GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND THIRTY-NINTH MEETING of the Society held on 24th, 25th and 26th JULY 1962, at the DEPARTMENT OF GENETICS, TRINITY COLLEGE, DUBLIN

THE IMPLICATIONS OF THE INTRODUCTION OF PLANT BREEDERS' RIGHTS

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The proposed scheme of plant breeders' rights, based on a system of financial rewards, has as its objective the encouragement of the private breeder of agricultural and horticultural plants, but it is considered that agriculture and horticulture will benefit directly. Government has decided to give effect to most of the recommendations contained in the Report on Plant Breeders' Rights, and plans are being made for appropriate legislation.

Any such legislation will necessarily result in the introduction of a special organisation through which separate schemes for individual crops will be handled. It is probably inevitable that there will be a general review of plant breeding research and activities, and it will be necessary to consider the position of state-aided research, while international collaboration and reciprocity are relevant to the problems involved.

It is essential that proper consideration be given to the maintenance and expansion of long-term fundamental research on plant breeding which is concerned with the more recalcitrant problems in the improvement of agricultural and horticultural crops. If the scheme is to provide a better service for agriculture and horticulture, as is assumed it will, it will be necessary to match expansion with the highest standards of scientific research and investigation, and this presumably will have to be financed, as far as private breeding is concerned, by the rewards of breeders' royalties.

PLANT BREEDERS' ROYALTIES: THE COMMERCIAL BREEDERS' POINT OF VIEW

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In recent years, varieties produced by overseas commercial breeders have been very much more successful and important in Great Britain than have those of commercial breeders at home. This arises, one may suppose, from the fact that schemes of protection for breeders in various European countries have encouraged large-scale activity in breeding, which has been generally impossible in Britain. The introduction of a royalty scheme, if the rewards are adequate, should in the course of time correct this balance and also stimulate an export trade in improved varieties. The machinery required to administer the scheme need not be elaborate or costly and the increase in cost of seed to the farmer would be repaid by only a very tiny increase in the quantity or quality of his produce. Only successful breeders would reap a reward and foreign as well as British commercial breeders and also official plant breeding institutes would be eligible to earn royalties. Therefore it is visualised that competition would be intense and this would lead to a more rapid improvement in the varieties available to the farmer or horticulturist.

ON PLANT BREEDERS' RIGHTS: LEGISLATION AND ARRANGEMENTS IN VARIOUS COUNTRIES

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In 1954 the European Productivity Agency of the former Organisation for European Economic Co-operation arranged a conference on seed production, and seed trade. During the conference the subject of plant breeders' rights was discussed and this led to recommendations for the protection of the plant breeders' products. Such a protection was at that time already established in certain countries, while other countries did not offer any form of protection of the plant breeders' rights. Furthermore, the mode of protection varied, and still does vary, greatly from one country to the other, and this leads to certain difficulties in trying to get an establishment of reciprocity between the countries concerned.

During the years since this conference, considerable activity in this field has been developed in various countries. This has led to legislation in some countries, for example in Sweden in 1961, while in Denmark it is just being proposed to the parliament, and the recommendation for such legislation in Great Britain was made in 1960. With the aim of creating a convention for the protection of novelties of plants, the French Ministry of Foreign Affairs in 1957 assembled an international conference which finished its work in December, 1961.

A text was signed by five countries : France, Belgium, The Netherlands, Western Germany and Italy. The member states of the Union formed assume the responsibility of protecting novelties in a certain number of botanical species. The duration of protection is being regulated, etc. This recent development will be of the greatest importance for the development of plant breeding. It will lead to uniform principles for the protection of novelties of plants and it will without doubt strongly influence also the present national legislation and regulations on seed distribution. The importance of this is still further emphasised through the recent development of a Common Market.

ACRIDINE MUTAGENESIS AND THE GENETIC CODE

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A study of some r_{II} mutants of bacteriophage T4 showed that mutants induced by acridine dyes often revert to wild type by means of suppressors. These suppressors when segregated are themselves r_{II} mutations. By this means a sign (+ or −) can be attached to acridine mutations to indicate whether a pair of them suppress each other or not.

