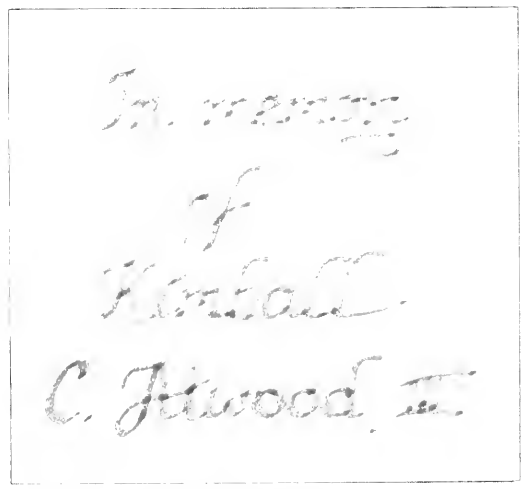
GENETICS TODAY

Proceedings of the XI International Congress of Genetics
The Hague, The Netherlands, September, 1963

Volume I

MBL/WHOI
LIBRARY



GENETICS TODAY

Volume 1

MBL/WHOI
0 0301 00163181 8

PERGAMON PRESS LTD.

Headington Hill Hall, Oxford
4 & 5 Fitzroy Square, London, W.1

PERGAMON PRESS INC.

122 East 55th Street, New York 22, N.Y.

GAUTHIER-VILLARS ED.

55 Quai des Grands-Augustins, Paris 6

PERGAMON PRESS G.m.b.H.

Kaiserstrasse 75, Frankfurt am Main

Distributed in the Western Hemisphere by

THE MACMILLAN COMPANY - NEW YORK

pursuant to a special arrangement with

PERGAMON PRESS LTD.

Copyright © 1963

PERGAMON PRESS LTD.

Library of Congress Card No. 63-19264

Printep in the Netherlands by A.W. Sythoff – Leyden

PREFACE

This volume contains the abstracts of contributed papers, demonstrations and films presented at the XIth International Congress of Genetics. The editorial policy has been to assemble the abstracts into sections not corresponding completely with the titles of the symposia (see vol. II and III). Within each section we have endeavoured to arrange the abstracts in a practical manner which may, however, vary from section to section. Those abstracts delayed in some way are printed at the end of their respective sections. The abstracts are numbered within each section; D refers to demonstrations and F to films. In the alphabetical index of authors at the end of this volume references are given to these numbers. Full addresses of the authors may be found by consulting the membership list supplied at the Congress and printed at the back of volume III.

S. J. GEERTS

CONTENTS

SECTION I

COMPLEX LOCI

1.1. Genetic Fine Structure of the rosy Cistron in Drosophila melanogaster. A. CHOVNICK, A. SCHALET, and R. P. KERNAGHAN (Storrs, U.S.A.).

A highly efficient crossover selector system[1] has been used to examine the fine structure of a single cistron in *Drosophila*. This system permits routine large-scale sampling approaching that used in microbial studies, and permits distinction between rare crossovers and spontaneous mutations. Such studies, involving a large number of mutants of the rosy cistron, permit the following general conclusions: (a) The mechanism of recombination in higher organisms, like that of microorganisms, does permit crossing over within a cistron. (b) The resolving power of such analysis of genetic fine structure in *Drosophila* approaches that of microbial systems.

---

1. CHOVNICK *et al.*, *Amer. Nat.* **96**, 281, 1962.

1.2. On the Phenotypic Manifestation of Mutations in the rosy Region of Drosophila melanogaster. A. SCHALET, R. P. KERNAGHAN, and A. CHOVNICK (Storrs, U.S.A.).

According to the suggestion of Welshons (1962) discontinuous mapping of complex loci in *Drosophila* may be a function of the type of mutant (recessive visibles) usually used. His evidence shows that *Drosophila* results may be reconciled with continuous mapping of mutant sites in microorganisms when methods are used to detect mutable sites for recessive lethals, recessive visibles with atypical phenotypes and possibly wild-type iso-alleles. While Welshons' proposal may apply to many of the complex loci in *Drosophila*, evidence from analysis of the rosy region reveals that within a single functional unit defined on the basis of a visible and enzymatic phenotype, recessive visibles give continuous recombination mapping of mutable sites upon appropriate sampling. The following considerations suggest that the range of phenotypes exhibited by mutations in the rosy region does not include complementary recessive visibles and lethals or non-complementary lethals: 1. Lack of complementation among all mutants at separable sites and failure to detect mutants complementing rosy[2] among 24 X-ray-induced rosy mutants selected over a deficiency for the entire rosy region. 2. Absence of rosy mutants which are lethal and unassociated with genetically detectable alterations involving closely neighboring loci among a sample of 13 rosy mutants with lethal effects localized to the vicinity of the rosy region. 3. Absence of complementary recessive mutations within the rosy cistron among a sample of 11 lethals and visibles selected within a small (0.5 map units) chromosomal segment which included the rosy region.

1.3. Notch Pseudoalleles in Drosophila melanogaster. W. J. WELSHONS, E. S. VON HALLE, and B. J. SCANDLYN (Oak Ridge, U.S.A.).

The Notch locus is a complex one composed of pseudoallelic recessive visibles superimposed upon an array of pseudoallelic recessive lethal Notch (N) mutants. The linear order from left to right on the X chromosome of all pseudoalleles is *fa* - fa^{no} - N^{40} - N^{Nic} - *spl* - N^{103} - N^{j24} - N^{Co} - N^{gll}-*nd*. The order of N^{gll} and *nd* is tentative but both are to the right of N^{Co}. The genetic length of the region has been estimated to be 0.11 units.

It was noted previously that no *N*'s had been discovered in the region to the left of fa^{no}, but it was presumed that they would eventually be found there. Recent experiments performed with N^{ell}, *spl*, and *fa* yielded results which placed N^{ell} 0.03 units to the left of *fa*, and increased the genetic length of this complex locus to 0.14 units.

Similar experiments utilizing N^{39}, *fa* and *spl* localized N^{39} around fa^{no}. Since N^{39} was known to be cytologically deficient for salivary band 3C7, 85,600 chromosomes were tested from N^{39}/fa^{no} heterozygotes, but no recombinants were found. It is probable that deficiency N^{39} includes the locus of fa^{no}.

Obviously the region left of fa^{no} is capable of producing recessive lethals as indicated by the localization of N^{ell}. Furthermore, the N^{39} experiments indicate that the single salivary band C37 cannot contain all the pseudoallelic loci comprising the functional Notch locus since mutants like N^{ell} and N^{Co} are localized at appreciable distances to the left and right of the deficiency.

as found by Green at the white locus, in which event the resulting duplication in the present case might be spectacle and the complementary deficiency a lethal.

This investigation was supported by U.S. Public Health Service Research Grant GM-8889-02 from the Division of General Medical Science.

1.4. An Apparent Non-equivalence in Crossover Rates between Lozenge Alleles in Trans and Cis Arrangement in Drosophila. LUOLIN S. BROWNING and EDGAR ALTENBURG (Houston, U.S.A.).

The rate of recovered crossovers between lz^{BS} and lz^{46} in *Drosophila* was found to be significantly greater from females heterozygous for these two lozenge alleles in *trans* arrangement as compared with *cis* as follows. Among 350,000 F_1 from the *trans* arrangement (lz^{BS} + / + lz^{46}), 116 crossovers (46 lz^{BS} lz^{46} and 70 + +) were recovered, or about 1 crossover in 3000 offspring; among 436,500 F_1 from the *cis* arrangement (lz^{BS} lz^{46} / + +) 75 crossovers were recovered (75 being the combined number of lz^{BS} + and + lz^{46}, both of lozenge phenotype as contrasted to the spectacle phenotype of the double mutant non-crossover class on the one hand and the complementary normal on the other), or about 1 crossover in 5820 offspring. It is not considered very likely that this difference in rates could be due entirely to the relative viabilities of the crossover classes as compared with the non-crossovers in the *trans* and *cis* arrangements. Work earlier reported indicated that the crossover rate between apricot (w^a) and white (w) was also greater for the *trans* than the *cis* arrangement. In explanation of these results, it is suggested that alleles might somehow differ structurally. One series of lz-locus experiments (involving a question as to the identity of the alleles) gave an unexpected result in that the two lz alleles in *trans* arrangement gave the apparent double mutant class (10 phenotypic spectacles in 70,000 offspring) but none of the complementary normal phenotype. Judd reported a result which we interpret as similar to this, insofar as in the F_1 from apricot and buff in *trans* arrangement, the only "exceptions" found were of white phenotype (no normals). We have no explanation for this kind of result except perhaps that it is due to some type of non-homologous pairing such

1.5. A Study of Recessive Lethals on the Dot-like Fourth Chromosome in Drosophila melanogaster. BENJAMIN HOCHMAN (Knoxville, U.S.A.).

A three-year study of 1352 fourth chromosomes, extracted from natural populations of *D. melanogaster*, has uncovered 15 which are lethal in the homozygous state. Allelism tests of 14 of the lethals (one was lost) demonstrate that they occupy eleven different loci. One locus is represented three times; a second locus twice; and nine have a single representative each. One of these nine is allelic to a lethal which has arisen spontaneously in two different laboratory stocks.

Crosses of the lethals to the six existing chromosome 4 dominant visibles have thus far localized one of them to the bt^D locus and a second to that section of the microchromosome delimited by the Minute-4 deficiency. None of the lethals permits pseudodominance of any of the seven non-allelic recessive visibles tested.

Several X-ray-induced lethal fourth chromosomes, generously provided by Drs. M. M. Green and H. Gloor, have been examined for interactions with the lethals of spontaneous origin. Three heterozygous combinations which are lethal have been observed to date.

If all of the spontaneous microchromosomal lethals are "point" mutations, the number of loci identified on chromosome 4 has been approximately doubled over the figure based heretofore solely on the known visible mutations. The total number of "potentially-lethal" loci to be expected on the microchromosome, as well as the developmental period during which each lethal acts, will be discussed.

This research was supported by Grant RG 9845, United States Public Health Service, National Institutes of Health.

1.6. A "Mutable" Gene in Drosophila melanogaster. WILLIAM M. HEXTER (Amherst, U.S.A.).

Attached-X females properly marked and heterozygous for a specific allele of garnet (g^{53d}) and any other garnet allele (g^{x}) were singly mated by appropriately marked males, and the female progeny were screened for the presence of wild type (non-garnet-eyed) females. In a total of 282,000 females, 15 were wild type. In a similar test differing only in the homozygosity of g^{53d}, 0 wild type females were recovered in 539,000 females. Of the 15 wild type females, 6 were non-diagnostic as to generating mechanism; of the remaining 9, 6 could be interpreted as due to single crossover between pseudoallelic loci. The remaining 3 are not so easily explained. If the pseudoallelic hypothesis were correct it should be possible to demonstrate a double mutant. Four of the presumptive 6 females were tested in a manner to reveal one of the two garnet alleles should the double mutant exist. The sum of these 4 experiments was 428,000 flies with 0 single garnet mutants recovered. An additional and independent test of a presumptive double mutant was to place the supposed double mutant chromosome (g^{53d} g^{x}) in apposition to an appropriately marked g^{53d} free-X chromosome. Due to homozygosity of g^{53d} such a test would not be expected to yield any wild type progeny. In fact, however, 22 wild types were recovered in 685,000 flies; of these 22, 11 were associated with recombination and could be simply interpreted as a crossover between pseudoallelic loci. The remaining 11 were non-recombinants for outside markers and are not as easily explained. The conclusion based on these results is that some, if not all, of the wild types were due to mutability of g^{53d} when heterozygous with other garnet alleles. Further genetic tests have not suggested, nor ruled out, the possibility that g^{53d} is a duplication.

Detailed data are in press in *Proc. Nat. Acad. Sci. Wash.*

Supported by a grant from The National Institutes of Health (CA-03114).

1.7. Evidence from Rod-ring Experiments for the Duplicational Origin of f^{3N} Reversions in Drosophila. EDGAR ALTENBURG and LUOLIN S. BROWNING (Houston, U.S.A.).

It was previously suggested that the reversion of f^{3N} (an allele located in the left subsegment of the forked locus) might be due to duplication of the right forked sub-locus at a pre-meiotic division, a suggestion which would account not only for the relatively high reversion rate of f^{3N} (to f^{3N+}), but also for the restriction of the reversions in large measure to diploid cells (oogonia and spermatogonia) in irradiated material. Since a ring X would get lost much more often than a rod as the result of a duplication in a dividing chromosome, the recovered reversion rate of f^{3N} would expectedly be higher for a rod than a ring X, provided the reversions were due to duplications (whether X-ray induced or spontaneous). In accordance with the above suggestion, it has been found that the spontaneous reversion rate of f^{3N} is much higher in a rod X than in a ring as follows. Among 380,000 females heterozygous for a rod and a ring (but homozygous for f^{3N}) there were 12 reversions recovered in the rod and one in the ring. Insofar as these results would indicate that the forked locus arose as the result of a duplication, they would support the Lewis theory of pseudoallelism in *Drosophila*.

This investigation was supported by U.S. Public Health Service Research Grant GM 08889-02 from the Division of General Medical Science.

1.8. The Genetic Fine Structure of the Mutants z^{m} and z^{l} in Drosophila melanogaster. B. H. JUDD (Austin, U.S.A.).

The zeste locus in *Drosophila melanogaster* is located at 1.0 on the X chromosome. The mutant z^{m} (zeste-mottled) was found by Green as a single male (*sc* z^{m}) from the cross *sc z ec ct*/ w^{bf} × *sc z ec ct*. Later z^{l} (zeste-light) was found by Becker as a single male in the *sc* z^{m} stock.

Analysis of z^{m} leads to the hypothesis that it does not represent a change at the zeste locus, but is the result of an asymmetrical exchange at the white locus. The white locus is very closely linked to zeste (1.5 on the X chromosome) and is functionally related. It is postulated that z^{l} arose as a similar asymmetrical exchange from z^{m}.

The change in the white locus can be localized within the two rightmost recombination sites of the locus. The phenotypic expression of this change leads to the conclusion that a portion of the white locus has been duplicated. Recombination experiments give support to this interpretation.

The duplicated nature of the two mutants

leads to further asymmetrical pairing and exchange within the locus when these chromosomes are used for crossover studies. A variety of unusual recombination products presumably resulting from asymmetrical exchanges have been analyzed.

1.9. Biochemical Division of the White Locus in Drosophila melanogaster. HUGH S. FORREST (Austin, U.S.A.).

Despite the fact that the *white* locus in *Drosophila melanogaster* has been known for some time to be divided into subloci separable by crossing over and other genetic tests, there is remarkably little knowledge of its fundamental biochemical role. It is believed by some workers, for example, that mutation at this locus results in an "incomplete" or "non-functional" pigment-synthesizing granule, resulting in loss of synthetic ability for both the red and brown pigments.

In a new attempt to investigate the biochemical function of the locus, it has been shown that all of the alleles at the locus so far tested can be divided into two groups—those that accumulate the compound xanthurenic acid, albeit in smaller amounts than wild type, and those that do not contain this compound at all. It is reasonable to assume that xanthurenic acid can be used as an index of the production of 3-hydroxykynurenine, an intermediate in the biosynthesis of the brown pigments. Thus the *white* locus may be separable into two functional areas, one of which, in the mutant condition, is unable to produce 3-hydroxykynurenine (measured as xanthurenic acid) and the other of which can produce limited amounts of this material. A theory relating this finding with the failure or partial failure of mutants of the *white* locus to synthesize the red eye pigments will be presented.

1.10. Complementation Groups at a Complex Locus in the House Mouse. DOROTHEA BENNETT and L. C. DUNN (New York, U.S.A.).

The study of three newly arisen alleles has provided the opportunity to compare the effects of lethals of independent origin, and especially to compare members of the same complementation group with respect to secondary effects. Complementation groups are defined as consisting of alleles from different sources which are lethal in combination. Alleles in the same complementation group are in general indistinguishable; they produce similar syndromes of abnormalities at similar times in embryogeny, and have similar effects on male transmission ratio and recombination. Combinations of members of different complementation groups are of course viable to some extent, but the proportion of viable compounds found varies greatly depending on the alleles concerned. The three new alleles reported here were found to belong to three different previously established complementation group. Their characteristics regarding embryonic effects, transmission ratio, and recombination followed the general pattern of being in each case indistinguishable from other members of their group. However, one of the new alleles was found to be clearly distinguishable from other members of its group on the basis of degree of complementation with another lethal allele. This suggests that alleles in the same complementation group may not have identical structures, and that quantitative measurements of the degree of complementation amongst different alleles may be used as a basis for assessing their similarity or dissimilarity.

1.11. Genetical Analysis of the R Chromonemal Region of Mormoniella. P. W. WHITING and DORIS J. BUSH (Philadelphia, U.S.A.).

Two eye-color factors, *O* and *S*, mutating with relatively high frequency to oyster-white and to scarlet respectively, make the "*R*" region of *Mormoniella* especially favorable for genetical analysis. These colors serve as markers for many other *R*-locus changes including semilethals and lethals, steriles and near-steriles. Complementation tests identify factor homologies. For factors such as lethals and male-steriles which cannot be transmitted through haploid males, diploid males heterozygous for these deleterious factors are used. Two different female-steriles have been found, masked in lethal-bearing genes. Two different lethals, complementary to each other and, therefore, non-homologous, have proved non-complementary, "homologous", to a third lethal. It is suggested that the last impairs some process essential to the normal functioning which is impaired by each of the other two lethals. Formula of each *R*-locus gene is given in terms of its factor states. By use of the gene formulae it is possible to predict the phenotypes and breeding behavior of the different compounds. It is postulated that the complexity of the *R* region is not greater than that of other chromonemal regions. Its complexity has been explored and revealed by

use of oyster and scarlet mutations, acting as markers of each pleiotropic gene of the series. Data in full are at present being published in *Genetics*.

1.12. Partial Complementation of Six Multiple Alleles in a Chlorophyll Mutation Locus in Barley. GERHARD HOLM (Lund, Sweden).

A locus in chromosome 1 contains at least 6 recessive vital viridis alleles which, when intercrossed, give F_1's exhibiting different degrees of partial complementation judging from the visually examined leaf colours. A spectrophotometrical analysis of 8 of the F_1's verified this interpretation and also showed that the degree of complementation in an F_1 apparently depends upon the relative position in the locus of the two alleles involved in the cross. The 6 alleles probably belong to the same cistron and overlap each other in a linear order. The less two alleles overlap, the higher the complementation in their F_1.

The pigment content in 5 of the alleles is the same, 1.23 mg/g fresh weight, but in the 6th (no. 4 in the linear order) it is significantly higher, 1.44 mg/g fr.w. Normal plants contain 2.00 mg/g fr.w.

The 6 multiple alleles appeared in a material of 82 vital viridis mutations of which the resulting 76 most probably belong to single loci. 865 diallelic crosses were made in the group and the result indicates a very low frequency of multiple allele loci among the vital viridis mutations.

1.13. Fine Structure of Genes Conditioning Resistance to Erysiphe graminis hordei at the Ml_a Locus in Hordeum vulgare. JOHN G. MOSEMAN (Beltsville, U.S.A.).

Several genes conditioning the resistant reaction of barley *Hordeum vulgare* L. to infection with the ascomycetous fungus *Erysiphe graminis* DC. f. sp. *hordei* Em. Marchal have been found at the Ml_a locus on chromosome 5. Genes Ml_a, Ml_a2, and Ml_a3 at this locus were studied. When genes Ml_a and Ml_a3 were homozygous they conferred a similar degree of resistance, but when heterozygous with a recessive gene conditioning susceptibility they conferred a different degree of resistance. Gene Ml_a2 conditioned a lesser degree of resistance than genes Ml_a and Ml_a3. The degree of resistance conferred when the genes were Ml_aMl_a2, Ml_aMl_a3, or Ml_a2Ml_a3 also was determined. For each gene conditioning the reaction of the host, a corresponding gene has been found conditioning the pathogenicity of the pathogen. Pathogenically different cultures differentiated the genes at the Ml_a locus. The relation of the pathogen genes corresponding to host genes was determined by the pathogenicity of haploid progeny cultures derived from crosses between selected cultures. Pathogen gene A_a corresponding to host gene Ml_a was inherited independently of pathogen genes A_a2 and A_a3 corresponding to host genes Ml_a2 and Ml_a3, respectively. Pathogen genes A_a2 and A_a3 were found to be at or near the same locus. The utilization of degree of resistance conferred, relation of corresponding pathogen genes, and other host-pathogen relations in studying fine structure of genes conditioning resistance to *E. graminis* f. sp. *hordei* at the Ml_a locus in *H. vulgare* were discussed.

1.14. Complementation, Recombination, and Biochemical Relationships within the Td locus in Neurospora crassa. ANN MATTHEWS LACY (Baltimore, U.S.A.).

The present study of non-indole-utilizing (NIU) tryptophan synthetase deficient mutants indicates that the complementation and recombination maps of the *Td* locus are not co-linear. The clustering on the recombinational map bears some relationship to the biochemical characteristics of the mutants; however, the biochemical relationships between mutants grouped together by complementation appears somewhat obscure.

The twenty NIU complementing mutants tested are distributed among three of the four complementing groups previously established for NIU mutants (the fourth group is still represented by *Td*7 only).

The ability to form cross-reacting material (CRM) is not a prerequisite for complementation. Four CRM^- mutants are in the *Td*71 complementation group. These mutants, however, are not located near *Td*71 on the recombination map, but are located in the vicinity of *Td*3, 7, and 24 (i.e. "profound region").

The ability to catalyze the formation of indole from indole-glycerolphosphate is not limited to members of the *Td*71 complementation group, but can be detected at varying levels in crude extracts of at least 90% of the CRM^+ NIU mutants tested (including *Td*3, 7, and 24). CRM^- mutants tested exhibit no detectable activity in this reaction.

Of three temperature sensitive mutants studied (mutant phenotype at 25°C, not at 37°C),

two are in different complementation groups and exhibit different levels of ability to form indole; the third neither complements nor forms indole.

The relationships between the NIU mutants will be described in detail and their possible significance in terms of gene structure and function will be discussed.

Supported by grants from the National Science Foundation.

1.15. Further Analysis of Interallelic Complementation at the leu-2 Locus of Neurospora crassa. S. R. GROSS (Durham, U.S.A.).

A previous analysis of the complementation behavior of a large number of *leu*-2 mutants of *Neurospora crassa* yielded a complementation map that was linear, overlapping and continuous.(1) Evidence was presented that indicated that the polypeptide whose structure was determined by the *leu*-2 gene was at least one of two different polypeptide structural units of an enzyme β-carboxy-β-hydroxyisocaproic acid isomerase, an enzyme which catalyzes the isomerization of β-carboxy-β-hydroxyisocaproate and α-hydroxy-β-carboxyisocaproate, intermediates in leucine biosynthesis. An analysis of the complementation interaction between *leu*-2 and *leu*-3 alleles as well as enzymological analyses indicated that both the *leu*-2 and *leu*-3 loci were involved in the determination of the structure of β-carboxy-β-hydroxyisocaproic acid isomerase. The evidence obtained was interpreted as indicating that complementation between *leu*-2 mutants resulted from a protein-protein interaction in the formation of a polymer consisting of α chains coded by the *leu*-2 gene and β chains coded by the *leu*-3 gene, and that at least in the case of enzymes synthesized as a result of complementation, the isomerase consisted of at least two α and two β polypeptide chains. A recent study of the physical chemistry of the isomerase obtained from several complementing pairs of *leu*-2 mutants has revealed that the molecular weight of the normal enzyme as well as that of the heteroallelic enzymes are the same, *ca.* 80,000. The enzymes obtained by complementation are remarkably similar to the normal enzyme with respect to the functional area of the protein. Several physical properties of the heteroallelic enzymes differ markedly from that of the isomerase enzyme. These differences, however, are probably confined to areas not directly involved in substrate binding.

