normal cells are easily

detected among the vast majority of nonincorporating variant cells. Where there is contact between variant and normal cells the variants show an intermediate amount of labelling. Even cells in contact with these intermediately labelled variant cells show some labelling.

This metabolic co-operation is apparently obtained only where cells are, or have been, in contact. It is probably mediated by transfer from cell to cell through cytoplasmic connections of one or more of the following: (*a*) nucleotide, (*b*) labelled RNA, (*c*) enzyme, (*d*) messenger RNA for enzyme production, (*e*) enzyme " inducer "

Some general implications of these findings will be discussed.

ENZYMIC DEFICIENCIES IN THREE BIOCHEMICALLY MARKED VARIANTS OF THE MAMMALIAN CELL LINE BHK21

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Three types of stable genetic variant of cell line BHK21 have been obtained. The first is highly resistant to 6-thioguanine, 6-mercaptopurine and their ribosides and fails to incorporate hypoxanthine into nucleic acid. Inosinic pyrophosphorylase activity was not detectable in cell free extracts.

The second is highly resistant to 8-azaadenine, partially resistant to 2, 6-diaminopurine and 6-methylpurine but sensitive to 8-azaadenosine and 7-deazaadenosine. *In vitro* studies suggest a deficiency in adenylic pyrophosphorylase activity.

The third shows considerable resistance to 5-bromodeoxyuridine, 5-bromodeoxycytidine and 5-iododeoxyuridine. The thymidine kinase activity in cell free extracts is less than 10 per cent. of that of normal cell extracts. Autoradiography shows that *in vivo* thymidine incorporation is reduced to 30 per cent. of that of normal cells.

THE GLYOXYLATE CYCLE IN *NEUROSPORA CRASSA*

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The enzymes of the glyoxylate shunt and certain citric acid enzymes were induced when *Neurospora crassa* was transferred from a medium with sucrose as the sole source of carbon to one in which acetate was the sole carbon source. Isocitrate lyase and malate synthetase activities increased approximately 20-fold and acetic thiokinase 10-fold.

Mutants were isolated which could not utilise acetate as sole source of carbon but which could grow on sucrose. These mutants map at 8 different loci.

Enzyme assays of crude extracts prepared from the mutants after transfer to acetate have shown one group to lack isocitrate lyase activity; another group lacks acetic thiokinase activity. In other mutants none of the glyoxylate shunt enzymes are significantly induced by acetate. A fourth group of mutants in which isocitrate lyase is uninduced or only partially induced by acetate while the other enzymes of the cycle are induced like wild type.

The levels of the glyoxylate cycle in these and certain other mutants in *Neurospora crassa* will be discussed with a view towards understanding the control of the cycle.

THE INTERPRETATION OF COMPLEMENTATION DATA

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Comparison of numbers of mutants and numbers of complementation groups comprising complementation maps in microorganisms has shown that non-linear

complementation maps are constructed using larger samples of mutants, and have larger numbers of complementation groups and units, than linear complementation maps. This is taken to indicate that many loci would have non-linear complementation maps if the number of mutants comprising the map were increased. This was confirmed by constructing sample complementation maps using mutants with known complementing properties selected by means of random numbers, from amongst those already described at the *leu-2* locus in *Neurospora* (*Proc. Nat. Acad. Sci. U.S.*, *48*, 922, 1962). Theories of complementation must be able to account for complex (non-linear) complementation maps as these are the largest and presumably the most complete, and many more maps would probably be of this kind if sufficient mutants were used in constructing the maps. A general interpretation of the *leu-2* complementation map will be given in terms of the theory of Crick and Orgel (*J. Mol. Biol.*, *8*, 161, 1964). In this interpretation the " circular " complementation map is considered to represent a " circular " interface between two protein monomers.

APPENDIX (see pp. 183-190)

EFFECTIVE POPULATION SIZE WITH OVERLAPPING GENERATIONS

Consider an allele at a locus and denote its frequency by p in a population in which generations are overlapped. Following Kimura and Crow (1963), the variance effective size is defined as

$$N_e = \frac{p(1-p)}{2V_{\delta p}}$$

where $V_{\delta p}$ is the variance of the change in gene frequency in a period of time equal to one generation. Let $N_m dy$ be the number of individuals who are born during a time interval dy and able to reach the mean reproductive age or, more accurately, participate in the reproduction. The variance of the change in gene frequency during dy is then given approximately by

$$p(1-p)2N_m dy/4(\tau N_m)^2$$

assuming, as Kimura and Crow (1963) did, that gene frequency changes are slow enough that p is nearly the same for the period of one generation. In the above expression τ represents the generation time. Note that the denominator $4(\tau N_m)^2$ is approximate since individuals of all age groups do not mate at random. In practice, marriages are contracted more frequently between individuals whose ages are close with each other than those whose ages are far apart. However, if generations are completely overlapped for a sufficiently long time, the genes possessed by young and old individuals in a certain generation are finally mixed up. Therefore, the above approximation appears to lead to no serious error, though the variance of the change in gene frequency is slightly overestimated.

At any rate, the above expression should be equated to $V_{\delta p} dy/\tau$. Thus,

$$V_{\delta p} = \frac{p(1-p)}{2\tau N_m}$$

Hence,

$$N_e = \tau N_m.$$

In the derivation of an equivalent formula, Kimura and Crow (1963) used the total population number in place of τN_m. Since, however, $V_{\delta p}$ has been defined as the variance of the change in gene frequency during one generation time, the total number cannot be used. This is especially so when the effective size referring to a long time change in gene frequency is considered.