Double mutant bacteriophages containing two mutations of the *same* sign preserve the r_{II} character. However, triple mutants of the same sign can have the wild phenotype. It is believed that acridines add or delete bases to the DNA of the phage. It is therefore suggested that in producing a protein the bases on the DNA are read from one end in groups of three. A + mutation puts the reading out of phase, and a − one (or two more + ones) restores it.

Among other experiments to test this theory, use has been made of a deletion mutant covering parts of both the r_{II} genes (the A and B cistrons). It was found that the introduction of a + or − mutation into the A cistron removed the function of the B cistron although a +− pair in the A cistron preserved the B function. Thus the deletion appears to join the cistrons when they are read.

RIBONUCLEIC ACID AND RIBOSOMES

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There is evidence for the existence of two forms of high-polymer RNA (*i.e.* molecular weight 250,000 upwards). One is relatively stable and is the major component of ribosomal particles. The other, labile form—messenger RNA—has been detected in bacterial and bacteriophage systems. Two questions arise. (1) Is the RNA of the ribosomes genetically active or genetically inert? and (2) What is the relationship of the two high-polymer forms of RNA to DNA?

TRANSFORMATION OF *BACILLUS SUBTILIS* IN RESPECT OF MOTILITY AND AUXOTROPHY

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Bacteria of a tryptophan-exacting (*ind*$^-$), non-motile (non-flagellated, *fla*$^-$) *Bacillus subtilis* mutant were exposed to DNA from an *fla*$^+$, *ind*$^+$*his*$^-$ (histidine-exacting, linked to *ind*) strain, then to DNase, then incubated in medium with or without tryptophan; the number of *ind*$^+$ transformant clones did not increase in the first 4 hours. In medium without tryptophan bacteria of *ind*$^+$ phenotype were recognised on microscopy by their elongation into filaments, and in medium with tryptophan bacteria of *fla*$^+$ phenotype by their motility. Neither type was seen until 3-4 hours after DNA treatment. Delay in multiplication and expression probably both result from an inherent metabolic inactivity of bacteria which have taken up DNA; exposure of DNA-treated cells to penicillin, which kills only growing bacteria, did not reduce the number of *ind*$^+$ transformants, though it killed 90 per cent. of the whole recipient population (E. Nester and B. A. D. Stocker, unpublished observations).

Bacteria of *ind*$^+$, *fla*$^+$ or *ind*$^+$*fla*$^+$ phenotype were isolated by micromanipulation. Most but not all of them produced transformed progeny. Many clones were mixed, comprising usually a single recombinant type together with the recipient parental type. A few clones yielded two recombinant types (*ind*$^+$*his*$^-$ and *ind*$^+$*his*$^+$) in unequal proportions. Unilinear transmission of motility was observed; but no "abortive transformation".

CHROMOSOME MANIPULATION AND THE WHEAT CROP

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The hexaploid wheat of commerce, *Triticum æstivum* (2n = 42), is peculiarly tolerant of aneuploidy. Nullisomics, monosomics, trisomics and tetrasomics are available for each chromosome of the haploid complement. By the use of aneuploids in appropriate breeding procedures intact chromosomes can be transferred between varieties.

Moreover single chromosomes, or pairs of chromosomes, from related species can be added to the full complement of wheat chromosomes or can be substituted for wheat pairs. This is one way of introducing genetic variation from species with chromosomes that do not recombine with wheat chromosomes in normal hybrids.

In addition, this meiotic obstacle to the introduction of alien variation can be overcome by the removal of wheat chromosome V, the activity of which is responsible for the diploid-like meiotic behaviour of the polyploid. This activity is also responsible for the failure, in hybrids and synthetic allopolyploids, of meiotic pairing between wheat chromosomes and those of other species in related genera.

Attention will be given to the types of chromosome manipulation possible in wheat and to some of the results of these procedures.