1. GROSS, *Proc. Nat. Acad. Sci.* **48,** 922, 1962.

1.16. Homology Tests on X-ray-induced Recessive Lethal Mutations in the ad-3 Region of Neurospora crassa. F. J. DE SERRES (Oak Ridge, U.S.A.).

Forward-mutation experiments on a balanced heterokaryon(1) have shown that 76 per cent of the *ad-3* mutations are recessive lethal (*ad-3*^{RL}) on adenine-supplemented medium and that 40 per cent of these were *ad-3A ad-3B*^{RL} double mutants. Atwood and Mukai(2) have shown that such recessive lethal mutations can be analyzed by homology tests. Such tests on the *ad-3*^{RL} mutations show that they form an inclusive complex; all combinations show homology except some of the *ad-3A*^{RL} + *ad-3B*^{RL} combinations. The apparent homology of certain *ad-3A*^{RL} + *ad-3B*^{RL} combinations is of particular interest since the trikaryon involving two such mutations in combination with a viable double mutant (from a cross of two viable mutants *ad-3A*^{V} × *ad-3B*^{V} = *ad-3A*^{V} *ad-3B*^{V}) is capable of growth on minimal medium. The behavior of these trikaryons shows that there is an intact essential region (designated region X) present in the *ad-3A*^{V} *ad-3B*^{V} double mutant that is nonfunctional in any of the *ad-3A*^{RL} or *ad-3B*^{RL} mutations showing homology. The simplest assumption is that region X is located between the *ad-3A* and *ad-3B* loci, and present data are consistent with this hypothesis. The interaction patterns of such *ad-3*^{RL} mutations in homology tests offer a unique opportunity for the analysis of the genetic composition of the *ad-3* region, and the results of these tests will be described in terms of a complementation map.

1. DE SERRES and OSTERBIND, *Genetics* **47,** 793-796, 1962.
2. *PNAS* **39,** 1027-35, 1953.

1.17. Complementation and Genetic Mapping of pan-2 Mutants Induced in a Reversion of a Primary pan-2 Mutant. MARY CASE (New Haven, U.S.A.).

Twenty-nine secondary *pan-2* (pantothenic acid-requiring) mutants have been obtained following X-irradiation of a reversion induced by X-rays in a complementing primary *pan-2* mutant *B5*. Thirteen of the 29 mutants complement with at least one primary mutant, and all 13 can be positioned on a linear complementation map of the locus. The behavior of the secondary mutants establishes the existence of an additional complementation unit at the *pan-2* locus. All

mutants were crossed to *B5* to determine whether the mutants are identical to *B5*. In addition, crosses were made to five other *pan-2* alleles located at various points on the genetic map of the locus. Two of the mutants map at the same site as *B5*; however, one of these mutants is a non-complementing type while the other exhibits a different complementation pattern from *B5*. Fourteen of the mutants are located near *B5* in the proximal region of the genetic map. Ten are located near the distal end of the map, while the three others are located at different sites near the middle of the map. The relationship between complementation and genetic maps of primary and secondary mutants will be discussed further.

Supported in part under a contract, AT (30-1)-872, with the U.S. Atomic Energy Commission.

1.18. Allelic Recombination and Complementation in Adenine-requiring Mutants of Schizosaccharomyces pombe. C. RAMIREZ, J. FRIIS and U. LEUPOLD (Bern, Switzerland).

Allelic recombination and complementation have been studied in several groups of mutants blocked in adenine biosynthesis, including two groups accumulating a red pigment (ad_6 and ad_7) and one group blocked in an early reaction preceding the pigment blocks (ad_1).

In the ad_7 group, 152 u.v.-induced allelic mutants have been demonstrated to represent mutations at 33 different but closely linked sites, recombination frequencies ranging from 1 to 77⁰ prototrophic recombinants per 10^6 ascospores. The mutant distribution is characterized by "hot spots", one particular site having contributed as many as 26 per cent of the mutants. Complementation has not been observed in any of the pairwise mutant combinations tested.

In the ad_1 group, however, complementation leading to a more or less prototrophic phenotype has been found in many of the diploid combinations tested. The complementation pattern of 138 mutants of u.v.-induced, diethylsulfate-induced and spontaneous origin may be represented by a linear complementation map in which eight types of complementation behaviour define five different "complementation units". The complementation and recombination maps show a considerable degree of co-linearity.

A more complex complementation pattern has been found in allelic combinations of 158 u.v.-induced mutants of the constitution ad_6. The results of complementation tests involving 21 different types of complementation behaviour may be represented in a complementation map in which both ends are closed to circles. With a few notable exceptions, the complementation and recombination maps show again a significant degree of co-linearity.

1.19. Studies on the Genetic Fine Structure of the ad_7 and ad_6 loci of Schizosaccharomyces pombe. H. GUTZ (Berlin, Germany).

Leupold has done a very extensive intragenic recombination analysis in the ad_7 and ad_6 loci of *Schiz. pombe*. The ad_6 locus shows the phenomenon of allelic complementation. In addition to the intragenic recombination analysis Leupold has constructed a complementation map of this locus. All mutants analysed by Leupold have been induced by ultraviolet irradiation. The ad_7 and ad_6 mutants are not randomly distributed in the maps. There are a few "hot spots", and the mutation sites appear to be concentrated in several parts of the map.

Taking the maps of Leupold as a basis, I have analysed the intragenic distribution and the complementation pattern of mutants which were induced by nitrous acid and X-rays. In both loci (ad_7 and ad_6) the intragenic distribution of the nitrous acid-induced mutants is quite different from that of the u.v.-mutants. They show some pronounced "hot spots". Above half of the sites induced by nitrous acid are not present on the u.v.-map. From the non-random distribution of the mutants it is concluded that a direct deamination of the DNA-bases is not to be considered as mutation mechanism. All X-ray mutants map on single sites, and no intragenic deletions have been found.

According to the results of Leupold, the complementation map of the ad_6 locus is non-linear. Seventy-nine per cent of the nitrous acid-induced mutants show complementation. Among these mutants at least 6 complementation types were found which are not present in the u.v.-mutants.

SECTION 2

RECOMBINATION

2.1. Factors Affecting Distributive Pairing between Nonhomologues in Drosophila melanogaster. RHODA F. GRELL (Oak Ridge, U.S.A.).

Meiotic associations that lead to the regular separation of nonhomologous chromosomes occur with high frequencies in *Drosophila melanogaster* females of particular genotypes. The relation of the size of the nonhomologues to the regularity of their association has been investigated by measuring the nondisjunction frequencies between a free fourth chromosome and a series of X-duplications in the progeny of females of the genotype $y^2/y^2/\mathrm{Dp}(1;\mathrm{f}),y^+;\ \mathrm{T}(3;4)$ 86D, *red*/T(3;4)86D, *red*; ci^D mated to $_{\mathrm{XY}}$, *yB*/Y males. The duplications were obtained and characterized by Cooper and Krivshenko and vary in size from $\sim$ 0.3. to 3.0 times the metaphase length of chromosome four. The percent of nondisjunction between chromosome four and the duplication, given in ascending order of duplication size, are, / $\sim$ 0.3 = 4.49 $\pm$ 0.19, / 0.5 = 0.39 $\pm$ 0.07, / 0.7 = 0.12 $\pm$ 0.06, 0.9 = 0.08 $\pm$ 0.03, 1.0 = 0.13 $\pm$ 0.05, 1.1 = 0.02 $\pm$ 0.02, 1.1 = 0.08 $\pm$ 0.03/ 1.4 = 0.38 $\pm$ 0.08, 1.6 = 0.39 $\pm$ 0.08, 2.0 = 0.42 $\pm$ 0.07, / 2.5 = 2.03 $\pm$ 0.39, 3.0 = 1.89 $\pm$ 0.24 /. The lowest frequencies of nondisjunction ($\sim$ 0.08 per cent) occur with those duplications closest in size to chromosome four (0.7 - 1.1) whereas the highest frequencies occur with the smallest ($\sim$ 0.3) and largest (2.5 and 3.0) duplications.

The results indicate size alone is not responsible for pairing efficiency since the progressive change in duplication size is not associated with correlated changes in nondisjunction frequency. Instead, the duplications fall into five discrete classes (designated by slashes in the sequence above) within which size variation causes no apparent related change in segregation behavior. This suggests the possibility of sites that participate in nonhomologous associations as has been postulated by Gershenson and Cooper for homologous associations.

2.2. Nonrandom Assortment of Compound Chromosomes with Nonhomologous chromosomes in Oocytes of Drosophila melanogaster. E. H. GRELL (Oak Ridge, U.S.A.).

The requirements for nonrandom assortment of nonhomologous chromosomes was studied by R. F. Grell (1962). For noncompound chromosomes it was determined that a chromosome must not have been a crossover. (The term compound chromosome is intended to include chromosomes formed by the joining of two homologous chromosome arms to one centromere.) If two nonhomologous chromosomes are noncrossovers they may pair and pass to opposite poles of the first meiotic division spindle. The pairing in which nonhomogolous elements may participate has been termed *distributive pairing* to distinguish it from *exchange pairing* which occurs prior to crossing over and only involves homologous chromosomal regions.

The studies reported here deal with distributive pairing of nonhomologous chromosomes when at least one member of the pair is a compound chromosome. A familiar compound chromosome is the attached X. The two arms are attached in reverse order on either side of a centromere. Crossing over occurs between the two arms with almost the same frequency as for normal free X chromosomes. Yet, from females of the genotype $_{\mathrm{XX}}$/O; SMl, *Cy*/T(2;3)A, 90 per cent of the recoverable gametes have the attached X or SMl, *Cy* and only 10 per cent have both or neither chromosome. Despite the fact that more than 95 per cent of the tetrads contain an exchange, the attached X shows a very highly nonrandom assortment with a second chromosome. Experiments that compare compound X's with an exchange and those without an exchange indicate that in contrast to free X's, exchange has no influence on the extent of nonhomologous pairing. All compound chromosomes tested (various compound X's and the attached 4's) have shown nonrandom assortment with nonhomologous chromosomes.

2.3. On the Nature of the Event that leads to Recombination of Pseudoalleles of the m-dy locus in Drosophila. ALLAN B. BURDICK, ROBERT A. SHLESER, EVELYN BARBOUR BENDBOW, and ROSANNE ABBADESSA (Lafayette, U.S.A.).

The recombination map of the *m-dy* locus

shows an unambiguous sequence of five separable mutant elements extending over about 0.07 map units. The sequence is *v* . . . m^{61e}, m^{59a}, *m*, *dy*, dy^{60k} . . . *g* with several other elements being inseparable from one or more members of the sequence. A number of insertional-type crossover products (double and triple crossovers with respect to the outside markers) have been obtained from free-X recombination tests. These have involved nine different elements of the locus, and their explanation poses a problem as to the nature of the event that leads to recombination of the elements of the locus. Although the *cis-trans* test of the most distal elements (m^{61e} and dy^{60k}) indicates that they occupy separate functional sites, complementation tests among the various elements indicate that they could all belong to the same cistron. The m^{61e} dy^{60k} *cis*-phase double mutant has been tested in *trans*-phase with all of the internal elements of the locus and has never yielded a wild-type recombinant—that is, no double crossing over takes place within the locus—although m^{61e} dy^{60k} has broken down several times to yield dy^{60k} types. Attached-X recombinations of m^{61e} and *m* yielded 31 events of which 14 were reciprocal. One of the m^{61e} *m cis*-phase double mutants has been *trans*-phase tested to m^{61e}, m^{59a}, and *m* and has not given any wild-type recombinants. Our present opinion is that recombinations within this locus are of the same classical reciprocal kind that have been postulated for interlocus recombinations, and that the insertional-type crossovers we have obtained can be explained on a classical basis.

Supported by a grant from the National Science Foundation.

2.4. Genetic Ultrafine Structure of the T4rII Region. IRWIN TESSMAN (Lafayette, U.S.A.).

Recombination frequencies as low as 10^{-6} per cent have been measured in the *r*II region of phage T4. At this sensitivity, hot spots in the *r*II region have been resolved into two or more genetic sites, separable by recombination. No lower limit to recombination frequencies is apparent. Therefore, lack of recombination cannot guarantee identity of genetic sites.

The largest recombination frequencies in the *r*II region are greater than 1 per cent and the lowest less than 10^{-6} per cent. The ratio, 10^6, is greater than the number of nucleotides in the entire phage. Therefore, in this range there must be extreme deviations from even rough additivity of recombination frequencies. Within the range of very low recombination frequencies, marked deviations from additivity are, in fact found.

2.5. Effect of Negative Interference on Genetic Map Concepts and Its Consequences for Intra-allelic Recombination Studies. W. D. HANSON (Raleigh, U.S.A.).

Experimental studies involving intra-allelic recombination have yielded results which are not entirely compatible with classical recombination concepts. Negative interference noted in intra-allelic recombination studies suggested that given a genetic recombination at a point one could expect additional points of recombination or a "cluster" of points of recombinations (one or more) within some interval. The products of meiotic division were described in a probability space so that the probability of chromosome types could be formulated. The products of meiosis for linked loci were formulated for classical recombination studies and for intra-allelic recombination studies assuming that cluster recombinations could occur in some interval. The results involved the probability of an "odd cluster", length of cluster interval, and genetic map distance between linked loci. Cluster recombinations within a relatively short interval should not upset classical recombination measures. The frequency of chromosome types (with respect to marker genes) with selection for intra-allelic recombinations reflected a bias identified with cluster effects. Relative reverse mutation rates could also be a factor; however, for crosses involving a locus bounded by two markers a contradiction existed for linear order of alleles vs. relative mutation rates. A positive bias in map distance involving a non-selected marker was associated with cluster recombinations which was a maximum for a closely linked marker and decreased with increased map distance. Published data supported cluster recombination concepts, as compared to mutation, with a positive bias in recombination being a consistent feature in these data.

2.6. Polarized Intragenic Recombination in Neurospora crassa. NOREEN E. MURRAY (Stanford, U.S.A.).

Methionine-independent progeny from crosses between many pairs of combinations involving

nineteen alleles at the *me-2* locus were classified with respect to the markers present on both sides of the *me-2* gene. One of the two classes of methionine prototrophs having markers recombined occurred in excess over the other; when the markers entered the cross in the opposite phase, a similar excess was found in the reciprocal class. The linear order of *me-2* alleles determined from these asymmetries was consistent with a map based on prototroph frequencies.

Pronounced asymmetries were also observed in the numbers of the two parentally-marked classes of prototrophs; when the markers entered the cross in the opposite phase these asymmetries were reversed. The asymmetries between the two parentally-marked classes were correlated in direction with the asymmetries between the two classes having markers recombined.

The results may be interpreted in terms of multiple exchanges within small, discontinuously distributed, regions of effective pairing—the asymmetries would result from a reduction of coincident exchanges in the region proximal to the selected interval.

The coincident exchange frequency in the proximal region is influenced by the position of the more proximal of the two *me-2* alleles, but is independent of the recombination frequency between the two alleles. The reduced coincidence in the proximal region could be explained if the *me-2* region is situated immediately distal to some discontinuity which imposes a nonrandomness of exchanges by reducing the chance that the effectively paired region could be proximal to, yet extend into, the *me-2* region.

Supported by Public Health Service research grant E1462.

2.7. Conversion and Crossing-over as Recombination Mechanisms in Ascobolus immersus. W. GAJEWSKI, A. KRUSZEWSKA, A. MAKAREWICZ, A. PASZEWSKI, S. SURZYCKI and H. BIELAWSKA (Warsaw, Poland).

Crosses between mutants with white ascospores from one series give wild type, dark recombinants due predominantly to conversion. The frequencies of conversion-type asci are roughly proportional to the distances between the sites involved which enables one to map them. When mutants from one series are crossed with wild type strain the frequencies of 2:0 asci increase in percentage from one end of the series to the other approaching the value of recombination between the extreme sites of the series.

This is interpreted that the frequency of 6:2 asci in crosses between two white mutants represents only that part of copy choices which begin or end in between two sites. It seems that the beginning of the switches is random along the analysed unit whereas the return to the original matrix is rather nonrandom.

One mutant, namely 186, crossed with wild strain shows very high frequencies (10-12 per cent) of 6:2 and 2:6 asci of conversion type. Besides, less frequent asci 5:3, 3:5, 7:1 and 1:7 also appear. They are not due to aberrant chromosome segregation or mutation. The asci 5:3 and 3:5 result probably from copy errors of half-chromatids. The 7:1 and 1:7 asci may be the result of two copy errors it two successive replications. The tetrad analysis of visible ascospore mutants in *Ascobolus immersus* on sufficiently large scale reveals the predominant role of conversion as a mechanism of recombination within a small piece of genetic material.

2.8. Effect of Crossing-over on the Process of Spontaneous Mutation. G. E. MAGNI (Pavia, Italy).

It has been recently observed that the rates of spontaneous mutations occurring during meiotic division are higher than those occurring during vegetative reproduction (mitosis) in the same type of cells (yeast).[1]

Further experiments have shown that most spontaneous back-mutations at locus his_{1-1} during meiosis, in either one of the two identical alleles carried by diploid yeast cells, are connected with crossing-over in the specific locus.

Data regarding the amount of negative interference in the adjacent chromosome regions and the polarization of the intragenic crossing-over will be presented.

The hypothesis of unequal crossing-over within the locus leading to back mutant chromatids will be discussed.

---

1. MAGNI and VON BORSTEL, *Genetics* **47**, 1097, 1962.

2.9. Further Investigations on Somatic Gene Conversion in the Tomato. RUDOLF HAGEMANN (Gatersleben, Germany).

In heterozygous $sulf^{+}sulf$ plants a green yellow variegation occurs (*sulf* means any mutan allele of the chlorophyll deficient *sulfurea* series either of the $sulf^{pura}$ or of the $sulf^{vag}$ group)

The genetical analysis demonstrated that the *sulf*$^+$ allele, stable in homozygous condition, becomes unstable in presence of a *sulf* allele. This type of allele-induced instability in somatic cells is termed somatic gene conversion.[1]

Further experiments have shown:

(1) A two-element-system (as found in maize) is not involved.

(2) In hybrids between *sulf* homozygotes and taxa of the subgenus *Eulycopersicon* the percentage of variegation is only determined by the "conversion activity" of the special *sulf* allele present. The environmental conditions (temperature, nutrition, illumination) do not significantly alter the percentage of variegation. Conversion-table *sulf*$^+$ alleles have not yet been found.

(3) In hybrids between *sulf* homozygotes (*Lycopersicon esculentum*) and *L. hirsutum* (subgenus *Eriopersicon*) or *Solanum pennellii* variegation occurred very seldom, thus indicating an influence of the genotypic milieu.

(4) The somatic conversion is confined to the *sulf* locus. But as to the different alleles, conversion is not highly directed. The *sulf* allele, converted from *sulf*$^+$, need not be identical with the *sulf* allele already present; e.g. in a *sulf*$^+$ *sulf*$^{pura-90\ per\ cent}$ plant the *sulf*$^+$ allele is converted to *sulf*^{vag} more frequently than to *sulf*^{pura}, and even the *sulf*^{pura} alleles arisen may differ in their conversion activity from *sulf*$^{pura-90\ per\ cent}$.

1. *Z. Vererbungsl.* **89**, 587, 1958.

2.10. Preliminary Experiments on the Chemistry of Crossing-Over. SHELDON WOLFF and FREDERICK J. DE SERRES (Oak Ridge, U.S.A.).

Experiments with metabolic inhibitors have indicated that protein synthesis is necessary for the rejoining of radiation-induced chromosome breaks. Because of these experiments Wolff has postulated that the bonds formed at rejoining are in protein. Although crossing-over may in some ways be likened to breakage and rejoining there are some differences. We, therefore, treated *Neurospora crassa* with specific metabolic inhibitors at the time of crossing-over to see if recombination is affected when certain cellular synthetic processes are blocked. In particular, the experiments were designed to see whether or not protein synthesis inhibitors which can inhibit the rejoining of radiation-induced breaks can affect crossing-over.

Several experiments of two different types were performed. In the first type the marker asco that gives rise to white ascospores was used. The asci were dissected and second division segregations scored. In the second type biochemical markers that were separated by various distances were used and recombinants scored.

The protein synthesis inhibitor, chloramphenicol which inhibits rejoining and also inhibits growth of *Neurospora*, did not affect recombination. We interpret this to mean that protein synthesis is not involved in crossing-over.

The only inhibitor that gave consistently higher recombination values when added at the time of meiosis was 5-fluorodeoxyuridine (FUdR) a compound that, in some systems, has been shown to be a specific inhibitor of DNA synthesis.

Bromodeoxyuridine (BUdR) a compound that is structurally similar to FUdR but is a thymidine analog that becomes incorporated into the DNA did not have any affect on recombination nor did uridine which is usually added with FUdR to prevent effects on RNA.

The simplest explanation for these experiments is that genetic recombination, which entails the exchange of chromatids, is specific for DNA and that a functional mechanism for DNA synthesis is required when the bonds rejoining the two pieces are synthesized. This is unlike the rejoining or repair of X-ray induced breaks that are non-polarized and are inhibited by chloramphenicol.

2.11. Tetrad Analysis of Crosses of Neurospora crassa Grown on Media Containing Various Concentrations of 5-bromouracil and Cytidine Sulfate. S. F. H. THRELKELD (Hamilton, Canada).

Crosses of two *Neurospora crassa* strains *A, arg-3* (30300), *cr, tryp-2* (75001), *ylo* (Y30539y) and *a, pyr-1* (H263), *pdx-1* (37803) were made on various media, by conidiating 5-day-old cultures of the protoperithecial parent, *a pyr-1 pdx-1*, with conidia from the other strain.

Standard *Neurospora* reproductive medium was supplemented in the following ways to give four types of media: (i) cytidine sulfate 100 mg/l., 5-bromouracil 0 mg/l.; (ii) cytidine sulfate 100 mg/l., 5-bromouracil 100 mg/l.; (iii) cytidine sulfate 45 mg/l., 5-bromouracil 100 mg/l.; (iv) cytidine sulfate 45 mg/l., 5-bromouracil 0 mg/l.

Crosses grown on media (i) and (ii) showed no significant differences with respect to ascus patterns or recombination frequencies. Com-

pared with the crosses grown on media (i) and (ii), crosses grown on media (iii) showed a highly significant increase in asymmetrical tetrads, and in the recombination frequency between the marker *ylo* and the respective centromere. Crosses grown on media (iv) were infertile.

These results will be discussed.

2.12. Changes of the Recombination Frequency by Means of Different Concentrations of Ca and Mg in Barley. MILOŠ ONDŘEJ (Prague, Czechoslovakia).

F_1 generation of hybrids between cultivar Stupicky plnozrný and the new-breeding Solary 38-50 were cultivated in sand cultures and watered with these kinds of nutrient solution:

1. with normal content of Ca and Mg (N)
2. with 100 × decreased content (—)
3. with 2 × increased content (+)

The last batch of plants were cultivated in field conditions (C). The content of the nutrient medium influenced all the quantitative characters of the analysed plants.

In F_2 generation the recombination frequencies for rough-smooth and long-short rachila hairs were tested.

The lowest recombination frequency was found in the progeny of plants C, higher in N, more higher in (+) and highest in (—) nutrient solution.

The results are in controversy with those authors which used EDTA to influence the recombination values. It is possible to explain it in two different ways:

1. The meiotic cells were not influenced by the decreasing or increasing content of Ca and Mg. They were influenced only by the general physiological changes of the whole organism.
2. EDTA acts on crossing over another way other than direct binding of ions Ca and Mg from chromosomes and their environment.