BREEDING MARROW-STEM KALE (*BRASSICA OLERACEA* VAR *ACEPHALA*)

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Application of the double-cross hybrid method to kale should give vigorous and more uniform varieties. Self-incompatibility in kale is sporophytically controlled and for the production of 100 per cent. hybrid seed each of the four inbred lines must be homozygous for a different *S* allele. Methods for the recognition of inbreds, homozygous for *S* alleles have been developed but may be laborious. A close linkage between seedling pigmentation factors and the *S* alleles facilitates the recognition of plants, homozygous for *S* alleles.

Objectives at the Plant Breeding Institute include the production of a short type, suitable for strip-grazing with electric fencing and also the breeding of high-yielding, medium height and tall varieties. Special emphasis has been placed on selection for greater edibility, winter hardiness and recently for early vigour and lodging resistance.

BACTERIAL GENETICS

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Some current problems in the genetics of bacteria will be discussed.

THE CONTROL OF MUTATION IN *SALMONELLA TYPHIMURIUM* BY EPISOMES

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Unstable reversions at the *su-leuA* locus in strain *leu-151* of *Salmonella typhimurium* have been studied. Experiments will be described which demonstrate that the instability is best interpreted in terms of the movement to and from the locus of units similar to controlling elements, called controlling episomes, which induce mutation at any *su-leuA* site to which they are attached.

PATTERNS OF REVERSION AT THE *TRYA* LOCUS IN *SALMONELLA TYPHIMURIUM*

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Ability to synthesise enthranilic acid is regained by mutants of the *tryA* locus in *Salmonella typhimurium* both by suppressor mutations and by back mutations at the *tryA* locus. Suppressed strains of one mutant which are phenotypically similar to wild type have been shown jointly to suppress the mutant and the functionally related *tryB-4*.

Of particular interest were the back mutations which mapped at the *tryA* locus itself. Over 50 such reversions have been extensively analysed for intralocus suppression ; these have included fast, semi-fast and slow growing reversions but in no instance was the site of the " back mutation " separable by recombination from the original mutant site.

From the slow growing back mutants which map at the *tryA* locus two further types of reversions are obtainable, which have either intermediate or extremely low growth rates. The former have been shown to be due either to suppression at unlinked loci, or to intralocus suppression within the *tryA* locus. The extremely slow strains are highly unstable, reverting to the original slow growing types. The cause of the instability is located in the *try-cys* region of the linkage map and may well represent episomic interference.

THE GENETIC STRUCTURE OF THE RECOMBINANT CHROMOSOME FORMED AFTER CONJUGATION IN *ESCHERICHIA COLI*

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Evidence from a variety of sources indicates that the bacterial chromosome consists of DNA which is made up of two genetically identical sub-units. When the chromosome replicates the sub-units separate and the daughter chromosomes formed each contain one parental and one newly synthesised unit. If the interaction between chromosomes which gives rise to a recombinant structure takes place via the same type of semi-conservative duplication process (" copy-choice ") then the recombinant chromosome should be heterozygous, containing one parental and one recombinant sub-unit. Determination of the number of generations elapsing between the occurrence of the recombination event and the segregation of bacteria giving rise to pure recombinant clones indicates that the chromosome formed in the recombination process is not heterozygous. This result is in agreement with similar observations of Dr J. Tomizawa.

THE CONTROL OF MULTIPLICATION OF *F-LAC* IN MALE CELLS OF *ESCHERICHIA COLI*

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The sex factor (*F*) of *E. coli* exists in the autonomous state in F^+ donors whilst in *Hfr* donors it is integrated in the chromosome. An F^- strain carries no sex factor. *F* factors can incorporate a marker from the bacterial chromosome, *e.g. F-lac*. An F^- strain infected by *F-lac* acquires the specific donor properties associated with this factor.

Lac$^-$ *Hfr* and F^+ strains were infected with an *F-lac*$^+$ factor and the resulting colonies analysed. Autonomous multiplication of the *F-lac* was found to be suppressed in *Hfr* cells. Cells of F^+ origin permit the *F-lac* to multiply but unlike the F^- they produce sectored colonies. An *F-lac* factor infecting cells already harbouring autonomous *F* is therefore unable to keep pace with cell division during colony formation.

These results indicate the existence of a control mechanism regulating the multiplication of autonomous *F* factors and of a mechanism present in *Hfr* cells by which multiplication is inhibited completely.