2.13. Effect of Temperature on Recombination in Sphaerocarpus. W. O. ABEL (Heidelberg, Germany).

The effect of temperature on recombination in *Sphaerocarpus* was tested in 3- and 4-factor crosses. The position of the loci used in the crosses enabled an analysis of recombination almost in the whole now known left arm of the linkage group I. The sporophytes grew during the (for the recombination) important stages of development in temperatures of 10, 18, 26 or 33°C. The temperature of 33°C had to be reduced for physiological reasons to 19°C during the night. The temperatures of 10, 26 and 33°C caused for all chromosome sections higher recombination frequencies than the temperature of 18°C. We found therefore a minimum at 18°C for the recombination frequency. This minimum was very striking and statistically significant in the chromosome section near the centromere. The double crossing over frequency was higher in the experiments at 10, 26 and 33°C than at 18°C. It is very likely that the increase of the double crossing over frequency was caused through a decrease of crossover interference. This demonstrated the alteration of the second division segregation for the locus "crassa" from 91 per cent at 18°C to 69 per cent at 33°C.

The observation of a minimum for the recombination dependent on temperature, the reaction of crossover interference and the high sensitiveness to temperature of the chromosome region near the centromere are comparable with the findings of Plough and other authors in *Drosophila*.

2.14. The Genetic Effect of Mitomycin C. ROBIN HOLLIDAY (Hertford, Great Britain).

All mutagenic agents which have so far been tested are able to induce somatic recombination in diploid strains of fungi. Apart from their specific mutagenic properties, such agents may have a more general metabolic effect, in particular the temporary inhibition of DNA synthesis relative to RNA and protein synthesis. In order to determine which of these properties is responsible for the induction of recombination, a heterozygous diploid of *Ustilago maydis* and a heteroallelic diploid of *Saccharomyces cerevisiae* have been treated with the antibiotic mitomycin C, which is known to be a specific inhibitor of DNA synthesis. It was found that in *Ustilago* mitomycin is somewhat more efficient than ultraviolet light in stimulating reciprocal mitotic crossing over; whereas in *Saccharomyces* it is less efficient than ultraviolet light in stimulating nonreciprocal recombination or gene conversion. Parallel experiments with haploid strains of *Ustilago* have shown that mitomycin has no mutagenic activity. It is proposed that agents which induce recombination but not mutation be termed *recombinagens*. Experiments are now in progress which will attempt to determine whether recombination can occur during mitomycin treatment, i.e. in the absence of genetic

replication, or whether it occurs only subsequent to the treatment.

2.15. Centromeres and Sites of Affinity Located on Saccharomyces Chromosomes. CARL C. LINDEGREN, YUH LIN HWANG, J. K. BHATTACHARJEE and GERTRUDE LINDEGREN (Carbondale, U.S.A.).

The analysis of data from more than 5000 yeast tetrads has revealed eleven independently segregating centromeres and five sites of affinity, confirming the data previously reported by Lindegren *et al.* (1962) concerning Chromosomes I to X. The centromeres (C), their markers and the number of linked genes, in parentheses, are:

C_I, ad-1 (1); C_{II}, ga-1 (8); C_{III}, le-2 (5); C_{IV}, tr-1 (1); C_V, ur-3 (14);

C_{VI}, hi-2 (4); C_{VII}, le-1 (3); C_{VIII}, ar-4 (1); C_{IX}, hi-6 (2); C_X, met-3 (1).

The new eleventh centromere-marker, met-14, is about 10 strains from Centromere XI. A cluster of 4 sites of affinity linked to 6 genes lies near Centromere V; a fifth, linked to 2 genes, lies near Centromere VIII. Four segments containing 11 genes are not yet linked to centromeres. The homogeneity of data between crosses has been examined and gene-to-centromere distances have been calculated to a high order of accuracy.

2.16. Comparison of Sexual Recombination in Escherichia coli with Gene Recombination in Streptomyces violaceoruber. S. G. BRADLEY (Minneapolis, U.S.A.).

E. coli cells and *S. violaceoruber* hyphae contain several to many sets of genetic information. In neither organism is the germ plasm separated from the cytoplasm by a well-defined nuclear membrane. Cellular or hyphal fusion therefore is tantamount to nuclear fusion. The resulting heterogenomic state is maintained during several cell divisions in *E. coli* and indefinitely in the mycelia of *S. violaceoruber*. Homogenomy is established in *S. violaceoruber* by serial plating of spores. In both microbes, parental types are recovered frequently from recombinant clones. Many recombinant phenotypes may be derived from a single heterogenomic clone. Different sublines of a heterogenomic clone often show disparate patterns of segregation for one or more characters. Recombinant clones may appear homogeneous for some expressions but heterogeneous for others. These results are best explained in terms of repeated opportunity for recombination during vegetative growth. The recombinational process in both microbes is essentially similar to parasexuality as described in *Aspergillus* by Pontecorvo. *S. violaceoruber* differs from *E. coli* in that the mycelial growth habit of the former stabilizes the heterogenomic state whereas the relatively regular cell division of the latter establishes homogeneity rather quickly. If pedigrees based on single cells and serial spore analyses are compared, this difference disappears. Another difference is that *S. violaceoruber* seems to possess two linkage groups capable of independent segregation whereas *E. coli* has a single linkage group.

2.17. Mitotic Recombination within the paba1 Region of Aspergillus nidulans. ALEXANDRA PUTRAMENT (Warsaw, Poland).

The results of analysis of 393 mitotic recombinants within the paba1 region of *Aspergillus nidulans* can be summarized as follows:

Chromatid segregation following intragenic mitotic recombination is not random. Wild-type (selected) strands segregate preferentially with non-recombinant ones.

Reciprocal, non-reciprocal and probably inexactly reciprocal exchanges can occur in intragenic mitotic recombination.

In intragenic mitotic recombination within one effective pairing segment exchanges can occur between three or even four chromatids.

Negative interference observed in intragenic mitotic recombination in *Aspergillus nidulans* is higher than that observed in meiotic recombination within the same gene (paba1). In fact it resembles rather the pattern of intragenic meiotic recombination observed in *Neurospora crassa*.

Polarity of recombination observed in the present work has the same tendency as that observed in meiotic recombination within the paba1 region; it is even more pronounced.

2.18. Mitotic Recombination in Translocation Heterozygotes of Aspergillus nidulans. ETTA KÄFER (Montreal, Canada).

A variety of rare segregants can be isolated from vegetative diploid cells of the normally haploid fungus *A. nidulans*. These segregants are of a few specific types and most of them

appear to be produced either by mitotic crossing-over or by mitotic non-disjunction. Unusual types of segregants have been isolated from certain diploids. All results obtained are consistent with the hypothesis that these diploids contain chromosomal aberrations, most probably translocations. Such aberrations cause consistent complete linkage of the markers of the two linkage groups involved in the haploids resulting from repeated steps of mitotic non-disjunction. This is expected if near-haploids with unbalanced chromosome complements, that is dis omic and nullisomic for translocated segments, are inviable. The frequency of haploids is therefore reduced (evident, e.g. relative to the frequency of diploid segregants resulting from more than one event of mitotic recombination).

Similarly, mitotic crossing-over in chromosome arms heterozygous for translocated segments regularly leads to the formation of unusual segregants. Suitably marked diploids were produced heterozygous for either or both of the translocations T(VI-VII) and T(I-VII), as well as normal and translocation homozygotes. Mitotic crossovers selected in one of the involved arms were found to be unstable and presumably unbalanced. They produce regularly, by further steps of mitotic recombination, better growing diploid and haploid sectors on complete medium. Segregation patterns specific for each translocation were observed and segregants of each type were found to show very characteristic phenotypes. Information on the position of the breakage points as well as the relative size of the translocated segments was obtained.

2.19. Somatic Recombination in Coprinus lagopus. K. M. Swiezynski (Warsaw, Poland).

In a vegetative mycelium, arising from a compatible or incompatible di-mon mating, frequently new nuclei arise, containing markers originating from two different nuclei.

Dikaryotic mycelia containing such nuclei fruit easily. In the progeny markers often appear in the proportion 2 : 1. The 1 : 1 proportion of alleles is also frequent.

In common AB heterokaryons, containing two types of nuclei with complementary growth requirements, occasionally prototrophic nuclei arise and may be isolated. *Mycelia* containing such nuclei mate with compatible strains. In the progeny of the fruibodies obtained from such matings again the alleles often appear in the proportion 2 : 1 or 1 : 1.

The simplest explanation is that in heterokaryons nuclei may fuse and become diploid. Such nuclei are unstable and turning back to the haploid state in vegetative mycelia give rise to somatic recombinants. Hypothetical diploid nuclei, containing different alleles of the A mating type locus, are apparently very unstable and were never isolated. On the other hand diploid nuclei homozygous for the A locus are comparatively stable and are able to form dikaryons with compatible haploid nuclei.

The results will be published in detail in the journal *Genetica Polonica.*

2.20. Somatic Recombination in Arabidopsis. Y. Hirono and G. P. Rédei (Columbia, U.S.A.).

Somatic segregation in heterozygotes may be brought about by: deletion (breakage), sorting out of extranuclear elements, non-disjunction (reduction), mutation (gene conversion), translocation and crossing over. In higher organisms, the distinction among these possibilities is difficult partly because the lack of special cell markers and mainly because of the determinate differentiation of the cells. In *Drosophila* convincing positive evidence is available for somatic crossing over, though without breeding test but with supporting cytological observation of somatic pairing. Until now, only in certain fungi has it been possible to demonstrate the occurrence of mitotic recombination with progeny test, though the responsible chromosomal mechanism is not amenable to cytological analysis. In *Arabidopsis* plants (crucifer) heterozygous for three good linked markers (ch^1: chlorophyll-b free; gi^2: late-vigorous; and *pa*: dark green, dwarf) somatic segregation was induced by X-raysand the seed obtained from the phenotypically different two sectors has been subjected to further genetic analysis. Though the irradiation caused multiple hereditary alterations, in the case of one plant the experimental data can be interpreted only by genetic exchange at the four-strand stage. Observations on somatic metaphases of wild type plants indicate that juxtaposition of the homologous chromosomes occasionally occur in *Arabidopsis*. Full details will be reported in *Genetics*.

Contribution from the Missouri Agricultural Experiment Station. Journal Series No. 2543. Approved by the Director.

2.21. Mitotic Recombination in Yeast. B. S. Cox (Liverpool, Great Britain).

A diploid yeast is described with which it has been possible to apply three of the selective

techniques described by Pontecorvo for detecting mitotic recombination, namely the use of (a) a morphological gene and (b) a recessive resistance gene in heterozygous combination with their wild-type alleles, and also the use of recombination between "heteroallelic" mutants in the same cistron to produce a heterozygote of wild phenotype. Roman's (1956) finding, that selection using the last system results in the detection of exclusively non-reciprocal events best explained by a copy-choice theory of recombination, is confirmed, but it is also found that some of the recombinants so selected cannot be explained so simply and may provide evidence of either four-strand or inexact reciprocal recombination at mitosis.

When homozygous recombinants from heterozygous loci are selected, for in this diploid they are found to fall into two classes: 90 per cent or more are found to be homozygous at all the linked marked loci, both proximal and distal to the "selected" locus; the remainder are non-recombinant at any other marked locus, either proximal or distal.

It is proposed that the second class arise by a recombination process similar to that, already described, which is observed in selecting for recombination between heteroalleles. The other, majority, class may be the result of chromatid non-disjunction rather than mitotic crossing-over. That marker genes unlinked to the selected genes remain heterozygous in all these recombinants indicates that this non-disjunction is not general, that is, that the recombination is not due to the occurrence of the first division of meiosis.

2.22. U.V.-induction of Somatic Recombination in Yeast. DAVID WILKIE (London, Great Britain).

In diploid yeast cells the change from the heterozygous Ad_2 ad_2 (white colony, prototrophic) to the homozygous recessive ad_2 ad_2 (red, adenine-requiring) takes place spontaneously with a low frequency and is usually detected as a red sector in a white colony. The change can be induced by u.v.-irradiation and irradiated cells when plated out give rise to colonies classifiable as follows:

(1) all white, showing no recombination;

(2) half-sectored, or otherwise showing sectoring from the centre of the colony, indicating segregation at the first mitotic division;

(3) all red. The relative frequency of this class increases with dose suggesting the reciprocal white cell had a lethal defect;

(4) *a.* showing a small red sector or sectors mainly at the periphery,
b. mottled red and white.

(4) *a* and *b* are interpreted as showing an unstable condition of partial induction which is apparently transmissable. In other words, it appears that the u.v.-induction is not an all-or-none event and cannot be attributed directly to a photochemical change. Further evidence of the complexity of the recombination process is seen in the results of preliminary experiments which show that u.v.-induction is dependent both on the intensity of the irradiation and on the temperature at which it is administered. It is suggested a comparison along these lines be made with heteroallelic systems where recombination appears to involve a different mechanism.

2.23. Extrabasidial Nucleic Recombinations in a Basidiomycete Coprinus radiatus. NICOLE PRUD'HOMME (Gif-sur-Yvette France).

It is possible to make triheterokaryons of *Coprinus* from three monokaryotic strains. In these heterokaryons new nuclei have been demonstrated, formed through an exchange of genetic material between two of the original nuclei. The study of these nuclei leads to the following facts:

(1) High frequency of aneuploid nuclei (single or multidisomic).

(2) Possible recombinations of the genes located on non-homologous chromosomes of the original nuclei.

(3) Apparently total linkage between genes located on homologous chromosomes, even if these genes are 35 c.o. units apart from each other.

(4) More frequent appearance of some nuclear types than others.

These data suggest a mechanism of parasexual recombination analogous to that described in various *Ascomycetes*, associated with possible selection of some nuclear types.

2.24. The Purple Suppressor System in Coprinus lagopus. D. H. MORGAN (Hull, Great Britain).

It has been established that certain suppressor mutations in *Coprinus* may be functionally allelic with one another, as judged by a complementation test, but at different chromosomal locations as judged by recombination data.[1] Further work on the purple-suppressor system is now reported.

When different cultures, in which spontaneous suppression of the mutant gene *purple* has taken place, are crossed with wild type, the proportion of unsuppressed purples appearing in the progeny varies (between suppressors) from 0 per cent to about 19 per cent. The scatter of values is continuous rather than discrete. Identification of the expected reciprocal recombinant class has been attempted, but unequivocal evidence for its occurrence and for the 1 : 1 segregation of suppressors has not been obtained. Evidence from the resolution of dicaryons formed between suppressed and unsuppressed cultures shows however that suppression is *not* due to cytoplasmic factors. Suppressed cultures are quite stable and recombinant suppressed cultures, from crosses with wild type, have exactly the same characteristics as their parents with respect to segregation of purples and complementation reactions. Infertility is frequent in crosses involving suppressed purples. It is possible that the spontaneous suppression of purple involves chromosomal changes, in which case non-complementation between suppressors which have different characteristic purple segregations would be less surprising.

1. D. Lewis, 1960; D. H. Morgan, 1961.

SECTION 3

MOLECULAR AND MICROBIAL GENETICS

3.1. Further Studies on Incorporation of Homologous DNA into the Mouse Germ Cells in vivo. CHAI HYUN YOON (Chestnut Hill U.S.A.).

DNA in the thymus glands was tagged with thymidine-H^3, and the extracted thymus-DNA-H^3 was injected into the gonads of mature male mice. The germ cells of recipient mice were studied with both squash and section methods for the evidence of thymus-DNA-H^3 incorporation *in vivo*. Quantitative data will be presented.

3.2. Evidence for the Source of Messenger RNA and the Control of Its Production in the Regulation of Gene Activity. ALFRED MARSHAK (Philadelphia, U.S.A.).

Ribonucleic acid from cytoplasm (c-RNA) is so constituted that on treatment with weak alkali all of its constituents are released as nucleotides. In contrast, ribonucleic acid from nuclei of the same or similar cells (n-RNA) when similarly treated yields adenine, guanine, and cytosine and adenosine in amounts equal to the nucleotide released. The release of these bases is correlated with the presence of material extractable in lipid solvents which renders the ribosidic bonds susceptible to alkali hydrolysis. When both components of n-RNA are accounted for, it is found to have base ratios resembling those of DNA. The component of n-RNA not associated with lipid has ratios similar to those of c-RNA. Previously it was shown that n-RNA is the precursor of ribosomal RNA. The present experiments indicate that it contributes to the ribosomes that portion of its nucleotides which are not associated with lipids. The n-RNA thus has the properties required of a source of messenger RNA. It is proposed that the association of lipid or lipoprotein with the n-RNA provides a mechanism for the control of gene activity through regulation of the release of messenger RNA from n-RNA or by other means.

3.3. The Structure of Adapter RNA. GEOFFREY ZUBAY (Upton, U.S.A.).

Adapter RNA is the carrier of amino acids to the template for protein synthesis. Part of its function is to arrange the amino acids in a specific order prior to peptide synthesis. It does this by the following means: each adaptor combines with one—and only one—amino acid; and each adaptor is presumed to have a trinucleotide sequence which hydrogen-bonds to a complementary site on the messenger RNA template. Physico-chemical measurements indicate that adapter RNA is a single polynucleotide chain about 67 nucleotides long. It has been proposed that the polynucleotide chain has a bend near the middle, with the two halves of the chain interacting in double helical fashion.[1] In a more recent and detailed model, it has been suggested that the only nucleotides not in the double helix configuration are the three in the bend, and the two on the nucleoside end which combines with the amino acid.[2] This structure will be discussed with regard to (1) stereochemical problems of protein synthesis[2,3] and (2) chemical methods for demonstrating the location and composition of the coding nucleotides.[4]

Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

1. G. BROWN and G. ZUBAY, *J. Mol. Biol. 2*, 287, 1960.
2. G. ZUBAY and J. BERGERON, Symp. A-11-1, 8th Intern. Microbiology Congr., Montreal, 1962, in press.
3. G. ZUBAY, *Science*, in press.
4. G. ZUBAY, *Biophys. Biochem. Res. Commun.*, in press.

3.4. The Genetic Implication of the Methylated Bases in Transfer RNA. ERWIN FLEISSNER, P. R. SRINIVASAN and ERNEST BOREK (New York, U.S.A.).

Transfer RNA is characterized by the unique presence of a small number of methylated purines and pyrimidines. The origin of these methyl derivatives has been obscure until recently. We have shown that the methylated bases

are not incorporated *a priori* into the primary structure of s-RNA but are acquired by the methylation of the polynucleotide chain by a multiple enzyme system — RNA methylase— which uses s-adenosyl methionine as the methyl donor.[1]

Studies of the RNA methylases from different species revealed that the enzyme systems are endowed with great species specificity.

The s-RNA from a given source while fully methylated with respect to its homologous methylating enzymes can accept excess methyl groups from an enzyme from a different species. These findings tend to eliminate the possibility that the methylated bases are involved in the coding of amino acids. If it is assumed that the amino acid code is universal, the methylating enzymes of all species would be expected to be uniform. Yet we found that a presumably fully encoded s-RNA of one specieo can accept methyl groups from a heterologous enzyme system.

The species variation of the RNA methylases has also been adumbrated by the analytical data on the distribution of the methylated bases of s-RNA from various sources.

However, the biological function of the species variation of the enzyme and, indeed, of the methylation itself awaits elucidation.

---

1. E. Fleissner and E. Borek, *Proc. Nat. Acad. of Sci., U.S.A.* **48,** 1199, 1962.

3.5. Synthesis of Specific RNA on Different Sites of the Phage T2 Chromosome in vivo and in vitro. R. B. Kcesin, M. F. Shemjakin, G. M. Gorlenko, I. A. Bass and A.A. Prozorow (U.S.S.R.).

1. Different types of phage-specific mRNAs are synthesized during early and later stages of the intracellular development of the phage T2 in *E. coli* B. The phenomenon is in accordance with the synthesis of different proteins during those stages (early enzymes and phage particle protein). Experiments on the formation of complexes of C^{14}-mRNA and phage DNA show that the mRNAs, synthesized on different stages, correspond to different regions of phage DNA.

2. Experiments with chloramphenicol indicate that the regulation of the synthesis of different specific mRNAs depends on the synthesis of proteins.

3. *In vitro* experiments showed that phage DNA, added as a primer to an extract from uninfected *E. coli* cells, directed only the synthesis of a type of mRNA, which is similar to that synthesized *in vivo* during the early stages of infection. The same mRNA was obtained when native deoxyribonucleoprotein from T2 phage, DNA from phage-infected cells or renatured T2-DNA were used as primer.

4. Also in systems containing T2-DNA as a primer and partially purified enzymes from uninfected *E. coli* B or *B. prodigiosum* cells only early mRNA was synthesized. In the *in vivo* experiments with RNA-polymerase it was possible to obtain the synthesis of specific mRNAs formed on different regions of the phage chromosome (DNA) in the infected cells. The isolation and purification of DNA does not change the order of reading of the genetic information.

3.6. The Active Complex in Protein Synthesis. R. Haselkorn, V. Fried, and J. Dahlberg (Chicago, U.S.A.).

RNA from turnip yellow mosaic virus has been used to characterize the complex responsible for protein synthesis in cell-free extracts from *Escherichia coli.* Three types of experiment were performed, all utilizing zone centrifugation through sucrose gradients. In the first type, viral RNA and coli ribosomes are mixed, fractionated on the gradient, and the RNA located. Secondly, the RNA–ribosome mixture is fractionated, and the individual fractions are assayed for their ability to incorporate amino acids. Finally, the RNA–ribosome mixture is allowed to incorporate amino acids, then fractionated, and both the RNA and newly made protein located. All three experiments give essentially the same result, namely, that in the cell-free system 5-10 per cent of the ribosomes participate in protein synthesis and the active complex consists of one 70S ribosome and one molecule of viral RNA.

3.7. A Model System for Studying the Genetic Determinants of Protein Configuration. Charles J. Epstein and Robert F. Goldberger (Bethesda, U.S.A.).

It is generally accepted that the genetic information contained in linear DNA molecules is transferred, via messenger RNA, to linear polypeptide chains. However, the process by which these chains are converted to "native" proteins is poorly understood. The reduction and reoxidation of ribonuclease by Anfinsen, White and co-workers provided a model system for studying this process, and led to the conclusion

that all information necessary for the secondary and tertiary structure of this enzyme is contained within the primary amino acid sequence. More recently, similar results have been obtained with other proteins, indicating that this conclusion may be generally applicable. Because of the relatively long time required for the refolding process *in vitro*, in contrast to the rapid synthesis of native proteins *in vivo*, a catalytic system was sought and found in rat liver extracts. This system, consisting of soluble microsomal and supernatant factors, greatly accelerates the reactivation of reduced ribonuclease.

In order to study the effects of amino acid alterations on the configuration, as opposed to the "active center", of proteins, chemically modified, but still active, enzymes have been prepared. The effects of the modifications on folding of the extended molecules (following reduction) are now being studied.

Much of this work was done in conjunction with Dr. Christian B. Anfinsen.

3.8. The Evolution of the Fibrinogen Molecule* Russell F. Doolittle and Birger Blombäck (Stockholm, Sweden).

Fibrinogen is a protein molecule which is apparently found in the blood plasmas of all vertebrate species. During its conversion to fibrin by thrombin, certain segments of the parent molecule are proteolytically released. These peptides, usually termed the fibrinopeptides A and B, range from 13 to 21 amino acids in length, depending on the species from which they are obtained. Comparisons of the amino acid sequences of fibrinopeptides from a variety of vertebrates has permitted us to follow a flow of amino acid substitutions through these regions of the fibrinogen molecule. Complete sequences are now available for the A and B fibrinopeptides from ox, human, sheep, and pig. In addition, partial amino acid sequences of the peptides from several other mammals and the lamprey eel (*Petromyzon marinus*) are reported. The differences in sequence are greater than expected. Thus, the B fibrinopeptides of sheep and ox differ in ten of twenty residues. On the other hand, certain positions and segments of the peptides appear to have been maintained throughout all the species examined. Only one fibrinopeptide has been isolated from lamprey eel fibrinogen so far. Its partial amino acid sequence suggests that it has characteristics of both the A and B mammalian fibrinopeptides. For example, it contains tyrosine-O-sulfate, an unusual amino acid found in the B fibrinopeptides of several mammals, but its C terminal portion corresponds to the C terminal sequence of the mammalian A fibrinopeptides. Although our observations are restricted to those amino acid substitutions which have survived, as opposed to all those that may have occurred, it is possible to draw some conclusions about the plausibility of suggested amino acid codes.

3.9. Control of DNA Replication in an Organism with Synchronous Mitosis. E. Guttes and S. Guttes (Providence, U.S.A.).

The nuclei of the plasmodial slime mold, *Physarum polycephalum*, divide in synchrony every 12–14 hr. The replication of DNA occurs during a period of 3–4 hr immediately following mitosis. The role of the nuclei in the initiation of this process was investigated by examining their competence for DNA replication at various stages of the replication cycle. For this purpose, the normal correlation between the nuclear and the cytoplasmic replication cycle was disrupted by implanting nuclei representing one stage of the cellular cycle into plasmodia representing another. We found that hostplasmodia at a stage of the cell cycle at which they are able to sustain DNA biosynthesis and replication, cannot initiate DNA replication in implanted nuclei, unless these have undergone mitosis just prior to the implantation. This failure is not due to a lack of functional integration between the host plasmodium and the implanted nuclei, nor to unspecific damage to the latter as a result of the implantation procedure. DNA feeding with subsequent exposure to tritiated thymidine resulted in strong DNAse labile cytoplasmic labelling at all times of the intermitotic period. It is concluded that only at mitosis the nuclei acquire competence for DNA replication and that this event is a critically controlling factor in the timing of DNA replication. The structural alteration of the nuclei in acquiring of competence is not reflected in their *in vitro* behaviour. In an experiment involving exposure of fixed nuclei to a suitable DNA-polymerase preparation[1] we found that nuclei in ethanol-fixed smear preparations were at no time of the replication cycle able to serve as primers, whereas nuclei which were previously fixed with denaturing ethanol-acetic acid[2] were able to serve as primers. An investigation with the electron microscope[3] has shown that *in vivo* the onset of DNA replication coincides precisely with the time at which prenucleolar material is beginning

to be dissociated from the chromosomes after mitosis. We assume that the acquisition of competence at mitosis for DNA replication may result from a dissociation between the DNA double helix and a previously stabilizing (prenucleolar?) protein.

1. Unpublished results of R. C. VON BORSTEL, D. M. PRESCOTT, and F. J. BOLLUM; BOLLUM, personal communication.
2. Unpublished result of E. GUTTES and F. J. BOLLUM.
3. In collaboration with R. A. ELLIS, BROWN University, R. I.

3.10. Incorporation of H^3 Thymidine by K12 (λ) Induced by Streptonigrin. CHARLES M. RADDING (Michigan, U.S.A.).

K12(λ) is induced by streptonigrin (SN), an antibiotic and anti-tumor agent[1]. Like Mitomycin C, SN initiated a rapid breakdown *in vivo* of the DNA of *E. coli* to acid soluble products: 35 per cent of H^3 labelled DNA was rendered acid soluble in 2 hr. Vegetative multiplication of phage was also initiated, and, in spite of the rapid degredation of the bulk of cellular DNA, all cells could be induced to produce about 50 phage per cell.

The net incorporation of H^3 thymidine (in a 1 min pulse) into acid insoluble material was 90 per cent inhibited in 20 min by SN in lysogenic and non-lysogenic cells. Only in lysogenic cells was a recovery observed in the ability to incorporate H^3 thymidine. The peak of this recovery coincided approximately with the appearance of mature intracellular phage. Consistent with incorporation of H^3 thymidine into DNA, both before and after treatment with SN, was the sensitivity of the acid insoluble radioactivity to heating in acid, and stability to heating in alkali. Further studies will attempt to determine if the incorporation of H^3 thymidine by lysogenic cells induced by SN is equivalent to the specific synthesis of λ DNA. Preliminary study of defective λ lysogens indicates that they are separable into those which can or cannot incorporate H^3 thymidine after treatment with SN.

1. M. LEVINE, *Bacteriological Proceedings* 1963, in press.

3.11. Replication of T2 DNA. F. R. FRANKEL and L. INGRAHAM (New York, U.S.A.).

Bacterial cells infected with bacteriophage T2 contain phage particles and intermediate DNA structures. Chloramphenicol (CM) arrests the production of phage but permits continued synthesis of DNA. On removal of the inhibitor, most of the accumulated DNA is utilized in the formation of complete phage particles. We have observed that immediately upon addition of CM to an infected culture, not only does phage synthesis cease, but molecules of DNA like those contained in phage particles are no longer produced. Instead, about 25 per cent of the total DNA of these cells is recovered as unusual DNA structures, in many respects similar to, but apparently larger than, phage DNA.[1] This material is not a precursor of phage DNA, since on removal of CM (1) it does not diminish in amount, and (2) radiophosphorus enters phage DNA without passing through these structures. If fluorodeoxyuridine is added to a CM-treated culture, total DNA synthesis abruptly stops. However, the amount of these unusual DNA structures continues to increase. Therefore, this material may be derived from the actual phage DNA precursor, which itself resists isolation, and perhaps it reflects the unusual structure of the latter. In fact, subjecting the extracts to gentle shear further increases the yield of these unusual molecules. We are examining the hypothesis that phage precursor DNA is composed in large part of exceptionally large, fragile DNA molecules.

1. *Proc. Nat. Acad. Sci.* **49**, 1963.

3.12. Inhibitory Effect of Chloramphenicol on Mating Fragmentation of T4 Phage DNA. ANDRZEJ W. KOZINSKI (Philadelphia, U.S.A.).

In our previous paper we have shown that parental T4 DNA undergoes an extensive fragmentation in the vegetative pool. As a result, final progeny molecules contain only small fragments of parental DNA as semi-conservative sub-units[1,2]. In the present studies, we have shown that chloramphenicol inhibits fragmentation of parental DNA in the vegetative pool, allowing at the same time strictly *semi-conservative replication* and apparently unimpaired net synthesis of progeny DNA. We conclude that molecular matings in the DNA vegetative pool require concomitant synthesis of protein,

presumably facilitating the recombination between DNA molecules.

1. A. W. KOZINSKI, *Virology*, **13**, 124, 1961.
2. A. W. KOZINSKI and P. B. KOZINSKI, *Virology*, 1963 (in press).

3.13. The Arrangements of Nucleotide Sequences in T2 and T5 Bacteriophage DNA Molecules. C. A. THOMAS, Jr. and I. RUBENSTEIN (Baltimore, U.S.A.).

The genetic map of T2 and T4 bacteriophage is circular but the DNA molecule that is liberated by phenol extraction is a linear duplex of polynucleotide chains. If the genetic map is related to the physical structure of the DNA molecule the problem arises as to how a linear molecule can give rise to a circular map. An explanation can be made on the basis that the bacteriophage liberate molecules which have nucleotide sequences which are circular permutations of each other. Thus, markers which are most distant on one molecule are closest together on another. To test this hypothesis, the middles of T2 and T5 DNA molecules were mechanically deleted and the absence of certain nucleotide sequences was tested by "renaturation" or "reannealing" experiments using columns containing denatured DNA immobilized in agar beads. The results indicate that when the middles are deleted from the T5 DNA molecule, some special sequences are removed; whereas, when the middles are deleted from the T2 DNA molecule, no special group of sequences are removed. This would indicate that the T2 molecule had a random beginning point while the T5 molecule has a special beginning point. These results would predict that T5 should have a single linear genetic map.

3.14. The Action of Streptonigrin on Genetic Recombination between Bacteriophages. MYRON LEVINE and MARCENE BORTHWICK (Ann Arbor, U.S.A.).

The antibiotic streptonigrin preferentially affects DNA metabolism in bacteria and is an excellent inducer of phage production in inducible lysogenic bacteria[1]. Streptonigrin also has a striking effect on phage recombination. Short treatments (as short as 2 min exposures to 10rg/ml of the antibiotic) of mixedly infected *E. coli* cells results in 2–3-fold increases in the frequency of recombination between T4rII mutations. Of special interest is the finding of a critical time during which infected complexes must be treated in order to obtain this effect on recombination. Increases in recombination frequencies are observed following treatment of infected cells early in the latent period, within the first 10 min. Treatments at later times in the latent period result in normal recombination frequencies. The data suggest a change in susceptibility to the action of streptonigrin on genetic recombination at about the 8th to 10th minute of the infection. Similar effects have been observed with phage P22 of *Salmonella typhimurium*.

1. M. LEVINE and M. BORTHWICK, *Bacteriological Proceedings*, 1963.

3.15. Correlated Variation of Recombination Potential and DNA Content among T4 Phage Particles. GISELA MOSIG (Cold Spring Harbor, U.S.A.).

A population of T4 phages is heterogeneous with respect to the following properties which appear to be correlated:

(a) potential for genetic recombinants.
(b) buoyant density in CsCl
(c) size of their DNA molecules.

Phages from prematurely lysed cultures show slightly greater buoyant density in CsCl, and, when used as parents in crosses, yield a lower fraction of recombinants for given markers than phages from spontaneously lysed cultures.

DNA isolated from unselected phage particles falls into three classes differing in molecular weight (recognized by column chromatography, sedimentation rate, and susceptibility to hydrodynamic shear). If unit length is assigned to the majority (class I DNA), class II DNA measures 0.6, and class III DNA varies between 0.05 and 0.2. The DNA from these classes does not differ in base composition.

Phages of different buoyant density differ in DNA content. Those of average density (the majority) contain a single molecule of class I DNA. Less dense phages yield many recombinants and contain DNA of class II. Dense phages yield few recombinants and contain DNA of classes I and III, or of class III only. A small fraction of very low density phages also contain DNA of class III only.

The following working hypothesis is proposed: The dense phage particles yield few recombinants because they are diploid over a wide range of their genome and the diploid state interferes with heterologous pairing.

3.16. Recombination of Purified DNA of Bacteriophage ΦX 174 in Spheroplasts. H. S. JANSZ, C. VAN ROTTERDAM and J. A. COHEN (Rijswijk (Z.H.), The Netherlands).

The DNA-spheroplast system[1] for the determination of the biological activity of purified DNA of bacteriophage ΦX 174 was employed in a biochemical approach to the elucidation of the mechanism of gene action. In order to study genetic recombination in this system phage and bacterial mutants were derived from ΦX 174 wild type (Φo) and its host *E. coli* C (Co) according to the method of D. Pfeifer.[2]

Φo is designated genetically as $h1^+h2^+$ indicating that it is able to be adsorbed to and to infect Co but not the Φo resistant bacterial strains C1 and C2. Φ1 ($h1\ h2^+$) is able to infect Co and C1 but not C2. Φ2 ($hi\ h2$) is able to infect Co, C1 and C2. Φ3 ($h1^+h2$) is able to infect Co but not C1 and C2. In a cross Φ3 × Φ1 recombinants Φ2 were obtained (recombination frequency 10^{-4}). The DNA's isolated (using phenol extraction) from purified batches of Φ1 and Φ3 were infectious to spheroplasts of *E. coli* K12, producing approximately 10^7 phages/ml. Mixed infection of spheroplasts of *E. coli* K12 with DNA's from Φ1 and Φ3 produced approximately 10^3 recombinants Φ2. The effects of u.v.-irradiation and chain scission by DNAse of one of the parental DNA's on infectivity and ability to form recombinants were studied. U.V. irradiation causing a fall in infectivity of DNA down to 5 per cent of its original value did not weaken its efficiency in giving rise to recombinants. However, chain scission of Φ3-DNA by DNAse corresponding to an average of 3 hits per molecule destroys its recombining faculty.

1. Cf. G. D. GUTHRIE and R. L. SINSHEIMER, *J. Mol. Biol.* **2**, 297, 1960.
2. *Z. für Vererbungslehre* **92**, 3, 1961.

3.17. The Response of Mutants of the Bacteriophage f2 to a Bacterial Suppressor Gene. NORTON D. ZINDER (New York City, U.S.A.).

Garen and Siddiqi have isolated a suppressor gene for certain alkaline phosphatase negative mutants of the bacterium *Escherichia coli*. This same suppressor gene can also suppress specific mutants of the "r2" type of the bacteriophage T4 (Benzer and Champe). The assumption is that this suppressor gene can work on mutants in many different cistrons.

Following nitrous acid treatment of the bacteriophage f2, we have obtained mutants which respond to this same suppressor gene. These phage mutants are being studied for their effects upon the bacteria without the suppressor gene. If, as supposed, mutations which respond to this suppressor can occur in all cistrons, by determining at what stage in their life cycle these mutants are blocked we can infer the genetic functions of the phage. The determinations include the production of new enzymes, infective RNA, phage coat protein and phage particles. In addition, RNA from the mutants is added to *E. coli* extracts and the nature of the protein products it stimulates determined.

3.18. Studies on Plaque Mutants of the RNA Phage fr. F. KAUDEWITZ and P. KNOLLE (Berlin-Dahlen, Germany).

Phage fr is related to the RNA phages described by Loeb and Zinder.[1] Its physical characteristics have been described by Hoffmann-Berling.[2] The occurrence of plaque-size mutants has been reported in phage suspensions treated with nitrous acid (Kaudewitz and Knolle.[3] Turbid mutants have been obtained by the same method. These mutants are stable upon cloning. They are inactivated by wild type fr antiserum, their burst size is smaller than that of the wild type phage. The infection of cells of *Escherichia coli* K-12 strains by these mutants is inhibited by RNase as is the infection by phage fr. The analysis of recombination experiments obtained from mixed infections involving a number of different mutants of this type will be presented. The data will be published in full in the *Zeitschrift für Vererbungslehre*.

1. T. LOEB and N. ZINDER, *Proc. Natl. Acad. Sci.* **47**, 282, 1961.
2. H. HOFFMANN-BERLING, *Z. Naturforschg.*, in press.
3. F. KAUDEWITZ and P. KNOLLE, *Nature*, in press.

3.19. Host-range Mutations of the Bacteriophage ØX-174 and the Specificity of Its Host E. coli. G. A. VAN ARKEL and J. H. VAN DE POL (Utrecht, The Netherlands).

The host-range phenotype provides a useful tool in genetic studies with bacteriophage ØX-174, which, because of the single-strandedness

of its DNA, has been of vivid interest to both biochemists and geneticists. We therefore studied some properties of host–phage relationships with this phage.

Plating wild type *E. coli* C122 with an excess of wild type ΦX-174 will result in the selection of phage-resistant bacteria (designated by C/O). Among them three types of C/O mutants could be distinguished, on the strength of differences in the frequence with which plaques of host-range phage mutants (Oh) were found after plating C/O cells with wild-type phage.

The largest class, some 80 per cent of all C/O isolates, comprises the mutants for which no host-range particles (i.e. less than 10^{-11}) were observed in wild-type lysates, not even after heavy mutagenic treatment with nitrous acid. These bacterial strains (C/OR) are considered to be resistant to all first step host-range mutants. On a second type of C/O bacteria ("normal", C/ON) small, turbid host-range mutant plaques (Oh^n) were found with a frequency of 3×10^{-9}. From Oh^n, but not directly from wild-type phage, mutants yielding clear plaques could be isolated (Oh^c). The host-range patterns of independently isolated strains of Oh^n and Oh^c are identical for all C/O indicator strains. A third, extremely rare, type of C/O mutants ("sensitive", C/OS) was characterized by a higher frequency (4×10^{-7}) of large, clear plaques of host-range mutants (Oh^s) found after plating with wild-type phage. C/OS also served as a host for Oh^n and Oh^c. On the other hand, C/ON was resistant to Oh^s.

Results obtained, using *Shigella paradysenteriae* Y6R as the host bacterium, partly paralleled those with *E. coli*.

Our findings will be discussed in relation to the mechanism of phenotypic expression of bacterial resistance and host-range properties of the phage.

3.20. Studies of Proteinless Mutants of Tobacco Mosaic Virus. A. Siegel and M. Zaitlin (Tucson, U.S.A.).

Two mutants of tobacco mosaic virus have been isolated which are unable to induce the formation of complete virus particles upon infection. Strain PM1 induces the formation of no detectable virus-like protein. Strain PM2 induces the formation of a non-functional, low molecular weight protein serologically related to tobacco mosaic virus protein. This virus-like protein, although it aggregates into rods under slightly acidic conditions, will not reconstitute with virus nucleic acid to form complete virus particles. The PM2 protein differs from parent strain protein in one of the first forty amino acids, starting from the acetyl-N end. Infectious material present in phenol extracts of PM infected leaves can be reconstituted with virus protein to form highly infectious particles. It has also been found that infectious nucleic acid can be separated from the bulk of the leaf RNA on methylated albumin columns.

3.21. Hereditary Changes induced by the Host in Bacteriophage Lambda. L. Fischer-Fantuzzi and E. Caleff (Naples, Italy).

A particular strain of K12 sensitive to lambda (called U 173) upon infection with the hd_1 mutant of bacteriophage lambda yields wild type progeny phage or lysogenic derivatives which in turn will release wild type phage.

Both vegetative and induced phage appear to be mixed, wild type and mutant.

The wild type and the mutant obtained from the growth on strain U 173 breed true.

This hereditary modification is associated with the appearance of phages of non standard density.

3.22. The Genetic Nature of the Exclusion of Bacteriophage T2 by T4 with Mixed Infection on E. coli B. B. de Groot (Leyden, The Netherlands).

Cross products of bacteriophage T4 and T2 show the efficiency of plating (e.o.p.) of T4 on certain *E. coli* K strains; they have the same glucose-substitution of HMC as T4, and the frequency of T4 markers is about 90 per cent. This phenomenon, the exclusion of T2 by T4, has been ascribed to one gene ("Bar") by Streisinger and Weigle.(1) The genetic nature of exclusion was investigated in the following ways:

(1) Single bursts of T4 T2 crosses were analyzed completely. The majority of the progeny particles showed the T4 host range. A few of them showed either the u.v. sensitivity of T2, or did not exclude the markers of T2, or appeared to plate with a low e.o.p. on *E. coli* K strains, like T2.

(2) Simultaneous infection of *E. coli* B with heavily u.v.-irradiated T4 and unirradiated T2 resulted in marker rescue of the e.o.p. of T4, when plating the infective centres on *E. coli* K (λ h). An isolated marker-rescue product did not exclude T2 markers on crossing. Both these experiments suggest that the "Bar"-properties

result from more than one factor and cannot be ascribed to one gene with a pleiotropic effect.

1. *P.N.A.S.* 1956.

3.23. Mutants of Prophage P1 which Affect Host-induced Modification. S. W. GLOVER (London, Great Britain).

Bacteriophage λ plates with an efficiency of 1.0 on *Escherichia coli* strain C 600 (K), and with an efficiency of 10^{-4} on K made lysogenic for phage P1. This phenomenon is called restriction.

The λ phage which comes through K(P1) plates with an efficiency of 1.0 on both K and K(P1) and is designated λ (P1). This is called modification. After one cycle of growth in K the modified phage λ(P1) loses its modification.

By several different methods to be described derivatives of K(P1) have been isolated in which the carried P1 prophage is abnormal. So far two types of abnormal P1 have been characterized. One type has lost the ability to restrict λ but is still able to modify it. The other type has lost both the restricting and modifying functions of P1. The results of genetic crosses between these abnormal P1 phages will be discussed.

3.24. Different DNA Systems undergoing Host-induced Modification in Bacteria. J. SCHELL and S. W. GLOVER (London, Great Britain).

In the system of host-induced modification by bacteria carrying prophage P1, λ DNA is restricted and modified (see previous abstract, S. W. Glover).

Data will be presented and discussed concerning the restriction and modification of DNA from other sources, including:

(a) Chromosomal DNA in conjugation.
(b) Episomal DNA in Flac infection.
(c) Colicin factors in colicinogeny transfer.
(d) Phages other than λ.

The nature of the abnormal P1 prophage described in the previous abstract (S. W. Glover) has been investigated with these systems.

3.25. Affinity of Coliphage P2 for Prophage Sites. ERICH W. SIX (Iowa City, U.S.A.).

Affinity of P2 for its prophage sites (I, II, etc.) on the chromosome of *Escherichia coli* strain C was studied. Usually strain C derivatives with P2 at site I liberate phage with strong preference for this site, whereas phage from donors carrying P2 at site II show no strong site preferences.

The site occupied in the donor cannot be solely responsible for the site affinity of the phages: P2 may retain its low site I affinity even while occupying this site in the donor, and the site I affinity of phages from donors carrying P2 at site II or other sites ≠ I varies.

P2 from donors producing phage with strong preferance for site I occasionally looses this preference when lysogenizing another cell. Donors producing phage with low site I affinity also produce some phages with strong site I preference.

All P2-like phages, including P2 Hy dis and PK, show some affinity for site I, even if liberated from donors other than *E. coli* strain C. But P2-related phage P4 shows no affinity for site I.

Existence of P2 in at least two states: one with high preference for a particular chromosomal site, one without particular site preference, resembles the behavior of fertility factor F. Since only relative affinities were measured, loss of site I preference may indicate either decrease of affinity for this site or increase in affinity for other sites.

3.26. On the Induction of Lysogenic Bacteria by Halogenated Pyrimidines. E. *Geissler* (Berlin, Germany).

The paper summarizes results of experiments on the induction of *Escherichia coli* K12 (lambda) by halogenated pyrimidines. An induction results, if the lysogenic bacteria are incubated in SS-medium (containing Bacto vitamin-free casamino acids) supplemented with 0.5 μg/ml of either 5-fluorouracil (FU) or 5-fluorodeoxyuridine (FUdR).

The inducing activity of FUdR is greatly enhanced by 200 μg/ml 5-bromouracil (BU)—which itself does not induce—even if an apparently sublethal concentration of FUdR (0.05 μg/ml) is used. BU is ineffective in combination with a further reduced concentration of FUdR (0.005 μg/ml), or if its own concentration is lowered (20 μg/ml).

The inducing activity of FU seems to be nonspecific with regard to the later stages of nucleic acid synthesis: The effect can be reversed by 200 μg/ml BU, 0.5 μg/ml uracil, 25 μg/ml deoxycytidine (CdR) or 200 μg/ml thymidine. These substances revert the bacteriocidic action of FU too, but a nearly normal multiplica-

tion-rate was achieved only by addition of CdR. In modified SS-medium (containing normal Bacto casamino acids instead of the vitamin-free preparation) FU does not induce but still shows its bacteriocidic activity. Investigations with FU in SS-medium supplemented with vitamins are in progress. Preliminary results show that several vitamins are apparently able to reduce or to abolish the inducing action of FU without interfering with its bacteriocidic activity.

These results suggest that the halogenated pyrimidines used exert their inducing action by a rather unspecific disturbance of (nucleic acid) metabolism of the lysogenic cells so treated. There are no reasons for assuming a direct action of these agents on the deoxyribonucleic acid of the prophages or on DNA synthesis. Other experiments on the inducing effect of ultraviolet- and X-rays on BU-labelled lysogenic cells indicate that ultraviolet rays too do not induce by a direct action on DNA.

The paper will be published in *Acta Biologica et Medica Germanica* (Berlin).