Studies on the nature of these control mechanisms will be presented.

THE EFFECT OF FERTILITY FACTORS ON THE TRANSFER OF COLICIN FACTORS IN *ESCHERICHIA COLI* K12

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The genetic factor responsible for the production of colicin I (*col* I^+) differs from most other colicin factors in that, when present in strains of *E. coli* K12, it is able to initiate its own transfer and also the transfer of chromosomal markers, in the absence of the fertility factor, *F*.

The presence of the *F* factor in either the *col* I^+ donor or the *col* I^- recipient, has a profound influence on the kinetics of colicin factor transfer. In the absence of *F*, slow, linear transfer of *col* I^+ commences immediately at the time of mixing and reaches about 50 per cent. after 18 hours. The introduction of *F* factors into both the *col* I^+ and the *col* I^- strains has little effect either on the rate of *col* I^+ transfer or

on the final level of transfer. However, when the *col I*$^+$ strain is F^- and the *col I* strain is F^+, transfer of *col I*$^+$ is considerably more rapid and reaches 90 per cent. after 18 hours. In the reverse situation, using F^+ *col I*$^+$ and F^- *col I*$^-$ strains, there is initially a reduced rate of transfer followed by a more rapid increase approaching the control F^- *col* $I^+ \times F^-$*col* I^- level of 50-60 per cent. after 18 hours.

The bearing of these results on the mechanism by which autonomous genetic elements are transferred will be discussed.

ARE COLICIN FACTORS EPISOMES?

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The primary criterion of an episome is that it should be able to exist either as a chromosomal element or alternatively as an autonomous non-chromosomal (cytoplasmic) element. The control of temperate phage and fertility (*F*) factors particularly, in the bacterial strain *Escherichia coli* K12, are well established as being due to episomal elements. In contrast, although many experiments involving genetic factors responsible for the maintenance of several colicins support the concept of a non-chromosomal location, the sole evidence of chromosomal location is restricted to one series of experiments involving a single colicin factor, *col* $E_1{}^+$. These experiments are of two types. Firstly the ability of a series of non-colicinogenic *Hfr* donor strains to cause lethality to varying extents to the same recipient colicinogenic (*col* $E_1{}^+$) F^- strain, have been interpreted as being due to the transfer of the same chromosomal (*col* $E_1{}^+$) site to the recipient. Similar experiments will be described in which analogous lethal effects can be produced in the absence of any *col*$^+$ factor. Secondly, the ability of these same *Hfr* donor strains, now carrying the colicin factor, *col* $E_1{}^+$, to transfer this factor to varying extents to the same recipient non-colicinogenic F^- strain has been correlated with the transfer of a specific chromosomal (*col* $E_1{}^+$) site. Similar experiments using different *Hfr* strains do not support this conclusion. Possible alternative interpretations will be discussed.

SELECTION FOR LINKED LOCI IN *BACILLUS SUBTILIS* BY MEANS OF TRANSFORMATION

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In transformation, as in transduction, only a small fraction of the genome is transferred and incorporated into the chromosome of the recipient. The probability that two markers chosen at random will be linked is therefore very small, and attempts to build linkage maps in this way are unprofitable. The method outlined below, however, searches directly for loci linked to any given locus.

DNA from a prototroph is treated with nitrous acid until only 10-20 per cent. of the transforming activity remains. It is then used to transform an auxotrophic mutant to which linked markers are required. The DNA is used at a concentration low enough to ensure that not more than one molecule is incorporated per recipient cell, and the treated cells are plated on a medium containing all available growth factors except that required by the recipient strain. Colonies appearing on these plates are replicated on to minimal medium to detect auxotrophs. These auxotrophs are derived from the nitrous acid treatment. Since they are searched for only among transformants for a given locus, and since only dilute DNA was used, the only mutations which should be isolated are those occurring on the same molecule as the wild type allele of the locus we have transformed. Any mutations induced on other molecules should be automatically screened out.

Five pairs of linked markers have already been obtained by this method. They are being used to study the organisation of the DNA in the genome and for other genetical work requiring markers whose linkage relationships are known.