3.27. Genetic and Environmental Factors affecting Prophage Induction. MARGARET LIEB (California, U.S.A.).

A prophage has one or more genes whose product(s) apparently repress the function of other genes involved in phage replication. Although the repressor may be a protein, DNA and/or RNA are probably the sites affected by many treatments that induce phage development in lysogenic bacteria. In some lysogenic bacteria, the phage system(s) blocked by the hypothetical repressors are extremely sensitive to induction (derepression) by u.v. light. Methyl purines and acridines, which enhance mutation in u.v.-irradiated bacteria, also enhance induction of lambda prophage replication. These substances combine with DNA and presumably distort the molecules in previously denatured regions. In this way, they either prevent reversal of u.v. damage by a dark repair system in the bacterium, or otherwise increase the probability that an absorbed u.v. quantum will result in mutation or induction. These substances do not enhance prophage induction by agents other than u.v. and have little effect on killing of non-lysogenic bacteria.

We have also studied a lambda prophage that is unusually sensitive to induction by u.v. For this prophage, photoreversal of induction is less for a given dose of u.v. than in wild-type lambda. But if doses giving the same surviving fraction of lysogenic bacteria are compared, the u.v.-sensitive prophage is at least as susceptible to photorepair as wild-type lambda. Acridines and methyl purines do not enhance u.v. induction of u.v.-sensitive lambda. These results can be attributed to the inability of the u.v.-sensitive phage to undergo dark repair of u.v. damage to the nucleic acid region that controls production, or acceptance, of repressor.

When lysogenic bacteria are forced to incorporate 5-bromouracil, their prophages become more sensitive to u.v. induction. Acridines and methyl purines enhance u.v. induction in such bacteria, but there is no photoreversal of induction. Additional data indicate that the photo- and dark repair systems act on the same u.v. damaged sites in nucleic acid, but act independently.

3.28. Transfer of Information determining Lysogenization in Bacteria by Transforming DNA. B. GYÖRFFY (Budapest, Hungary).

Immunity to subsequent superinfection with closely related phages to that carried as prophage is a general property of lysogenic bacteria. This immunity could be transferred by the DNA isolated from the lysogenic strain 41/63-3/ of *Rhizobium meliloti* into the sensitive strain 41, on which a brief preliminary report was already published.[1] Further experiments were performed, involving bacterial DNA and phage DNA labelled with ^{32}P and/or ^{14}C, and transformations with DNA isolated from temperate phages, respectively, to gain more insight into the process of transfer of the capacity to establish the lysogenic complex in the recipient bacteria after transformation. The results obtained presenti reliable evidence to separate the transforming efficiency of the bacterial DNA and prophage DNA, respectively, involved in the transformation of immunity to superinfection by the DNA isolated from the lysogenic strain.[2]

Studies on this line also are in progress with *Bacillus subtilis*, where DNA from the lysogenic strain 3NT is used to transform the recipient strain 168, and the results on transformation

---

1. K. SZENDE, T. SIK, F. ÖRDÖGH and B. GYÖRFFY, *Biochim. Biophys. Acta* **47**, 215-217, 1961.
2. T. SIK, B. GYÖRFFY, K. SZENDE and M. CZAKÓ, to be published in *Acta Microbiol. Hung.*, 1963.

of the information determining lysogenization will be presented.[3]

The possible interpretation of the process of transformation of lysogenization and immunity in line with current ideas of the specific repressor control mechanism will be discussed.

3. B. Györffy, K. Hatnay and I. Kállay, to be published in *Acta Microbiol. Hung.*, 1963.

3.29. Bacteriophage Transformation. G. Veldhuisen, R. A. Oosterbaan, P. H. Pouwels, H. S. Jansz and J. A. Cohen (Rijswijk (Z.H.), The Netherlands).

Transformation of bacteriophage T4 with purified DNA has been demonstrated earlier.[1] Spheroplasts of *Escherichia coli* B were mixed with a purified DNA preparation obtained from T4rII$^+$ phages and infected with urea-disrupted particles of a T4rII-mutant. By plating on *E. coli* K 112-12 (λ h) # 3 and on *E. coli* B the yield of transformants and total phage yield were determined respectively; the rII$^+$/rII ratio (transformation frequency) varied between 10^{-6} to 10^{-7} for native DNA using a concentration of approx. 10 μg/ml.

We wish to report some further results:

(1) More reproducible results were obtained using spheroplasts prepared from cells that were grown to exhaustion in minimal medium plus 0.04 per cent glucose.

(2) Heating of the DNA preparation (5 min at 100° C) in 0.01 M phosphate buffer pH7 increases the transformation frequency and also the total number of transformants by about 5–10 times.

(3) The increase in biological activity after heating follows the increase in optical density at 260 mμ measured after rapid cooling of samples that had been heated at various temperatures.

(4) DNA molecules fragmented mechanically to a weight average molecular weight of about 275,000 were still biologically active. Degradation by means of limited amounts of DNA-ase gave similar results.

(5) The transformation of T4rII mutant is equally effective with DNA prepared from T2, T4 and T6. Among the transformants, obtained using T4rII$^+$-DNA and disrupted T6rII, some are able to grow on B/6 suggesting that other T4 markers may be inherited.

1. Cf. Veldhuisen *et al.*, *Biochim. Biophys. Acta* **61**, 630, 1962.

3.30. Electronmicroscopic Studies of Cells and DNA Molecules during the Genetic Transformation of Bacteria. Alexander Tomasz and Walther Stoeckenius (New York, U.S.A.).

Competence: The ability of certain bacterial cells to absorb highly polymerized biologically active DNA molecules in the process of genetic transformation must necessarily involve the adsorption of the molecules to the cell surface and their eventual penetration through the bacterial surface structures. Attempts are being made to study this process in the electron microscope. Conditions will be described under which the method of Kleinschmidt[1] can be adapted to visualize DNA strands in association with competent bacteria.

The particular question asked is whether there is any localization on the bacterial surface for the adsorption and entry of DNA molecules. Thin sections of pneumococcal cells[2] revealed the existence in these cells of unusual membrane-like structures which were often attached to the developing septum of dividing cells. In view of the suggested correlation between the division state of the cells and their competence[3] this observation directed our attention to the septum of dividing cells as possible sites for the entry of DNA molecules into the cell.

1. *Z. f. Naturf.* **16b**, 730, 1961.
2. Publication under preparation—Drs. J. Jamieson and E. Ottolenghi.
3. Hotchkiss, *Proc. Nat. Acad. Sci. U.S.* **40**, 49, 1959.

3.31. Some Factors affecting the Frequency of Streptomycin Resistant Transformants throughout the Evolution of Competency. M. Kohoutová, H. Kopecká, and J. Koníček (Prague, Czechoslovakia).

Two different transformation media for the transformation of the streptomycin sensitive strain of *Pneumococcus* (PMSIIB) to the streptomycin resistant one were used. (1) Neopeptone-heart infusion broth enriched with some ingredients and (2) the so-called NS-medium (H. E. Taylor). With both media we obtained similar results as in the type-transformation experiments previously performed in our laboratory, concerning the presence of salts with monovalent cations and their support in a good transformation yield.

The competent recipient culture was diluted into the appropriate transformation medium

with donor DNA. To the medium KCl salt was added to the final concentration of 0.075, 0.15, 0.2 and 0.3 M. The number of transformants rose 6, 60, 120 and 20 times respectively in comparison to the control without the added salt. The optimal concentrations of 0.15 or 0.2 M KCl were later used in the transformation reactions. It was found that the salt showed its effect only in the time of evolution of competency when the rising tendency of competency in the recipient culture was noticed and not during the falling one.

When small inocula of the recipient culture were used and sometimes two waves of competency were obtained, the presence of KCl salt was still more effective in the rising period of the second wave of competency. While in the first rising period the difference between the control without the added salt and the medium with the salt was 2 to 5 times higher, the yield of streptomycin resistant transformants in the second rising period was even 10 to 15 times higher.

This effect of salts with monovalent cations is not to be confused with the effect of divalent cations (Fox and Hotchkiss).

If we used in the transformation medium different bovine serum albumin preparations (BSA) in which various amounts of residual contents of deoxyribonuclease could be determined, we also found different answers to the presence of KCl salt added to the medium. Higher amounts of DNAse in BSA (or in the cultivation medium) require the presence of KCl salt for a good yield of streptomycin resistance transformants (1–10 per cent) while with a very low content of deoxyribonuclease the presence of KCl salt is inhibitory.

Series of our results may indicate that the presence of small amounts of DNAse in the transformation medium may play its active role in the transformation reaction when properly counterbalanced.

3.32. Genetic Transformation Among Living Pneumococci in the Mouse. Elena Ottolenghi and Colin M. Macleod (New York, U.S.A.).

Experiments were performed to determine whether deoxyribonucleate-mediated transformation could take place spontaneously among genetically distinguishable pneumococci growing side by side in the same living host, the mouse. It was found that when living, unencapsulated, nonpathogenic, streptomycin-resistant pneumococci and living, streptomycin-sensitive pneumococci which synthesized only a small amount of type III polysaccharide capsule were injected sequentially into the peritoneal cavity of mice, recombinants could be recovered from about 30 per cent (26/83) of the mice. The recombinants were streptomycin-resistant, synthesized type III polysaccharide in almost the full amount, and were more pathogenic for mice than either parental type. The recombination was most likely a result of deoxyribonucleate-mediated transformation since it could be completely prevented by exogenous deoxyribonuclease. It may be, therefore, that transformation can serve as a mechanism of recombination in nature for some microorganisms such as pneumococci.

This data will be submitted for publication in the *Proceedings of the National Academy of Sciences of the United States* in 1963.

3.33. Linkage of Genetic Factors affecting Similar Function and Derived from Different Bacterial Species. Arnold W. Ravin and Joscelyn de Sa (Rochester, U.S.A.).

Certain strains of streptococcus and pneumococcus are known to be capable of transforming each other by means of their respective deoxyribonucleic acids (DNAs). In pneumococcus, moreover, various spontaneous mutations that confer different levels of resistance to streptomycin (*str-r* mutations) have been shown to be genetically and physically linked. In DNA-mediated transformations of pneumococcus, for example, *str-r* mutations conferring high levels of resistance generally replace (i.e. are allelic with) *str-r* mutations conferring low levels of resistance. In order to determine whether *str-r* mutations arising spontaneously in streptococcus and pneumococcus were also genetically linked, therefore, a streptococcal mutant resistant to a low streptomycin concentration was exposed to DNA from a pneumococcal mutant capable of resisting a high antibiotic level. Conversely, pneumococcal mutants capable of resisting only low concentrations of the antibiotic were exposed to DNA from a streptococcal mutant capable of resisting high streptomycin concentrations. Transformants produced in such interspecific reactions were isolated, measured for level of streptomycin-resistance, and DNA prepared from them for test on streptomycin-sensitive strains. The latter test was undertaken to determine whether the genome of the transformed bacterium continued to harbor its original *str-r* mutation and, if so, whether the original mutation was linked to the

introduced transforming marker, or whether the original mutation had been replaced by the introduced marker. In inter-specific transformations involving two low-level and one high-level pneumococcal mutations, one low-level and one high-level streptococcal mutations, the results demonstrate that mutations affecting a similar function (streptomycin-resistance) but arising in different bacterial species are genetically linked.

3.34. Biochemical and Genetic Studies of Integration and Recombination in the B. subtilis Transformation. WALTER F. BODMER and A. T. GANESAN (Palo Alto, U.S.A.).

DNA was isolated from a multiple marked strain of *B. subtilis* grown on N^{15} H^2 medium with added H^3 thymidine. Competent cells were prepared in an N^{14} H^1 medium containing P^{32}.

Using the labelled DNA as donor, transformation was terminated at 30 min with deoxyribonuclease. The DNA purified from the donor-recipient complex was fractionated pycnographically. Donor and recipient DNA differed in buoyant density and were traceable by differential counting of P^{32} and H^3. Donor atoms were found in density strata corresponding to native donor but also in native recipient, hybrid, and "denatured" donor DNA. These DNA fractions were assayed for their genotypic content by transformation. Donor and recombinant genotypes were found in all of these strata but the last, which is biologically inactive.

The association of donor atoms (i.e. H^3) with a recipient stratum persisted on refractionation of the DNA, and was also found when transformation was terminated after only 10 min. The number of donor atoms found in the other strata decreases when the cells were allowed to grow after transformation. However, the H^3 count in the recipient DNA stratum remains approximately constant. The continuous distribution of H^{33} label over strata of varying densities implies the integration of donor DNA segments, which were considerably smaller than the average size molecule present in our preparations of transforming DNA.

3.35. DNA - mediated Transformations to Improved Enzymatic Activities. STEPHEN ZAMENHOF and LILO H. HELDENMUTH (New York, U.S.A.).

A mutation resulting in an improvement in enzymatic activity (and, sometimes, in survival or in adaptive value) over and above that of a wild strain, may be caused by a change in regulatory mechanisms or an "improvement" in the structural gene determining the enzyme. At least the latter should be a change in DNA and might be transferable by DNA-mediated transformation.

In the present study several spontaneous mutants of *Bacillus subtilis* were isolated having various enzymatic activities improved over those of the wild strain. The DNA of some of these mutants was able to transform the wild strains in a single step to strains with improved enzymatic activities. The transformable improvements studied to date were: (1) In utilization of citrate as the only carbon source; (2) In production of paraminobenzoic acid (PABA); (3) In production of phenylalanine. Other improvements will also be discussed. Sulfanilamide-resistant strains of *B. subtilis*, first isolated by S. Greer in this laboratory, did not require sulfanilamide for growth but were better PABA-producers than the wild-type; both features are transformed concomitantly, in both directions. Some of the β-thienylalanine-resistant mutants were better phenylalanine-producers; both features are also transformed concomitantly. The improvements that could not be transferred by a single step transformation were those which accumulated in different strains as a result of more than one mutation, e.g. slime production, or utilization of pyruvate as the only carbon source.

3.36. Participation of RNA in the Transfer of Genetic Information of a Virus of the DNA-type. S. GERSHENSON (Kiev, U.S.S.R.).

Injection into healthy larvae or pupae of *Bombyx mori* of RNA isolated from those infected with nuclear polyhedrosis virus causes a high frequency of the disease, whereas RNA from healthy *B. mori* is non-infectious. The technique used for isolating the infectious RNA (extraction with phenol, high speed centrifugation) makes it improbable that the infectivity of the preparations is due to an accidental admixture of the virus. This possibility is further disproved by the fact that these preparations are infectious for larvae of *Pieris brassicae* wich are completely immune to the virus itself. The virus developing in *P. brassicae* infected with RNA from virus-diseased *B.mori* is infectious for *B.mori* but not for *P.brassicae*. Infectivity of the RNA preparations from virus-diseased *B.mori* is destroyed by RN-ase but not by DN-

ase. Dilution of the preparations leads to an approximately exponential decrease of infectivity. Two alternative explanations are suggested: transfer of genetic information of the virus by means of RNA synthetized by infected host cells, or formation in these cells of an infectious RNA-DNA complex. Resistance of the infective principle to DN-ase and its susceptibility to RN-ase seem to speak in favour of the first explanation.

3.37. DNA Transformation for Oxytetracycline Resistance in B. subtilis. M. POLSINELLI (Pavia, Italy).

A strain of *B.subtilis* resistant to about 300γ/ml of oxytetracycline has been produced through multistep selection and the DNA prepared from it used for transforming a sensitive strain.

Transformation was carried out with such DNA and selection of transformed bacteria performed on media containing a minimal inhibiting concentration of oxytetracycline. The degree of resistance of transformed clones was individually tested. The population of the transformed bacteria shows a trimodal distribution of resistance suggesting that at least two genes are involved in the process.

Transformation by means of DNA taken from clones belonging to the first and second peak of the distribution shows that such clones are transmitting a single genetic factor for oxytetracycline resistance, while DNA from clones of the third peak transforms sensitive bacteria in a way similar to that of the original resistant strain. Further results will also be discussed.

3.38. Multi-site Mutations and Deletions. MINNA B. ROTHEIM and ARNOLD W. RAVIN (New York, U.S.A.).

By means of DNA-mediated transformations in pneumococcus 13 spontaneous mutations to streptomycin resistance have been mapped at only three sites, which may be designated A, B and C. Mutations occurring at site C replace, but never combine with, those at sites A and B. For this reason mutations at site C are considered to be multisite mutations; such mutations have generally been considered to be deletions. Marker *str-r41* has been mapped at site C. In one culture of *str-r41* a new mutant appeared having a hundred-fold lower level of resistance. Experimental data indicate that the lower level of resistance is not due to the action of a suppressor on a still present *str-r41* mutation, but due to a new mutation. This latter mutation has been called *str-r42*, and in transformation reactions may be replaced by, but never combines with, marker *str-r41*. Moreover, by recombining with site A and B mutations, the *str-r42* mutation is shown to occupy a new site (D). Therefore, marker *str-r42* lies within the region covered by the mutation *str-r41*, suggesting that marker *str-41* may partially revert to leave a small mutated segment which confers a lower level of streptomycin resistance. Defining deletions operationally as mutations incapable of back mutation within the same segment of genetic material that was involved in the original mutational event, these results establish *str-r41* as a multisite mutation that is not a deletion.

3.39. Transformation of Genetic Traits Associated with Sporulation in Bacillus subtilis. JOHN SPIZIZEN, BERNARD REILLY, and B. DAHL (Minneapolis, U.S.A.).

Studies in *Bacillus* species strongly indicate an association of a number of enzyme systems formed in the post-logarithmic growth period with the sporulation process. Thus the formation of proteolytic and cell-wall lytic enzymes appears to be associated with sporulation. A genetic approach to this problem has been initiated with the finding of a linkage group containing genes which control the synthesis of a proteolytic enzyme, a wall-lytic factor or enzyme, and sporulation. These studies were made with transformable *Bacillus subtilis*, strain 168. Asporogenous mutants unable to synthesize protease and the lytic factor could be transformed with deoxyribonucleic acid (DNA) isolated from sporogenous strains with the production of sporogenous organisms containing also genes for protease and lytic factor.

Partially asporogenous mutants have also been found which contain the genes for protease and lytic factor. The asporogenous defect in such mutants appears to have arisen from a separate locus, so that sporogenous recombinants could be obtained with crosses of the two asporogenous types. Evidence will also be presented which strongly suggests that certain partially asporogenous mutants are heterogenotic for some regions of the sporulation genome.

3.40. Genetic Studies on Sporogenesis of Bacillus subtilis. I. TAKAHASHI (Ottawa, Canada).

By the use of temperate bacteriophage PBS 1,

it was possible to demonstrate transduction of sporogenesis in an asporogenous strain (Sp^-1) of *Bacillus subtilis*.[1] It was also reported that the locus *sp-1* was linked to prototrophy and to streptomycin and erythromycin resistance loci at different frequencies. Examination of sporogenous (sp^+) recombinants obtained from a number of other asporogenous (sp^-) strains by transduction with SB19-ery (*prot*, sp^+, *str-r* and *ery-r*) as donor, revealed that three distinct *sp* loci were linked to amino acid markers (serine, phenylalanine and tryptophan-phenylalanine) at relatively high frequencies. By reciprocal transduction performed with fourteen sp^- strains, nine genes which govern spore formation in this organism were recognizable. The finding that certain *sp* loci are linked to amino acid markers or to antibiotic markers and the results of the reciprocal transduction experiments suggest that these sp^- characters are under the control of chromosomal determinants. In addition, an anomalous recombination pattern was observed with strain Sp^-HI2-3. This strain was never transduced to sp^+ even when a wild type donor was used, although this strain could act effectively as donor for other sp^- strains and was transducible for prototrophy and erythromycin resistance. The mechanism responsible for this anomalous recombination will be discusesd.

1. *B. B. Res. Comm.* **5**, 171, 1961.

3.41. Inheritable Changes in the Nitrogen Assimilation of Bacillus subtilis. István Turtóczky and Imre Fedorcsák (Budapest, Hungary).

The growing and the nitrogen assimilation of *B. subtilis* C-46 (Am^-) and C-46 (Am^+) as well as *B. subtilis* wild types have been studied in aerated synthetic medium containing different nitrogen sources.

The mutant C-46 (Am^-) is not able to the assimilation of ammonia. It requires glutamic acid to its growth. In a medium in which the sole nitrogen source is glutamic acid, autolyis takes place regularly in the 18th hour. In the filtrate of autolysat it has not been possible to find the presence of infective phage particles. The autolysis does not take place if $(NH_4)_2SO_4$ or $NaHCO_3$ is also present in the medium besides glutamic acid; or if 20–30 vol. per cent CO_2 is mixed to the air used for aeration. The autolysis does not occur if the nitrogen source in the medium is only glutamic acid but if the Mg^{++} concentration of the medium is reduced from 1.0 mM mg/l. to 0.1 mM mg/liter. In this case in the 18th hour the growth stops and in the next 24 hours neither autolysis nor further growth was to be observed. From the filtrate of such cultures it is possible to isolate a factor (probably polypeptide) which inhibits the growth of the producing strain. The inhibiting effect can be antagonized with proteins or with denaturated proteins.

The mutant C-46 (Am^+) and the *B. subtilis* wild types grow well on a synthetic medium in which the sole nitrogen source in $(NH_4)_2SO_4$. In these cultures autolysis does not take place.

The activity of ALA-dehydrogenase, which—according to literature—probably plays a role in the ammonia assimilation has been determined in the lysozym-lysat of cells.

In the lysate of (Am^-) cells active (100 per cent) ALA-dehydrogenase, in the lysate of wild type reduced (10 per cent) ALA-dehydrogenase have been found. It is not possible to find ALA-dehydrogenase in the lysozym-lysat of C-46 (Am^+) cells.

The results indicate that in the case of *B. subtilis* the ammonia is not assimilated by means of ALA-dehydrogenase. Presumably the repression of the process of ammonia assimilation is simultaneous with the induction of ALA-dehydrogenase.

3.42. The Initial Events in Transduction of Galactose Fermentation in Salmonella typhimurium Cell. J. Hubáček (Prague, Czechoslovakia).

The frequency of the transduced cells is reduced by the transfer of the *Salmonella typhimurium* recipient cells in the early period after the adsorption of the transducing phage from a complete to a minimal medium and subsequent cultivation for 90 min at 37°C. The potentially transduced cells are most sensitive to this treatment at the onset of transduction process (3 min after the adsorption of the phage) and then after cultivation in complete medium the sensitivity gradually decreases. After 90 min cultivation in complete medium the frequency of transduction is practically not influenced by the transfer of the cells to minimal medium.

In order to time the period of the incorporation of the transducing element its elimination in the non-integrated form has been followed using acriflavine. It was shown that the incorporation requires the multiplication of the cells and takes place during the period from the onset of growth up to 110–120 min of cultivation (2–3 divisions). The sensitive phase of transduced cells to the minimal medium precedes this incorporation event. We assume, therefore, that the reduced

frequency of the transduced cells in the minimal medium is due to the recovery of the cell from the transducing element when it is in the non-integrated form.

Further experiments have shown that the reduced frequency of transduction can be achieved not only by transferring the cells from complete to minimal medium, but also by chilling the cells in complete medium from 37°C to 0°C. When the cells were treated in such a way, 10^{-x}20 per cent of the amino acid pool was lost while some amino acids were removed quantitatively. Biochemical analysis of this fact leads to elucidation of the importance of amino acid pool in the genetic expression of a new marker.

3.43. The Biochemistry of Dissociation of Bacillus brevis GB. V. N. Stoletov, S. V. Shestacov, V. M. Glazer, V. D. Filippov (Moscow, U.S.S.R.).

The comparative biochemical examination of the dissociating forms of *Bacillus brevis* GB has been carried out. The chemical constitution of cells, the content of some significant metabolites, some enzymic activities, and the occurrence of some metabolic processes have been investigated in rough R-form, smooth S-form, and in two variants of mat flat M-form of *B. brevis*. R-form and variants of M-form are known to synthesize the gramicidin C in contrast with S-form and second M-form variant.

All studied variations of *B. brevis* have the same nucleotide composition and DNA content. Gramicidin-producing variants have been shown to content the higher amounts of RNA. Flat forms differ from R- and S-forms in their free amino-acid and polypeptide content and composition, and the content of free nucleotides and some phosphorus-containing fractions.

The role of cell membrane in metabolic changes in the time of dissociating transition are discussed with respect to the study of respiratory characteristics of intact cells and protoplasts of various dissociating forms of *B. brevis*. Specific differences in lipide and polysacharide composition have been found for three forms of dissociation.

The activity of some amino-acid metabolism enzymes of dissociating forms has been investigated in respect to their capability to enzymic induction, repression, and feed-back inhibition.

Obtained results are interpreted as being due to existence of correlation between certain biochemical and cultural features in various forms of *B. brevis*. The data of biochemical analysis of dissociants are regarded from the point of view of detection of regularity in metabolic changes in the time of bacterial dissociation. The latter is assumed to be a form of displaying the special adaptive variability of microorganisms.

3.44. Genetic Analysis of Streptomycin Dependence in Proteus mirabilis. Helmut Böhme (Gatersleben, Kreis Aschersleben, Germany).

By selection in the presence of streptomycin several different streptomycin dependent (str-d) mutants can be obtained from the same wild type strain of *P. mirabilis*. Most of these str-d mutants require for growth in minimal medium besides streptomycin the aminoacids methionine, cysteine and arginine. All str-d mutants can be transduced to nondependence by phages grown in wild type (str-s) cells. Transduction with phages propagated in str-r mutants results exclusively in str-r transductants; str-s, str-r and str-d thus being allelic. Since in transductional crosses between nine different str-d mutants no wild type recombinants were obtained, the str-d mutations seem to be multi-site mutations. By selection on streptomycin-free nutrient agar streptomycin nondependent revertants can be obtained from str-d mutants. In most of the str-d strains two types of revertants result: auxotrophic and prototrophic revertants. The numerical relation between these two types as well as the growth factor (isoleucine and valine, in other cases arginine) required by the auxotrophic revertants, are specific for each str-d mutant. The auxotrophic revertants arise as result of a mutation in a suppressor locus; it can be shown by transduction with phages grown in these revertants that they still contain the str-d allele. The suppressor could not be transferred by transduction into str-d strains. The auxotrophic revertants can be transduced to prototrophy using phages grown in wildtype bacteria. The resulting prototrophic transductants further contain the suppressed str-d allele. Thus in spite of the always simultaneous origin of the suppressor and the growth factor requirement, both can be separated by transduction.

3.45. A Genetic Basis for Changing Virulence in Two Plant Pathogenic Fungi. E. W. Buxton (Harpenden, Great Britain).

Mutants of physiologic races of *Fusarium oxysporum* and *Verticillium albo-atrum* resistant to acriflavine or actidione were used to show how heterokaryosis and asexual recombination

produce biotypes with increased virulence and wider host range. Heterokaryosis alone, without mitotic recombination, immediately increases virulence when two mildly virulent strains anastomose. The nuclear ratio in inoculum of heterokaryons containing nuclei of labelled pathogenic and non-pathogenic strains determines the extent of wilt in the host, the rate the rhizosphere is colonized and the ability to survive in soil. Haploid nuclei bearing genes conditioning virulence are selectively increased in heterokaryons during host infection and their rate of multiplication in the heterokaryon is directly related to the development of wilt symptoms. Similarly, material released from host roots into the rhizosphere selectively increase nuclei conditioning virulence, but a few nuclei from non-pathogenic strains also enter the host as components of a heterokaryon. In soil, a few nuclei conditioning virulence survive in heterokaryons largely composed of nuclei from purely saprophytic strains. Heterokaryotic chlamydospores, the resting bodies of *F. oxysporum*, have been isolated from soil.

The control of both the parasitic phase and survival in soil through adjustments of the nuclear ratio in heterokaryons provides the wilt fungi with a rapidly adapting mechanism that helps explain some of their variability.

3.46. Utilization of a Dikaryotic Organism (Coprinus radiatus) for the Study of Lethals. MADELEINE GANS (Gif-sur-Yvette, France).

A method is described which enables us to discover, preserve and study from a genetical point of view the compensable and non-compensable lethal mutations (according to Atwood) induced in a dikaryotic mycelium.

Preliminary results show that from about twenty lethals induced by nitrous acid, two only are compensable by the growth medium.

3.47. Genetic Control of Conidiation in Aspergillus rugulosus. R. W. TUVESON and D. O. COY (Chicago, U.S.A.).

A strain of *Aspergillus rugulosus* isolated from sand in the Indiana Dunes State Park produces abundant cleistothecia when grown on complex and minimal medium at 37°C. Under these conditions, conidial production is limited. When this isolate is incubated at 25°C for extended periods (14 days or more), conidia are produced abundantly at the colony periphery. These conidia when transferred to complex or minimal medium and incubated at 37°C give rise to non-conidiating, typical wild type colonies characterized by abundant cleistothecial formation. Ascospores of this isolate were exposed to ultraviolet light for a period of time such that 2.5 per cent of the ascosporessurvived the treatment. Among the surviving colonies, three strains were isolated which were capable of producing conidia abundantly over the entire colony surface at both 25 and 37°C. One of the conidiating mutants was selected for the isolation of conidial color, auxotrophic and antimicrobial agent resistant mutant strains. Since the conidial mutant retained the ability to form cleistothecia, the markers carried by the conidial strain have been mapped based on random ascospore analyses. Additional conidial mutants have been isolated from the original non-conidial parental strain. Each conidial strain has been marked genetically and intercrossed with other conidial mutant strains in an attempt to locate the gene or genes which control conidiation in this strain. Since the conidial strains differ from one another morphologically, it is probable that more than one locus is involved or that there exist several alleles of the same gene.

3.48. Sexual Polarity in Streptomyces. G. SERMONTI, I. SPADA-SERMONTI and S. CASCIANO (Rome, Italy).

A group of intersterile strains (R^-) has been found in *Streptomyces coelicolor* A3(2). They are fully fertile with all the other strains, which in turn are fertile among themselves. In summary:

$$R^+ \times R^+ = \text{fertile}$$
$$R^+ \times R^- = \text{fertile}$$
$$R^- \times R^- = \text{sterile}$$

In $R^+ \times R^-$ crosses, the contribution to the progeny of markers of the R^+ strain is, as a rule, reduced as compared with that of the R^- markers, although in rare cases the reverse is observed.

The transfer (or integration) of the R^+ markers of the two linkage groups appears to be correlated in the different zygotes. When defects are observed on the R^- side, the deficiencies in the two linkage groups also appear to be correlated.

3.49. Directed Mutation of the Mating Type Alleles as an Explanation of Homothallism in Yeast. DONALD C. HAWTHORNE (Seattle, U.S.A.).

Hybrids of homothallic *Saccharomyces chevalieri* and heterothallic *Saccharomyces cerevisiae* yield asci in which two of the spores give rise to heterothallic cultures, i.e. they remain haploid and show a mating reaction when mixed with

test strains. The other two spores form homothallic cultures which readily diploidize and then are capable of sporulation. The 2: 2 ratios in the asci reflect the segregation of a single gene, *D*, for diploidization or homothallism. Gene *D* segregates independently of the mating type locus. Although gene *D* is seemingly epistatic to the mating type alleles, *a* and *x*, it in reality acts by causing their mutation. Under the influence of gene *D*, either mating type gene mutates to the other allele at rates which range from one mutation per two cell divisions to one per thirty cell divisions. The haploid cells fuse as soon as the mutant allele is expressed and the zygotes give rise to stable diploids which are heterozygous for mating type. The heterozygous condition of the mating type alleles blocks any further action of the gene *D*.

3.50. Establishment of Respiratory Capacity during Zygote Formation between Complementing Respiratory Deficient Mutants of Yeast. H. JAKOB and A. SELS (Gif-sur-Yvette, France).

Two classes of respiratory deficient mutants are known: genic mutants called segregational "petites" and cytoplasmic mutants called vegetative "petites", both lacking several respiratory enzymes especially cytochrome-oxidase. The cross of two haploid complementing strains (ex. g. cytoplasmic petite genic one, or between two non allelic genic mutants) gives rise to diploid cells able to synthesize the complete respiratory system.

We have studied the kinetics of the establishment of respiratory capacity in such crosses involving p5, p7 and ρ^- neutral mutants, during the zygote formation, and immediately after it. Every cross shows characteristic kinetics which can be followed by the overall respiratory rate and/or by cytochrome-oxidase activity of the extracts. In certain crosses a greatly enhanced respiratory activity is brought about immediately after the zygote formation.

These experiments will lead to more precise knowledge about the interactions between the different genes or between these latter and the cytoplasmic factor.

3.51. The Tetrade Analysis of Some Bakers' Yeasts. L. SEDLÁROVÁ (Bratislava, Czechoslavakia.

In some hybrids and production strains of the bakers' yeasts a genetic analysis has been made of cell and giant colony shape, fermentation characters and the production of biomase. The results of this analysis will be discussed, together with the process of spore copulation.

3.52. Directed Hereditary Changes of Fermentative Properties of Yeast and Indirect Selection. K. V. KOSSIKOV and O. G. RAIEVSKAIA (Moscow, U.S.S.R.).

Mutational change of fermentative properties of yeast *Saccharomyces globosus* induced by specific substrate (sucrose) can be markedly enhanced by changing the cultivation conditions. The greatest effect is obtained by increasing the sucrose concentration in the media (up to a certain limit).

The joint results show that the hereditary changes of fermentative properties in yeast (in the present case the appearance of their ability to ferment sucrose) are associated with their metabolic activity; this fact does not allow to connect these results with the selection of spontaneous mutations.

In order to confirm the absence of spontaneous mutations of the character that is being studied in the above-mentioned experiments on directed changes, tests with indirect selection are being carried out.

The results obtained show the ineffectiveness in the present case of indirect selection. The results will be discussed with due regard of the additional experimental data.

3.53. Regulatory Mutations Concerning Threonine and Methionine Biosynthetic Enzymes in Saccharomyces cerevisiae. H. DE ROBICHON-SZULMAJSTER and W. SLY (Gif-sur-Yvette, France).

In *S. cerevisiae*, methionine and threonine share a common synthetic pathway which branches from homoserine. The common steps include:

$$\text{Aspartate} \xrightarrow{(A)} \text{Aspartyl-phosphate} \xrightarrow{(B)} \text{Aspartic semi-aldehyde} \xrightarrow{(C)} \text{Homoserine}$$

In addition to the control they can exert on enzymes of their own branch (after homoserine), each end product control its individual rate of synthesis, by repression and feedback inhibition of different enzymes in the common pathway. Thus, enzyme A is inhibited and repressed by threonine(1) and enzyme C is inhibited and repressed by methionine (2). Structural genes for these two enzymes are unliked(3).

An attempt has been made to find regulation

mutants, using resistance to amino acid analogues for selection. Haploid mutants, resistant to the methionine analogue, ethionine, have been selected. They fall into three classes, recessive, semidominants and dominants. Most of them are methionine (or methionine-precursors) excretors.

In one of the semi-dominant haploids, in which resistance to ethionine appears to be digenic, aspartokinase (enzyme A) is no longer repressed by exogenous threonine, but regulation of homoserine dehydrogenase (enzyme C) is unchanged. The inhibitibility of aspartokinase is not modified in this mutant.

These observations were unexpected since ethionine is not an analogue of threonine which is important in the repression of this enzyme. Since non-repressibility by threonine, which represents an apparent regulation mutation, was found in one of these mutants, the other members of the three classes of mutants are being examined enzymatically and genetically, in hopes of identifying and mapping the characters which fulfil the criteria of regulation mutations.

1. De Robichon-Szulmajster and Corrivaux, *Biochem. Biophys. Acta* (1963).
2. Karassevitch and de Robichon-Szulmajster, *Biochem. Biophys. Acta* (1963).
3. Mortimer and Hawthorne; de Robichon-Szulmajster, unpublished.

3.54. Transformation in Yeast. W. F. F. Oppenoorth (Delft, The Netherlands).

DNA has been extracted from bacteria without loss of its genetic characters. The transformation of yeast also was possible but to a much less extent. When an experiment was successful only few transformed cells were present.

The transfer of fermenting abilities was investigated. A yeast, a hybrid obtained by Winge (303-9), not capable of fermenting disaccharides was treated with DNA extracted from *Sacch. chevalieri*, able to ferment sucrose and raffinose 1/3. The DNA was added into malt extract and inocculated with the acceptor yeast. After some days the yeast was full grown and transferred into different sugar Durham tubes. After several transfers cells were isolated with the micromanipulator and the fermenting abilities were analysed. Different new combinations were found.

Several other yeasts were used later.

Using diploid yeasts all the transformed cells were heterozygous for the newly acquired abilities. Heterothallic haploid yeasts treated with DNA derived from homothallic diploid yeasts yielded diploid homozygous cells.

A hypothesis is presented which can explain these facts.

The mendelian laws still hold, the foreign DNA is localized exclusively in the nucleus. In our opinion the different hereditary characters, dominant or recessive, are transferred independently from each other.

Attempts to fractionate the DNA preparations by means of an Ecteola column so that only one or a few genes were present in each fraction were unsuccessful.

Very confusing was a phenomenon which is called pseudo-transformation, a temporary change of characters, which decreases after subsequent transferring into the same broth.

Our previous hypothesis was that the pseudo-transformation was induced by the RNA still present in the DNA preparations. Harris showed that also enzymes still were present. This RNA or the enzymes are adsorbed at the cell walls. Thorough washing, however, did not always remove the pseudo-transformation. It seems that sometimes the substances have reached inside the cells.

3.55. On the Possibility of Carrying Out the Functional Test for Allelism in the Ascomycete Ascobolus immersus. Jean Mousseau (Gif-sur-Yvette et, France).

In some crosses between independent mutants with colourless spores there appear asci containing 6 mononuclear spores and 1 binuclear spore. Genetic analysis of such asci shows that in some of them the binuclear spore is heterocaryotic possessing a nucleus from each parental type. These heterocaryotic spores are of wild type phenotype (brown red colour). This shows that complementation takes place between mutations of the parental strains and that the mutant allele is recessive with regard to the wild type allele. These double spores therefore make it possible to carry out a functional test for allelism.

SECTION 4

GENE ACTION

4.1. Studies on the Function of Histones and Similar Basic Proteins. I. LESLIE (Belfast, Great Britain.

Chemical similarities have recently been detected between the histones associated with DNA and the basic proteins associated with RNA in ribosomes. However, the function of this class of protein is not yet known. From their chemical and physico-chemical investigations, Stedman and his collaborators have proposed that histones act as gene-regulators.

Latent-nuclease activity in ribosomes is known to be associated with basic proteins or proteins having compositional similarities to histones. The hypothesis that "histone-nucleases" control gene-expression in metazoan cells (Leslie, 1961) is being investigated with the aid of biochemical and tissue culture techniques.

Fractionation on carboxymethylcellulose and electrophoresis on starch-gel has been applied to the basic proteins of nuclei and ribosomes. With two procedures for detecting nuclease activity, it has been found that ribosomal RNA is most readily degraded by proteins of the lysine-rich type. Degradation by the arginine-rich protein is negligible.

Pulse-labelling and chase experiments with a human cell strain (HLM) are being used to follow the synthesis of the different types of RNA and basic proteins, when the cultures are treated with either fluorodeoxyuridine, which selectively inhibits DNA formation, and actinomycin C, which partially inhibits RNA formation. The results will be described.

4.2. Data on the Control of Genetic Replication in Temperate Bacteriophages. R. THOMAS (Brussels, Belgium).

When a lysogenic bacterium is superinfected with a temperate bacteriophage which is closely related to the prophage but has a different pattern of immunity, the superinfecting phage multiplies normally. The question can be asked whether functions supplied by the superinfecting phage will induce the prophage.

Suitably marked derivatives of λ and 434 hy (Kaiser and Jacob, 1957) (1) were used as prophage and superinfecting phage respectively. The prophage is not induced, the few prophage markers found in the progeny are rescued by recombination. The same appears to be true when a "virulent" λ is used as the superinfecting phage. In superinfection of cells lysogenic for λ with another λ and 434 hy, the superinfecting λ is not multiplied: the recovery of the corresponding type in the progeny is below the multiplicity of infection. These particles derive their protein coat from the unrepressed 434 functions.

These results suggest either that the control of replication does not entirely depend on the repression of synthesis of "early proteins", or that the functions involved in replication are not interchangeable between λ and 434 hy. If this is the case, the genetic loci for these functions should map in a small region which includes cistron c_1 (Kaiser and Jacob, 1957) but *does not* extend as far to the left as the site of the defective mutation d_{22} (since healthy λ can be rescued from λ d_{22} by recombination with 434 hy).

---

1. KAISER, A. D. and JACOB, F. *Virology*, **4**, 509, 1957.

This research has been supported by Euratom-U.L.B.-contract 016-61-10 ABIB, by the Air Force Office of Scientific Research, OAR 62-17 (through the European Office Aerospace Research, United States Air Force) and by the Belgian Fonds National de la Recherche Scientifique.

4.3. Regulatory Function of Sorbose on Sugar Metabolism in Neurospora. WALTER KLINGMÜLLER (Berlin, Germany).

Growth of *Neurospora* on filter sterilized sucrose medium is blocked by sorbose. (1) By treatment with nitrous acid mutants have been produced that are not blocked. These mutants also grow on fructose/sorbose and on sorbose alone, the wild type does not. Growth on those media is in dense, small colonies, the growth rate somewhat retarded as compared to other media.

Growth on sorbose alone may be due to impurities in this sugar. Growth on fructose/sorbose

and on sucrose/sorbose point to a regulatory mechanism related to the β-galactosidase system in *E. coli*. The mutants are supposed to be constitutive for a sucrose splitting enzyme, which in the wild type can be repressed by sorbose.

A genetic factor for colonial growth has been crossed into the mutant strains, to check for correlations between the repressor function of sorbose and its ability to induce colonial growth.

1. De Serres, Kølmark and Brockman, *Nature* **193,** 556, 1962.

4.4. Genetic Studies on ur⁻ Mutants of Coprinus radiatus (Fr. ex. Bolt). Georges Prevost (Gif-sur-Yvette, France).

The species studied is a basidiomycete having dikaryotic phase. This work has been undertaken in order to understand physiological mechanisms underlging the regulatory systems which occur in dikaryotic organisms.

Four groups of alleles controlling the biosynthesis of uracil have been found: *ur 1.* (Chromosome VI), *ur 4.* (Chr. IV), *ur 7.* (Chr. VII) and *ur 13.* (Chr. III). On the other hand a gene *r*, linked to *ur 7* (max. 6 UCO) permits the utilization of orotic acid as a source of uracil by strains *ur 1.* and *ur 4.* but not by strains *ur 7.* and *ur 13.* The gene *r* is recessive, which means that the dikaryon (*ur 1. r* + *ur. 1. r*⁺) cannot utilize orotic acid. The *r* gene does not control a permease system but it permits the participation of orotic acid in the pathway of uracil biosynthesis. The very high sensitivity of the wild type strains to the action of 5-fluorouracil (inhibition of growth by concentration of 10^{-7} M of 5-FU) has led to the utilization of this product for selection of possibly derepressed or/and desinhibited mutants. Monogenic mutants resisting to 5-FU (10^{-4}M) have been obtained. These mutants can be classified in several allelic groups and are recessive or semi-recessive. Their relationship to mutants *ur*⁻ as well as the mechanisms of resistance to 5-FU have been analysed.

4.5. Further Studies with Methionine Mutants of Salmonella typhimurium LT2. D. A. Smith and J. D. Childs (Birmingham, Great Britain).

The results of growth response, cross-feeding and transduction experiments with 166 mutants suggest that at least 6 genes (*met A, B, C, E, F* and *G*) are involved in the synthesis of methionine via cystathionine and homocysteine. *MetA* mutants are unable to carry out one, and *metB* mutants the other, of two successive steps in the synthesis of cystathionine, *metC* mutants cannot synthesize homocysteine and *metE, F* and *G* mutants possess metabolic blocks between homocysteine and methionine. *MetE* mutants have an alternative requirement for vitamin B12. All *metC* mutants appear to be heat sensitive.

Colicine mediated genetic transfer results indicate the sequence *metB*—(*metF*—*metE*)—*metA*—*metC* for 5 of the 6 genes. Only *metB* and *metF* are close enough to be transduced by the same phage particle.

Intragenic abortive and complete transduction tests between all the mutants of each gene are being carried out. Mutants of each of the *metA, F* and *G* genes all fall into single complementation groups. Those of the *metB* gene fall into at least three and those of *metF* gene into two groups. The occurrence of suitable *metA* and *F* deletion mutants permit some mapping of sites of mutation within these genes.

4.6. The Gene-Enzyme-Complex Involved in L-arabinose Metabolism. Ellis Englesberg, Nancy Lee and Richard Cribbs (Pittsburgh, U.S.A.).

Forty-seven L-arabinose nonutilizing mutants sites of *Escherichia coli*, strain B/r, have been ordered between the markers threonine (thr) and leucine (leu) by transduction experiments with phage Plbt employing three factor crosses. The mutant sites fall into four different gene loci, as defined by both genetic and functional criteria and are arranged in the order thr—D, A, B, C—leu.

Genes A and B are the structural genes for L-arabinose isomerase L-ribulokinase, respectively. Gene D is probably the structural gene for L-ribulose-5-phosphate-4-emimerase. Gene C is characterized by mutants which are deficient in all three enzymes. Recent experiments (Helling, Weinberg, and Englesberg, unpublished), conducted with K12, using temporary merozygotes, have shown that C mutants complement both A and B mutants thereby eliminating the possibility, previously considered, that C is an operator locus. Mutations in the B gene have a dual effect causing alterations in the structure of the kinase and increased or decreased inducible levels of isomerase and epimerase. The mutant sites affecting high and low inducible levels of these two enzymes are distributed in a random fashion. The amount of inducible L-ribulokinase CRM formed by some B gene mutants is greater or less than the amount of L-ribulokinase (as deter-

mined antigenically) produced by the parent strain. These changed inducible levels of isomerase, epimerase and kinase CRM vary coordinately. It is proposed that coding in the B gene besides determining the structure of L-ribulokinase may set the rate of production of the messenger for kinase, isomerase, and epimerase.

4.7. Complementation of Arabinose Negative Mutants of Escherichia coli. R. B. HELLING (Pittsburgh, U.S.A.).

L-arabinose non-utilizing mutants of *E. coli*, strain B/r, fall into four groups. A mutant in group A, B, or D lacks one of the first three enzymes involved in arabinose breakdown, while gene C mutants lack all three enzymes. [1]

In order to study the complementation pattern of these mutants using sexual merozygotes, the mutant sites were transduced from *E. coli* strain B/r (which mates poorly) to *E. coli* strain K-12, which had the arabinose genes of strain B/r. Induction of arabinose enzymes was measured in merozygotes obtained from a cross involving two different arabinose negative mutants, under conditions where neither parent could produce the enzyme. Four complementing groups were found which corresponded to the four known genes. Each group complemented all other groups; thus C gene mutants are not similar to known operator or regulator mutants, and gene C produces a cytoplasmic product necessary for the induction of the arabinose enzymes, possibly a permease.