HETEROZYGOSIS AND RECOMBINATION IN PHAGE

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The progeny of a phage cross contains a fraction of phage particles which are heterozygous for a part of their chromosome. The phage heterozygote is a transient structure which segregates on replication producing genetically pure (homozygous) phages. Phage heterozygotes are interesting for two reasons. Firstly, because of the haploid nature of the phage chromosome it would be of interest to know if the heterozygous region is a region of diploidy or whether it represents a non-complementary region in a normally haploid chromosome. The second problem concerns the role of the heterozygote in the phage recombination process; it is still unknown whether the heterozygote is an intermediate or just a by-product of the mechanism of recombinant formation.

By the use of physical techniques and more refined genetical analysis the progeny of crosses involving two closely-linked markers in the *h* (host-range) region of phage T2 are being examined in an attempt to answer these questions.

EFFECT OF GRAFT AND SEXUAL HYBRIDISATION ON THE NODULATION OF *TRIFOLIUM AMBIGUUM*

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Trifolium ambiguum M.B. is a potentially important clover species but one limiting factor which has deterred its use as a forage plant is that it nodulates sparsely, or not at all, and the nodules formed are usually ineffective. Parker and Allen (1952) showed that 35 strains of *Rhizobium trifolii* were ineffective on *T. ambiguum* and it was not until Erdman and Means (1956) isolated strains of rhizobia from nodules on *T. spadiceum*, *T. ochroleucon* and from soils in Turkey where *T. ambiguum* was growing, that effective nodulation of this species was achieved.

In the course of some investigations on the use of graft compatibility as an indication of genotype and species compatibility in *Trifolium* it was observed that in some successful grafts of *T. hybridum* on *T. ambiguum*, effective nodules developed on the roots of the stock. In addition the sexual hybrid between these two species nodulated in the absence of *Rhizobium* specific for *T. ambiguum*.

The present investigation deals with the pattern of nodulation of the graft hybrid and the sexual hybrid, and also of *T. ambiguum* itself when inoculated with four different isolates—from the graft hybrid, the sexual hybrid, *T. hybridum* and from effectively nodulating *T. ambiguum*.

SOME EFFECTS OF ANEUPLOIDY IN WHEAT POPULATIONS

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Aneuploids are found in the progenies of euploid *Triticum æstivum* plants as a result of occasional pairing failure at meiosis. The fresh occurrence of aneuploids in each generation, together with a proportion of the progenies of the aneuploids from previous generations combine to give a stabilised level of aneuploidy after only a few generations. In addition, chromosome misdivision products can arise from the univalent chromosomes and become fixed in the progenies of monosomics.

This type of chromosome behaviour limits the standards of genetic purity that can be achieved in the crop. Furthermore pairing failure is apparently related to the level of heterozygosity, and the production of monosomics in the early generations of hybridisation programmes can result in the transmission of non-recombinant chromosomes or can limit the amount of recombination in chromosomes which become monosomic.

SELECTION FOR PLEIOCOTYLY AND CORRELATED RESPONSES IN INBRED LINES OF CULTIVATED TOMATOES

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Most of the so-called varieties of tomatoes cultivated in Britain are inbred lines, probably gene eroded. Using the polygenic character " pleiocotyly ", selection has been exercised on different tomato populations for several generations. Mono-, tri- and where possible tetracotyledon seedlings have been used as breeding parents. Accompanying the selection for cotyledon number have been various morphological and physiological changes in some plants of some of the progenies; several have shown little resemblance to a cultivated tomato. These experiments with inbred plants support the polygenic selections of Mather and Wigan within highly inbred *Drosophila melanogaster*, which they attributed to selection of invisible polygenic mutations that accumulate until recombinations give rise to more extreme variants. It is maintained that the abnormalities are not a pleiotropic effect of the pleiocotyly. Illustrations of the abnormal plants will be shown.

INDUCED REVERSIONS OF *ADENINE*-1 MUTANTS IN *SCHIZOSACCHAROMYCES POMBE*

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Ten of Dr U. Leupold's mutants, located at the *adenine*-1 locus of the haploid fission yeast *Schizosaccharomyces pombe* have been tested for ability to revert to prototrophy. The ten mutants were originally isolated following ultra-violet treatment of the wild-type strain. Recombination data show them to be located at ten different sites within the *adenine*-1 locus. Nitrous acid and U.V. were the mutagens used to induce reversions.