1. See LEE and ENGLESBERG, *Proc. Nat. Acad. Sci., U.S.* **48,** 1962.

4.8. Genetic Control of L-Histidine Biosynthesis in Salmonella. PHILIP E. HARTMAN and BRUCE N. AMES (Bethesda, U.S.A.).

Eight closely linked genes specify the structures of eight proteins involved in L-histidine biosynthesis. The gene order and the relative biochemical steps are: *E*(2?), *F*(4), *A*(3), *H*(5?), *B*(6 and 8), *C*(7), *D*(9), and *G*(1). A coding ratio of three has been calculated for the complete genetic region, about 1.3×10^4 nucleotide pairs long. [1]

L-histidine and thiazole alanine feedback inhibit reaction 1 by altering the conformation of the protein. [2] The sites of mutation in histidine-excreting mutants with feedback-resistant enzyme map within two separate segments of gene *G*. [3]

Enzyme production is coordinately repressed in the presence of L-histidine [4] or 1,2,4-triazole-3-alanine (TRA). Both compounds elicit pyrophosphate exchange reactions, activities which are not repressed by L-histidine. [5] TRA allows synthesis of afunctional proteins in histidine auxotrophs and is recovered from protein hydrolyzates. [6] Mutations at (a) gene(s) *outside* of the histidine region *simultaneously* confer TRA-resistance and a relative intensitivity to repression by L-histidine. [7]

The site of repression is part of, or adjacent to, gene *G*. Deletion of this "operator" region eliminates production of theenzymes by the histidine genes. For example, mutant *hisG-203* fails to recombine only with the seven most terminal sites of the *G* gene. Yet no histidine enzymes are produced. Translocation of the remaining seven structural genes into an episome, or further deletion of genetic material to the "right" of the histidine genes, allows return of coordinate gene function which is, however, now unaffected by the presence of L-histidine. [8]

1. AMES and HARTMAN, In: *The Molecular Basis of Neoplasia*, p. 322, 1962.
2. R. G. MARTIN, *J. Biol. Chem.* in press, 1963.
3. D. E. SHEPPARD and HARTMAN, *Fed. Proc.*, in press, 1963.
4. AMES and B. GARRY, *Proc. Nat. Acad. Sci.* **45,** 1453, 1959.
5. AMES and B. GARRY, unpublished.
6. A. P. LEVIN and HARTMAN, *Bact. Proc.*, in press, 1963.
7. J. R. ROTH, AMES and HARTMAN, unpublished.
8. AMES, HARTMAN, and F. JACOB, *J. Mol. Biol.*, in press, 1963.

4.9. Restoration of Operon Activity by Suppressors. J. R. BECKWITH (London, Great Britain).

The genes clustered in the Lac region of the *E. coli* chromosome are under the control of a single operator region adjacent to the gene for β-galactosidase. 0° mutants, located in this region, prevent the synthesis of all three enzymatic activities associated with the operon, β-galactosidase (z), permease (y), and galactoside transacetylase. It has been proposed that these mutations prevent the transcription of messenger-RNA from the operon. In this paper the isolation and characterization of suppressors of an 0° mutant is reported. By the use of melibiose, an α-galactoside which requires permease but

not β-galactosidase for its metabolism, it was possible to select for suppressed mutants of the type z^-y^+ in addition to z^+y^+ revertants selected on lactose. The chromosomal location of the suppressors for many of these strains has been determined. They fall into four classes: (1) intra-operon suppressors, and extra-operon suppressors which map near (2) the methionine, (3) the serine and (4) the tryptophan markers on the *E. coli* chromosome. None of the extra-operon suppressors involves translocations of the defective operon to another part of the chromosome. These results are difficult to interpret by the hypothesis that the original O mutant is blocked at the level of gene transcription. They are better explained by a model in which the mutation has resulted in an inhibition of translation of messenger-RNA into protein. Dominance tests with these suppressors provide further indication of their character.

4.10. On the Possibility of a Specific Repression at the Genetic Level. M. LUZZATI, L. CLAVILIER and G. PÉRÉ (Gif-sur-Yvette, France).

At present, no case of specific repression of a genetic event is known. Such repression as has been described—i.e. the repression of the formation of a set of biosynthetic enzymes by the final product of the pathway—has always been concerned with the phenotype of the cell.

We have discovered a phenomenon [1] which concerns a specific inhibition at a genetic level. We have studied gene conversion of the *ad*3 locus in *S. cerevisiae*. The *ad*3 mutation leads to a simultaneous requirement for adenine and histidine.

In a diploid heteroallelic for *ad*3 the frequency of prototrophic revertants due to gene conversion is of the order of 10^{-4}/day/cell. L-histidine (but not adenine) prevents some of 95 per cent of the gene conversion. The conversion of four different heteroallelic combinations within the same locus are also inhibited by L-histidine to the same extent. However, L-histidine has no effect on the gene conversion of another locus *th*2.

Various reconstitution experiments have permitted us to exclude explanations based on a selection mechanism.

Inhibition is not due to an effect of histidine on the expression of $ad3^+$ gene in a $ad3^-$ cytoplasm, for histidine has no effect on the back mutation of either a haploid or a homoallelic diploid for the same locus.

If the ad3 gene is a type of operator mutant, one could imagine that the cytoplasmic repressor formed in the presence of histidine might, by reacting at the gene level, inhibit gene conversion, which is known to be a mitotic nonreciprocal recombination.

1. L. CLAVILIER, M. LUZZATI, P. P. SLONIMSKI, *Compt. Rend. Soc. Biol.* **154,** 1970, 1960.

4.11. Genetic Control of 5-Fluorouracil Resistance in Saccharomyces cerevisiae. FRANCOIS LACROUTE (Gif-sur-Yvette, France).

Growth of the wild type is inhibited by a 10^{-6}M concentration of 5 FU. Many resistant mutants have been obtained with thresholds varying from 3×10^{-5}M to more than 10^{-3}M.

Sixteen independent mutants have been analysed. They show single gene segregation; they belong to four different and unlinked loci and are dominant or semi-dominant.

Allelic relationships of these 5 FU resistance genes with the four loci for uracil dependence already known in *S. cerevisiae* and the mechanisms of resistance will be discussed.

The release in the cultures of resistant mutants of a substance promoting the growth of uracil requiring mutants suggests that feed-back inhibition or repression has been relieved in these resistant strains.

4.12. Gene Control of Differential Cell Function. WILLIAM K. BAKER (Chicago, U.S.A.).

The pattern of pigmentation in variegated eyes of *Drosophila* produced by position effect forms a useful system for studying the ontogenetic differentiation of cellular function. A comparison of the size and distribution of twin sectors produced in the eyes by induced somatic crossing over in *D. virilis* and *D. melanogaster* with the size and distribution of pigment patterns caused by position effect indicates that the latter patterns also have a cell-lineage basis.

From the size of certain pigmentation sectors in the variegated eyes, it is concluded that pigment potentialities of a given sector of the eye can be determined in early larval development, many days before pigment formation commences in the eye. Evidence is marshalled that this early determination—in spite of the clonal sectors it makes—is not based on any type of somatic mutation in the strict sense. Rather it is a regulation of gene action whose level is rather stably inherited within the somatic cells that will comprise one of the sectors of the compound eye.

The validity of these concepts will be illustrated by studies on position effects causing *white* variegation in *D. melanogaster* and *peach* variegation in *D. virilis*.

4.13. Non-specific Genetic Control of Variegation in Drosophila melanogaster. JANICE B. SPOFFORD (Chicago, U.S.A.).

The *Su-V* locus (IIIL, 41.4) has already been described; one allele enhances, the other suppresses variegation of *white*, *facet* and *diminutive* induced by Dp (1 : 3) w^m 264.58a, a short insertion from the X into the proximal heterochromatin of IIIL. Neither allele at the locus is dominant. The extent of variegation depends on both the individual's own *Su-V* genotype and his mother's. This reports a study of the effects of the *Su-V* locus on position-effect variegation due to other rearrangements, all X chromosome inversions: sc^8 (*scute-8*), y^{3p} (*yellow-3 of Patterson*), w^{m4} (*white-mottled-4*), and rst^3 (*roughest-3*). For sc^8, the number of scutellar bristles was counted; for y^{3p}, the amount of black pigment in the core of the bristle was arbitrarily scored, the scutella being mounted in euparol and examined immediately at 120*x*; for w^{m4}, the total drosopterin per head was measured by densitometer after paper chromatography; and for rst^3, the proportion of eye which was rough was estimated. In all cases the results agree with those already described above for white mottling due to the duplication. Possible modes of action of the suppressor locus will be discussed.

4.14. A Genetic Oscillatory Mechanism in Arabidopsis. G. P. RÉDEI (Columbia, U.S.A.).

In homozygous condition the recessive X-ray induced *im* gene periodically and repeatedly causes the turning on and off of chloroplastid differentiation, and consequently of leaf pigment synthesis. Due to this oscillating function all parts of the mutant plants exhibit sectors. The individual cells are either all white or normal green without any intermediates. The frequency of the switch may be very high. The pattern of variegation indicates non-randomness. It appears that the altered cells transmit their temporary character to their several mitotic offspring. Though there is some resemblance to certain mutable systems observed in other organisms, there are several obvious differences. The change of state of the cell lines dividing parallel to the axis of differentiation is fairly well synchronized. The cell phenotype is not yet fixed at the time of the replication. The expression is irreversible, however, after the completion of cell differentiation. The alternative functions of the *im* allele can be induced or repressed by various physical and chemical agents (temperature, irradiation, metabolites of the cysteine pathway, etc.). The transmission of this gene through the "germ line" is clearly Mendelian and linkage has been established. Spontaneous mosaicism in heterozygotes was not observed. Seeds harvested from white or green fruits are genetically identical and repeat the mosaicism. Apparently the *im* alteration is not due to mutation in a „structural" gene but rather to a stable change of a "regulatory" element. A detailed report will be published in *Genetics*.

Supported by National Science Foundation.

4.15. The Mode of Transmission of Exogenotic Fragment in Bacterial Crosses. E. CALEF and L. FISCHER-FANTUZZI (Naples, Italy).

Structural organization in heterogenote of K12 can be explored by interrupted mating.

The analysis of results of some crosses between Hfr heterogenote and haploid F- show that markers on the exogenotic piece are transferred with the same timing as those of the endogenote. Such mode of transmission is suggestive of an organization involving some kind of attachment between the exo- and the endogenote.

4.16. Thermosensitive Mutations affecting the Replicating Capacity of the Sex-factor of E. coli. F. CUZIN and F. JACOB (Paris, France).

It has recently been suggested that in bacteria a self-replicating unit, or replicon, such as a chromosome or an episome, determines its own replication system. [1] This hypothesis predicts the existence of mutations affecting specifically the replication of a given element such as an episome or a chromosome. From bacteria carrying an F-Lac$^+$, mutants have been isolated in which the episome replicates normally at 30° but not at 40°. At 40°, the episome is diluted out by the bacterial multiplication. It is not destroyed, however, since, when bacteria grown at 40° are shifted down to 30°, the multiplication of the episome in those cells, which will carry it, is resumed. This thermosensitivity is genetically

controlled by the episome as shown by transfer experiments.

When the thermosenisitve *F-Lac* episome, however, is integrated in the chromosome as Hfr, it replicates normally at 40°, suggesting that in this case *F* is no longer reproduced by the *F* system of replication but by the chromosomal one. In bacteria carrying both a thermosensitive *F-Lac*, and a normal F-Gal factor, the replication of the *F-Lac* is normal at high temperature, a result which suggests that the thermosensitive factor is diffusible.

1. F. JACOB and S. BRENNER, *C.R. Acad. Sci.* **257,** 298-300 1963.

4.17. The Mechanism of Chromosome Mobilization by F-lac in Escherichia coli K12. JOHN SCAIFE and JULIAN D. GROSS (London, Great Britain).

Male cells of *E. coli* K12 harbour the episomic sex factor, *F*. During conjugation these are able to donate genetic material to female cells, which do not carry the *F*-factor. *F*-episomes incorporating genes from the bacterial chromosome have been isolated, e.g. *F-lac*.

The chromosome of *E. coli* is circular; in conjugation, however, it is transferred as a linear structure. The opening of the circular chromosome and its subsequent transfer are associated with an interaction between the sex factor and the chromosome.

In the case of a cell harbouring *F-lac* this interaction occurs in the vicinity of the *lac* region of the chromosome, which suggests that the two structures synapse and interact within the region for which they are genetically homologous. It is proposed that the interaction is, in fact, a *genetic exchange* in the homologous region, causing the insertion of the *F-lac* into the chromosome, which then acquires the ability to be transferred as a linear structure. A prediction of this model is that *lac* will be transferred as the first chromosomal marker.

We have made crosses using male cells bearing different *lac* markers on the chromosome and the *F-lac* factor. Analysis of the progeny from crosses of this type has yielded results which are consistent with this prediction from the model for chromosome mobilization outlined above.

4.18. High Frequency Resistance Transfer System in Escherichia coli. TSUTOMU WATANABE (Tokyo, Japan).

Cells of *Escherichia coli* K-12, which have just received R factors (episomic resistance factors), are able to transfer them in unusually high frequencies unlike usual R^+ cells. HFRT populations which contain high ratios of such competent donor cells can be obtained following the procedure for obtaining HFCT (or HFC) of Ozeki and Stocker (1960). We have recently found that HFRT contains a large number of cells with higher drug resistance than usual R^+ cells. The R factors of the clones which grew on higher drug concentrations were found to have mutated resistance markers which confer higher drug resistance. Jacob *et al.* (1960) have shown that partial polyploids carrying multiple copies of F^1-lac synthesize unusually large amounts of β-galactosidase. In view of their finding, our results may be interpreted as due to a gene dosage effect by the unusually vigorous replication of R factors in HFRT cells. Transfer of R factors is very frequent because of the many copies of R factors in cytoplasm and also of the increased production of RTF-specific mating substance. The gene dosage effect is also exerted on the biochemical mechanisms of drug resistance and HFRT cells are thus phenotypically more resistant than usual R^+ cells. HFRT cells are able to divide for several divisions on higher drug concentrations until the R factors are reduced to "stable autonomous state" or integrated state. Accordingly, the probability of mutation of R factors per cell is higher in HFRT cells than in usual R^+ cells.

4.19. Linkage of Colicinogenic Factors with an F Agent and with Nutritional Markers in the Chromosome and in an Episome of Escherichia coli. PIERRE FREDERICQ (Liège, Belgium).

Some wild strains *E. coli* producing colicins V or B have been found to carry their colicinogenic factors closely linked to an F agent of sexual polarity. Almost every recombinant obtained in crosses with F- derivatives of *E.coli* K12 are F+ or F- according to whether it receives or not the colicinogenic factor. In such crosses, an unusual type of recombinant has been obtained, where the F agent has been integrated in the chromosome. Genetical analysis of that recombinant revealed that a B colicinogenic factor is still linked with the F agent and integrated with it in the chromosome. In that Hfr strain the leading markers is *mal* and the most distal *str*. There is no transfer of the Hfr and colicinogenic properties when selection is made for transfer of the proximal marker *met*. There is however a linked transfer of both properties when selection

is made for more distal markers. The rate of transfer of both properties increases from 0.01 when selection is made for transfer of *pro* to 0.70 when selection is made for transfer of *str*, the most distal marker.

Linkage of colicinogenic factors with nutritional markers has also been observed in an F^1 episome. In a cross of a wild strain *E. coli*, carrying V and B colicinogenic factors linked to an F agent, with a deletion mutant *tryABDC*, *T1-r* of *E.coli* B, some recombinants, selected for transfer of try^+, had a very peculiar behaviour towards phage T1, being partially susceptible. They were found to be partial diploids, carrying the wild *try* region, including *cysB* and *T1*, linked to the F agent and to the V and B colicinogenic factors in an F^1 episome. These recombinants have been further crossed with different *cysB* or *try* mutants of *E.coli* B or K12. The new recombinants appear to carry, like the male parents, an episome in which F is linked to the V and B colicinogenic factors and to the entire *cys try* region. Preliminary transduction experiments by phage Plkc grown on such a diploïd strain reveal that genetic material from either the chromosome or the episome can be transferred. In many transductants the introduced fragment is not integrated and remains in the episomic state, giving rise to stable diploids. Among them a few received all the known markers from the episome, including the colicinogenic factors. From the data, the order (F), *cysB*, *tryA*, *tryB*, *tryD*, *tryC*, *T1*, (colV), (colB) can be inferred.

The research reported in this document has been made possible through the support and sponsorship of the U.S. Department of Army, through its European Research Office, University of Liège, Belgium.

4.20. Controlling Episomes. G. W. P. Dawson (Dublin, Great Britain).

Instability at a number of loci in *Salmonella typhimurium* have been studied and their instability appears to arise by an attachment of genetic particles to the locus. To point the similarities between these particles and controlling elements in higher organisms and episomes in bacteria they are called controlling episomes. At some loci controlling episomes suppress gene expression as well as causing instability. Different loci have different avidities for particular controlling episomes. Different strains have different frequencies of controlling episomes. Controlling episomes can transpose from one locus to another

4.21. Controlling Episomes at the su-leuA locus of Salmonella typhimurium. P. F. Smith-Keary (Dublin, Great Britain.

Slow growing reversions of the leucine requiring strain *leu-151* of *Salmonella typhimurium* are due to mutations at one of a number of sites within a linked suppressor locus *su-leuA*; many of these reversions are highly unstable. This instability is caused by a particular type of episome called a controlling episome which becomes transposed from some unknown location to a site within *su-leuA* where it induces a high frequency of mutation. The instability is retained at this site, through successive back and forward mutations of su-leuA, until the controlling episome becomes transposed to another location.

Some strains of *leu-151* revert to the unstable types at a much higher frequency than other strains. This is attributed to these strains having a higher frequency of controlling episomes available for transposition to *su-leuA*; in these strains the frequency of unstable reversions can be greatly reduced by treatment with acridine orange. The frequency of stable reversions is the same in all strains of *leu-151* and is unaffected by acridine orange treatment.

A controlling episome located within *su-leuA* can be transposed to a locus modifying proline requirement, resulting in instability for proline independence and concomitant stability at *su-leuA*.

4.22. Transposed Inducer Function in E. coli. K. C. Atwood (Urbana, U.S.A.).

From a K-12 stock carrying F-lac, a mutant was screened that requires an inducer of B-galactosidase for growth on medium containing glycerol as sole carbon source. Inducing levels of the nonutilizable inducers, methyl-thiogalactoside or isopropyl-thiogalactoside satisfy the requirement. The inducer requirement is also present with amino acid mixtures or succinate as carbon sources, but not with any hexose or pentose that can be used by the parent strain. Neither induced nor uninduced cells of the mutant produce B-galactosidase. The mutant is interpreted as a transposition of controlling elements such that the system normally in control of B-galactosidase synthesis now controls a different enzyme, apparently an enzyme involved

in the synthesis of hexoses and pentoses from smaller compounds.

4.23. Discrimination among Copying, Plasmid, and Episome Models for Conversion-type Inheritance in Maize. E. H. COE, JR. (Columbia, U.S.A.).

Specific models for conversion-type inheritance or paramutation can be based on gene-extrinsic or gene-intrinsic mechanisms. The basic observations in regular conversion-type systems (failure of normal segregation of parental phenotypes in certain hybrids contrasted with normal segregation in others) can be conceived to depend upon (1) an independent but gene-maintained material, (2) a gene-associated but transferrable material, or (3) a gene-intrinsic feature that may be forcibly copied. These conceptions are respectively parallel to (1) the properties of plasmids in many organisms, (2) the properties of episomes in bacteria, and (3) special crossing-over models. Criteria by which these systems might be distinguished in eukaryotes include time of conversion, concurrence of chromosomal deletion with loss of the conversion effect, infectivity, synchrony in replication of the chromosome and the unorthodox system, association of the system with particular alleles, and the mechanics of origination of the phenomenon. Because the regular conversion-type event at the *B* locus in maize is (a) completed late in the life cycle, (b) eliminated by X-irradiation concurrently with linked chromosomal markers, (c) apparently not infective, (d) replicated synchronously with the chromosome, (e) unrelated to allele function, and (f) originating from a gene-independent system, a dual model (a gene-associated but transferrable material) is invoked. Specific predictions of the dual model are being tested. A full report is in preparation.

4.24. Control of Activity at a Mutable Locus in Maize. PETER A. PETERSON (Ames, U.S.A.).

Control systems as formulated from a study of mutable loci in maize are characterized by the following features; the definable elements that comprise the system vary in time and rate of action upon a locus, undergo transposition to various sites in the genome, and affect the qualitative expression of a locus.

The mutable locus described here (a_1 colorless, to A_1 color on chromosome 3) consists of two elements—*I* (Inhibitor) that controls the action of the gene in question when intimately associated with it and *En* (Enhancer) that controls the action of *I* by removing or inactivating *I*. The expression of the *I* pattern is dependent on the presence of *En*, however *En* cannot be recognized unless there is an *I* controlled locus. Each of these elements is identified by a pattern of mutability as determined by the time (manifest in size of mutant area) and frequency (manifest in number of mutant areas) of mutation events.

Recent experiments show that these elements can also control the qualitative expression of the locus. This is manifest in the wide assortment of gene expression derived from certain of the mutable alleles that have reduced levels of gene action. The phenotypes in these cases range from very light pales to dark pales that are heritable and remain stable at each level in future generations. Experiments also indicate that additional differences among the elements may be expressed in their differential rate of change to other pattern types or to colorless forms.

These results show that a particular gene locus can be affected both quantitatively and qualitatively by the controlling elements in the genome.

4.25. Transposition of Mutability among Components of a Compound Allele of the A^1 Locus in Maize. M. G. NEUFFER (Columbia, U.S.A.).

An unstable condition has arisen at the site of the β component of the compound A^b allele (α β) on chromosome 3 in maize. The mutability is expressed as the loss of β function which permits expression of the α phenotype (dilute seed, red brown plant), since β is dominant to α in these characteristics, and as frequent recovery of β action to give sectors of the β phenotype (full colored seed, purple plant). The system is apparently autonomous as all known elements are located at the A_1 locus. Localization the effect at β was confirmed by separation of the unstable β (β^m) from x by crossing over in a heterozygote of α β^m with a homologous segment carrying only a null component of the A_1 locus. The β^m crossover has a recessive phenotype (colorless seed, brown plant) with frequent sectors of β phenotype.

Included among several variations from the original case are those in which (1) the mutability has left β and appeared at α to produce α^m β and (2) the mutability remains at β but also has appeared at α to produce α^m β^m. Kernels expressing the latter are phenotypically colorless with dots of both dilute (α) and of full color (β). On these seeds it can be observed that changes

occur independently at α and β each having its own particular frequency and character.

From these observations it is clear that a type of instability of obscure origin is able to appear at a compound locus and then to move back and forth between the components of this locus, affecting each as though it were a separate gene.

4.26. Variegation following Hybridization between Nicotiana tabacum and N. otophora. D. U. GERSTEL, and JOYCE A. BURNS (Raleigh, U.S.A.)

Different mechanisms seem to cause chlorophyll, bud anthocyanin and flower anthocyanin variegations obtainable after hybridization of *N. tabacum* with *N. otophora*. The first two types can be recognized only in later generations after recombination has taken place. Carmine-coral flower variegation occurs as a rule in first generation hybrids between coral *N. tabacum* and *N. otophora*; this type has been sufficiently investigated for a preliminary report. It differs as follows from the variegations described in the literature: Coral spots on carmine background are not due to chromosome loss since chromosome numbers in variegated and self-coral petals of the same plant are identical; furthermore, coral branches on a variegated plant produce variegated test-cross progeny. Certain similarities with v-type position effect can be noted, but structural rearrangement is not required for coral spotting to occur. Paramutation is obligatory in certain heterozygotes of maize and the changed allele is passed on to the progeny while carmine-coral variegation seems to occur haphazardly and the changed form is not transmitted sexually, as stated. As yet incomplete genetic studies indicate that a single factor, co^v, from *N. otophora* is involved which is dominant over coral (*co*) of *N. tabacum* and which thus far appears to be autonomous since no controlling element could be separated by backcrossing and stable carmine types could not be obtained. One may hypothesize that there exists in *N. tabacum* (chromosomes or cytoplasm) a repressor element which at times inactivates co^v in somatic cells. During the reproductive cycle the potentiality of co^v for pigment formation is restored.