The results indicate (1) that the induced revertants scored are due to true reverse mutation or to very closely linked suppressors, (2) that these ten U.V.-induced mutants will revert, in general, with HNO_2 but not with U.V., (3) that the presence of methionine in the plating medium will suppress the appearance of revertant colonies of both spontaneous and induced origin.

GENETIC CONTROL OF PHENOTYPE

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The simple one-gene-one-enzyme view of the structure of an organism is inadequate to deal with enzyme sequences. Analysis of such sequences reveals that the kinetic structure of the organism may play a decisive role in deciding whether a given locus can influence the phenotype. On this view the organism is divided into genetically effective units, rheons. The arginine pathway in *Neurospora* is analysed in these forms.

THE NATURE OF THE METAGON IN *PARAMECIUM AURELIA*

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A brief account is given of the action of various substances on the " metagons " —gene-determined cytoplasmic particles in *Paramecium*, controlling maintenance of *mu* particles. The principle substances used were ribonuclease and 8-azaguanine. In addition, attempts have been made to extract " metagons " from Paramecia and to " re-infect " them into other cells.

THE POSSIBLE GENETIC SIGNIFICANCE OF CYTOPLASMIC DEOXYRIBONUCLEIC ACID

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That the substance of the gene is deoxyribonucleic acid (DNA) rests on four main lines of evidence. One of these, namely the exclusively nuclear localisation of DNA in normal cells, has been questioned by Chayen; moreover, indications that this substance is synthesised in the cytoplasm come from Chèvremont *et al.*, and from Plaut. Evidence in support of the cytoplasmic localisation and synthesis of DNA in *Allium cepa* is presented and the significance of these results discussed.

It is suggested that much of the evidence concerning the genic nature of DNA can be re-interpreted on the basis that in meristematic and other cells which are at a critical stage of development the DNA acts as the " messenger " of the gene.

CHROMOSOMES OF THE ANTHROPOIDEA

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Studies on the somatic chromosomes of the anthropoid apes is reported and discussed with particular relevance to the mechanisms involved in karyotype evolution within the anthropoidea.

THE METABOLISM OF THIOPYRIMIDINES IN TASTERS AND NON-TASTERS OF P.T.C.

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The drugs methyl-thiouracil and thiopentone are commonly used in clinical medicine. They both contain the chemical linkage $S = C{<}^{N=}$ which is the part of the phenyl-thio-carbamide (P.T.C.) molecule which detects the " taster " status of an individual. The same phenotypic classification has been found (by previous workers) to result from allowing subjects to taste solutions of these drugs instead of P.T.C.

The metabolism of these two drugs has been investigated in human subjects and compared with their P.T.C. taste titre.

The findings will be discussed.

ISONIAZID METABOLISM BY HUMAN LIVER *IN VITRO*

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Human subjects are polymorphic in their metabolism of isoniazid. Rapid inactivation is a dominant mendelian character to slow inactivation of the drug.

Studies on the urinary excretion products following drug administration have been performed by previous workers. Rapid inactivators produce a much higher percentage of the isoniazid in the acetylated form, than do slow inactivators. There is also evidence from previous work that sulphonamides and p-amino-salicylates which are also acetylated, do not detect the isoniazid polymorphism.

Small pieces of liver have been obtained from human subjects undergoing abdominal operations. Liver homogenates have been incubated in an acetylating

system with sulphanilamide and isoniazid. The amounts of unconjugated drug have been estimated before and after incubation. Following recovery from the operation the isoniazid inactivator phenotypes of the subjects have been determined.

The findings which are discussed add to the evidence pointing towards the enzymic mechanism underlying this polymorphism.

TRACING ANCESTRY BY DNA ANALYSIS

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The estimation of DNA content in genomes of known or probable ancestors of cultivated plants provides a method for investigating the evolution, by hybridisation and polyploidy, of the cultivated forms.

Results are presented for wheat and oats.