4.27. Studies on Amylase in Drosophila melanogaster by Agar-electrophoresis. HIDEO KIKKAWA and ZENICHI OGITA (Osaka, Japan).

It has been found by the senior author (1960) that the amylase activity in *D. melanogaster* is controlled by a semidominant gene located at about 80 on the second chromosome. However, the amylase activities differ from one another according to the strains used.

By using an agar-electrophoretic method modified by the junior author, amylases in various strains have been analysed. Seven amylase bands were found as a whole on a zymogram. But each strain showed a characteristic pattern in amylase bands. For instance, Amy^+ strain showed only one clear band (No. 1), whereas Amy^s strain showed two clear bands (No. 2 and No. 6).

F_1 individuals between any two strains which showed different patterns in amylase bands, gave a mixed type of parental patterns on a zymogram. No additional band has been detected except the parental ones. This result suggests that each allelic gene produces its own amylase protein in the cytoplasm, and also that no hybridization occurs between components of the enzyme proteins.

4.28. Studies on the Genetic Control of Xanthine Dehydrogenase in Drosophila melanogaster. R. P. KERNAGHAN and A. CHOVNICK (Storrs, U.S.A.).

Xanthine dehydrogenase activity in *Drosophila melanogaster* is controlled by at least three functional units (ry, ma-1, and bz). Mutation in any of these units produces a pleiotropic phenotype including absence of xanthine dehydrogenase activity (Forrest, Classman, and Mitchell, 1956; Glassman and Mitchell, 1956; Glassman and Pinkerton, 1960; Hadorn and Schwinck, 1956; Nawa, Taira, and Sakaguchi, 1958). The inactivation of antibody specific to xanthine dehydrogenase suggests that $ma\text{-}1^1$ contains greater amounts of cross reacting material (CRM) than ry^2 (Glassman, and Mitchell, 1958). The data on the fine structural organization of rosy (Chovnick *et al.*, 1962) and the description of $ma\text{-}1^2$ (Schalet, 1962), which complements neither $ma\text{-}1^1$ nor bz, requires a re-examination of the CRM question.

Extracts of wild type and mutant stocks were partially purified with Sephadex. Enzyme activity was determined as the increase in optical density at 395 mμ due to the reduction of added thionicotinamide—adenine dinucleotide mediated by the conversion of 2-amino, 4 hydroxpteridine to isoxanthopterin. Mutant extracts were tested for their ability to remove specific anti-enzyme activity from antibody preparations. $Ma\text{-}1^1$ and bz are positive while $ma\text{-}1^2$ is negative with respect to CRM activity. Several rosy mutants (ry^1, ry^2, ry^8, ry^9, and ry^{23}), were substantially negative. An anti-$ma\text{-}1^1$ antibody

preparation inactivated the wild type enzyme whereas an anti-ry^2 antibody preparation was ineffective. A discussion of these data will be presented.

4.29. A Possible Explanation for Some of the Differences between Drosophila Mutants lacking Xanthine Dehydrogenase. EDWARD GLASSMAN (Chapel Hill, U.S.A.).

Both the maroon-like (*ma-1*) and the rosy (*ry*) eye color mutants of *Drosophila melanogaster* lack detectable amounts of the enzyme, xanthine dehydrogenase. However, when extracts of these two mutants are mixed together and incubated for 90 min at 30° C, xanthine dehydrogenase activity is produced in the absence of protein synthesis. [1] It is postulated that there is a $ma\text{-}1^+$-complementing substance in *ry* extracts and a ry^+-complementing substance in *ma-1* extracts, and that these complementing substances interact to produce active xanthine dehydrogenase. Preincubation of each of the mutant extracts at various temperatures has shown that the $ma\text{-}1^+$-substance is very much more stable to mild heat treatment (35° to 50° C) than is the ry^+-substance. If this difference in stability occurs *in vivo*, it would explain some of the differences between the *ma-1* and *ry* mutants. Thus, there is no maternal effect at the *ry* locus because the ry^+-substance is too unstable to persiss in the egg. [2] In addition, the difference in effectt of gene dosage at these two loci can also be explained by the relative instability of the ry^+-substance. The more stable $ma\text{-}1^+$-substance will pile up in the cell to such an extent, that even if its level drops in response to lower doses of $ma\text{-}1^+$, it will still be in excess, and no change in xanthine dehydrogenase activity will occur. Thus only the *ry* locus has a gene dosage effect on the enzyme activity; the *ma-1* locus does not.

This research was supported in part by a research grant (GM 08202-03) from the National Institutes of Health.

1. *Proc. Nat. Acad. Sci.* **48**, 1491.
2. *Proc. Nat. Acad. Sci.* **48**, 1712.

4.30. Gene Control of Eye Pigments in Mormoniella. G. B. SAUL (Hanover, U.S.A.).

Wild type eye color results from the presence of at least three pigments separable by chromatography. Two of these, a yellow and a red pigment, are present in scarlet-eyed mutants but not in a black-eyed mutant type; the other, a purple pigment, is absent from scarlet-eyed wasps but present in the black-eyed type. Spectrophotometry and chemical tests indicate that the yellow and red pigments may be pteridines; the nature of the purple pigment is unknown, but it does not appear to resemble the brown ommochrome of *Drosophila*.

Three loci contain mutations for black eye color. Mutants of one of these contain only purple pigment and accumulate isoxanthopterin and 2-amino-4-hydroxypterine; the second group of mutants contain all eye pigments of wild type and do not accumulate the pteridines; and wasps containing mutations at the third locus contain all eye pigments of wild type and also accumulate the pteridines.

Scarlet-eyed mutants result from changes at any of four known loci. They cannot be distinguished from each other by color or chromatography, but have separable phenotypes when also containing mutant genes for black eye color.

4.31. The Genetics of Esterases in Drosophila melanogaster. THEODORE R. F. WRIGHT (Baltimore, U.S.A.).

Ogita [1] reports the existence of a genetic factor (ali^-) on the 3rd chromosome which lowers the esterase activity (methyl butyrate used as the substrate) of homogenates of whole adult flies. Homogenates from ali^-/ali^- individuals have only 15 per cent as much activity as homogenates from ali^+/ali^+ individuals. Heterozygotes, ali^+/ali^-, have intermediate activities; approximately 55 per cent of ali^+/ali^+. Wright [2] reports that two codominant alleles which control the structure of an esterase designated as Esterase 6 are located at 36.8 ± on the 3rd chromosome. Starch gel electrophoresis of homogenates show that homozygotes for the allele, Est 6^F, produce a form of Esterase 6 which migrates faster then that produced by homozygotes for the other allele, Est 6^S. The heterozygote, Est 6^F/Est 6^S, produces both fast and slow forms.

Because both of the above genetic systems are concerned with esterase production and because both are located on the 3rd chromosome, it became desirable to investigate whether or not the two systems are interrelated. Three of Ogita's strains, one ali^+/ali^+ and two ali^-/ali^-, are homozygous for Est 6^S. Using p-nitrophenyl acetate as a substrate, the total esterase activities of the Est 6^F and Est 6^S strains (which are equal to one another) are comparable to that of the ali^+/ali^+ strain. These data are not conclusive, however, and data comparing not total esterase activities,

but Esterase 6 activities of the various strains and of offspring from crosses, will be presented in an attempt to show whether or not the two systems are interrelated.

1. Botyu-Kagaku, *Scientific Insect Control* **26**, 93, 1961.
2. *Amer. Zool.* **1**, 476, 1961.

4.32. In Vitro Interconversion between Mutant Forms of the pH 7.5 Esterase in Maize. Drew Schwartz (Cleveland, U.S.A.).

Six esterase isozymes distinguished by electrophoretic mobility are formed by the three alleles of the E locus in maize. Three isozymes FF, NN and SS are formed by the E^F, E^N and E^S alleles when in homozygous condition and in addition three hybrid isozymes FN, NS, FS occur in the three heterozygous combinations. The FS hybrid isozyme migrates at the same rate as the NN band. Recent results indicate that the difference in the migration rates between the isozymes are due to a positively charged group associated with the enzyme. By treating extracts with sodium borohydride, the single pH 7.5 esterase band found in homozygotes is converted into a series of bands, all less positively charged. These new bands show migration rates identical to those produced by the various E alleles in homozygous and heterozygous combinations. In addition a new band migrating slower than the SS band is also seen. Treatment of FF extracts yields bands which are electrophoretically indistinguishable from the FF, FN, NN (or FS), NS and SS isozymes. Treatment of NN extracts reveals four bands,NN, NS, SS, and the new slow band. Treatment of the SS extracts yields only two bands, the SS and the new slow band. When a mixture of E^F/E^N and E^N/E^S extracts which contain all five isozyme types are treated, no new bands are seen except for that at the new slow position. These data are consistent with the hypothesis that F, N and S are associated with decreasing numbers of the positive charge group and that sodium borohydride removes varying numbers of the charged groups thereby converting the more positively charged isozymes into the less positively charged enzyme types. The other esterases and proteins in the same extracts are not effected by the borohydride.

4.33. Two Forms of Tyrosinase from Each of Two Different Mouse Melanomas. Jean B. Burnett and George F. Wilgram (Boston, U.S.A.).

The early work of Brown and Ward which resulted in the preparation of a soluble tyrosinase from mouse melanotic tumor has been the basis for current studies leading to the further purification and elucidation of the properties of this enzyme.

Harding-Passey and B-16 tumors are grown in Swiss white and C-57 black mice respectively. Harding-Passey and B-16 tumors each contain tyrosinases which are electrophoretically separable into two components; T_a^1, a tyrosinase of greater anodic mobility and T_a^2, a tyrosinase of lesser anodic mobility. At pH 8.6, T_a^1 (H-P) and T_a^1 (B-16) are tyrosinases of identical mobility while T_a^2 (H-P) and T_a^2 (B-16) are tyrosinases of also identical but of lesser anodic mobility. T_a^1 and T_a^2 of both tumors are also separable by column chromatography. An additional purification o fapproximately 10-fold is obtained using a DEAE-Sephadex ion exchange column. Characteristically, the enzymes have the same Michaelis constant and maintain characteristic differences in specific activity during the course of purification. Tryptic or chymotryptic digests of the purified enzymes (i.e. fingerprinting) suggest differences in amino acid sequence and composition.

These properties of the tyrosinases suggest that differences in primary structure are most likely in the inactive protein moiety of the enzyme while the primary, and probably tertiary, structure of the active sites are the same or very similar.

4.34. Studies on the Biosynthesis of Tyrosinase in Neurospora. Helen Macleod, Marguerite Fling, and N. H. Horowitz (Pasadena, U.S.A.).

The tyrosinase of *N. crassa* is a crystallizable, copper-containing enzyme of molecular weight 30-35,000. It has been the subject of a number of genetic and biochemical studies. (1) The enzyme is not produced in rapidly growing cultures, but is formed during the sexual phase of the life cycle, or in vegetative cultures when growth is inhibited by starvation or by amino acid analogs (e.g. ethionine, fluorophenylalanine, D-aromatic amino acids). When wild-type cultures growing on minimal medium are starved by transferring them to phosphate buffer, tyrosinase synthesis starts after a lag period, attains its maximal rate at about 24 hr after transfer, and ceases at about 60 hr after transfer. At this time, tyrosinase constitutes from 1 to 5 per cent of the extractable proteins. This induction of tyrosinase synthesis is much reduced in a mutant (*ty-1*) which is not

linked to the structural gene (*T*) of tyrosinase. The induction is pH dependent. It occurs optimally at pH 6 and is inhibited at pH 8. Inhibition at pH 8 is due to the loss of amino acids and calcium from the cells. The induction is inhibited only slightly by actinomycin-D, a drug which blocks DNA-dependent RNA synthesis and which strongly inhibits the growth of *Neurospora*. This result suggests that messenger-RNA synthesis for tyrosinase occurs during the period of active growth, but is not used for tyrosinase synthesis while growth is occurring.

1. See *Cold Spring Harbor Symp. Quantitative Biol.* **26**, 233, 1961.

4.35. Comparative Genetic Studies of Tyrosinase Synthesis in Neurospora crassa and Bacillus subtilis. K. E. Fuscaldo, V. Del Vecchio and W. Kaczmarczyk (New York, U.S.A.).

The genetic control of tyrosinase production in *Neurospora crassa* is being studied. Two strains, 15300-131a and 15300-138A, had been isolated by other workers and characterized for their ability to produce tyrosinases. Three electrophoretically separable enzymes were found which, however, do not differ with respect to their Michaelis constants or relative turnover numbers. It appears from previous work that the three forms of the enzyme were present in homocaryons. In order to clarify this system, matings were made between the two strains and extensive tetrad analyses carried out to establish the mode of transfer of the genetic information regulating tyrosinase synthesis. It has been suggested that the T locus in *Neurospora* controls the equilibrium among several interconvertible tyrosinases and therefore is concerned with the terminal stages of tyrosinase synthesis (Fox *et al.*). Other workers have interpreted their results to conclude that the T locus determines the structure of the enzyme and two other loci, ty-1 and ty-2, exert a regulatory control on the tyrosinase system (Horowitz *et al.*).

A corollary study was undertaken to examine the genetics of tyrosinase production in another organism. *B. subtilis* was chosen for this investigation since it had been reported that a variety (niger) produced a black pigment when grown on tyrosine supplemented media. The decision to pursue the tyrosinase studies on this organism was predicated on the fact that bacteria through their ability to participate in the phenomena of transduction and transformation offered excellent tools for the genetic analysis of an apparently complex system. Further, since these organisms possess a "chromosome" which is relatively simple when compared to those found in higher organisms, it may be possible to distinguish the two control mechanisms on that basis.

4.36. A Number of Loci associated with the Reduction of Nitrate to Nitrite in Aspergillus nidulans. B. M. Rever and D. J. Cove (Cambridge, Great Britain).

In a sample of 1200 mutant strains produced by u.v. irradiation of conidia of a prototrophic strain of the fungus *Aspergillus nidulans*, 40 were unable to grow on nitrate, but were able to use nitrite as a nitrogen source. Pairs of mutant strains were tested for complementary growth in heterokaryons. On this basis, the mutants fell into nine separate classes. The study of diploid growth complementation has now begun. The results of preliminary experiments suggest that there may be a difference between heterokaryon and diploid complementation with respect to certain pairs of mutants.

It is known that all forty mutants lack normal nitrate reductase activity. In the few cases so far investigated, complementary heterokaryons and diploids show detectable nitrate reductase activity.

Linkage studies on representatives of the nine complementation groups indicate that at least six separate loci are involved, none of which is closely linked to another. The loci may be allocated to their linkage groups using haploidization techniques.

A discussion of these results will be made in conjuction with the discussion of the biochemical aspects of this system in our later paper.

4.37. Biochemical Genetical Studies on Nitrate Reduction in Aspergillus nidulans. D. J. Cove and B. M. Rever (Cambridge, Great Britain).

The reduction of nitrate to nitrite is thought, in many organisms, to be catalysed by a single enzyme, nitrate reductase. This enzyme activity has been detected in extracts of mycelium of prototrophic strains of *Aspergillus nidulans*, and can be assayed quantitatively, in two ways. The rate of oxidation of $NADPH_2$, with which the enzyme is linked, may be observed spectrophotometrically, or the nitrate produced may be estimated colorimetrically. The enzyme activity is inducible.

The isolation and genetical analysis of 40 mutant strains unable to grow on nitrate, but able to use nitrite, has been described in a previous paper. These 40 strains involve mutations in at least 6 loci. All have been shown to have abnormal nitrate reductase activity, when grown in conditions inducing appreciable activity in prototrophs.

These findings may be explained in several ways. The conversion of nitrate to nitrite may proceed through a number of intermediates, each conversion being affected by a separate enzyme. Alternatively, the reduction may be catalysed by a single complex enzyme consisting of several polypeptide sub-units, the structure of each sub-unit being determined by a separate gene. It is also possible that some of the loci have a regulatory function, or control the transport of nitrate into the cell.

These hypotheses are not mutually exclusive, and combinations of any are possible. However, it appears that the mechanism controlling the conversion of nitrate to nitrite in *Aspergillus* cannot be of the one regulator—one operon type already known in bacteria.

4.38. The Inhibitory Effect of Sucrose-Sorbose-Agar on a Possible Invertase Mutant of Neurospora crassa. T. H. Pittenger and T. G. Brawner (Manhattan, U.S.A.).

One of the most widely employed techniques of the *Neurospora* investigator is the use of 0.1 per cent sucrose and 1 per cent sorbose to induce colonial-like growth of the mycelium. Although the conidial viability of many strains is quite high on such media, a mutant strain has recently been isolated that is inhibited by this media. The inhibitory effect of conidial germination and growth of this mutant is one compounded of three components of the media: 0.1 per cent sucrose, 1 per cent sorbose, and 2 per cent agar, and the full effect is expressed only when these components are autoclaved together with the basic salts. Some of the characteristics of this inhibition follow. If glucose is substituted for sucrose in the media, there is no inhibition. In the absence of sorbose there is no inhibition. The sucrose-sorbose medium is not inhibitory in the absence of agar. Furthermore, the inhibition may be relieved if the sucrose, sorbose, and basic salts are autoclaved separately from the agar and then combined. Similarly, if these components are autoclaved separately from the agar, and the agar is then added and the mixture autoclaved again, there is no inhibition. If mutant conidia are germinated in liquid media supplemented with either sucrose or glucose and then plated on sucrose-sorbose-agar medium, they do not form colonies if the incubation period is less than 20 hr. If, however, the pretreatment is extended to 24 hr or more, then inhibition is relieved. A hypothesis to account for these and other observations will be discussed.

4.39. Neurospora Studies of Pantothenates produced by Enzymatic and by Chemical Syntheses. Robert Fuerst, Lilli Li-chun-Li and Carolyn McFall (Denton, U.S.A.).

The *in vitro* synthesis of pantothenate with wild type mycelium of *Neurospora crassa* Em 5256A was demonstrated by Wagner and Guirard (1948). Acetone-dried mycelium of pantothenicless mutants were found to possess the same enzyme system as wild type for the synthesis of pantothenic acid from pantoyl lactone and beta alanine. A new homolog of pantothenic acid was prepared by incubating acetone-dried powder of either *Neurospora crassa* pantothenicless 5531A, 74A-Y153-M66, or commercial brain extracts, with a substrate consisting of pantoyl lactone and gamma-aminobutyric acid. The resulting active metabolite was named gamma-pantothenate. Wild type enzyme was not active in the production of this compound. Purifications of the active enzyme were successful, as well as its separation from an enzyme cofactor. Gamma-pantothenate supported the growth of *Lactobacillus arabinosus* as well as a pantothenicless *Neurospora* strain. Its chemical and physical characteristics were determined. Also metabolically active were chemically prepared synthetic calcium-gamma pantothenate and in part sodium-gamma-pantothenate. A *Neurospora* mutant, able to grow on calcium-gamma-pantothenate, was crossed to other strains. The results of these matings will be discussed.

Supported by the N.I.H., U.S. Public Health Service research grant, CA 03853-06 CY.

4.40. Glutamine Metabolism and Glutamine-requiring Mutants of Neurospora crassa. E. Reich and S. Silagi (New York, U.S.A.).

A number of mutant strains of independent origin, requiring glutamine for growth, have been recovered from conidial populations undergoing "inositolless death". No complementation has been observed between the six different

strains obtained to date. Concentrations of 500 mg/l. L-glutamine (D-glutamine is ineffective) are required to sustain optimal growth of all strains bearing the glm⁻ allele. Even at these glutamine levels there is an abnormally long lag phase, and the growth rate is less than wild-type at all stages, although the final yield of cell mass may be normal.

Since glutamine functions as a nitrogen precursor for purines, amino acids, vitamins and glucosamine, the potential sparing action of these metabolites on the glutamine requirement has been investigated. No significant sparing action has been observed for any glutamine-related metabolites, singly or in combination.

Through appropriate crosses a number of strains have been obtained containing combinations of the glm⁻ allele with other gene-con-trolled deficiencies affecting biosynthetic steps in which glutamine normally participates. The nutritional properties of these strains will be described and discussed. The level of glutamine synthetase, the enzyme responsible for glutamine synthesis, has been recorded in various strains containing mutational alterations which might be expected to affect intracellular glutamine concentration. These results will also be described.

The glm gene is located on linkage group V, where it is very closely linked to inos (approximately 2 per cent recombination) and probably distal to it.

4.41. Some Characteristics of the Protein Determined by the am locus in Neurospora crassa. R. R. BÜRK and J. A. PATEMAN (Cambridge, Great Britain).

Amination deficient (*am*) strains of *Neurospora crassa* lack both NADP linked glutamate dehydrogenase and alanine dehydrogenase activities. The purified glutamate dehydrogenase protein has alanine dehydrogenase activity. This is interpreted to mean the *am* locus determines a protein which has both glutamate and alanine dehydragenase activity.

When the protein is dissolved in 0.05 M pH 7.4 *o*-phosphate buffer the glutamate dehydrogenase reaction rate is initially low and subsequently increases to a level dependent on the assay system. In contrast the alanine dehydrogenase activity is initially high and decreases. When the protein is in pH 7.4 *o*-phosphate buffer of 0.1 or greater molarity the initial rate of the glutamate reaction is high. This suggests that at pH 7.4 in a buffer of molarity 0.1 or greater the protein is in a form which favours glutamate dehydrogenase activity while in 0.05 or less the form of the protein favours alanine dehydrogenase activity.

It is possible to distinguish three components in purified glutamate dehydrogenase by inverse filtration on a 5 per cent agar column. One of these probably corresponds to the 28,000 molecular weight sub-unit of Fincham and Coddington. It does not possess enzyme activity. Another component corresponds to their 220,000 m.w. component, and the other behaves as if its molecular weight is greater than 800,000. The relative amounts of the two larger components seem to be affected by the concentration of the buffer in which they are applied to the column, higher concentrations favouring the larger component.

4.42. Partial Revertants at the am locus in Neurospora crassa. J. A. PATEMAN and R. R. BÜRK (Cambridge, Great Britain) and J. R. S. FINCHAM (Hertford, Great Britain).

The amination deficient, *am* mutants *N. crassa* do not possess normal NADP linked glutamic dehydrogenase or alanine dehydrogenase activity. Backmutant strains were produced from *am-3* by ultraviolet irradiation and then assayed for glutamic dehydrogenase activity. A total of sixteen strains were found to possess less than 20 per cent of normal enzyme activity. A genetic analysis has shown that all the backmutations are in or close to the *am* locus. A biochemical investigation of the glutamic dehydrogenase produced by each of the partial revertants showed that the partial revertants can be classified into six distinct groups. The members of each group produce a unique variety of glutamic dehydrogenase with respect to the following criteria: thermostability, thermal activation, substrate affinities and substrate activation. A study was made of enzyme complementation between each of the partial revertant groups and a number of the original *am* mutants. In some cases the partial revertants show different enzyme complementation to that shown by the *am-3* mutant from which they were derived. The partial revertants have been assayed for alanine dehydrogenase activity and none of them possess wild type alanine dehydrogenase activity. There are significant differences between some of the partial revertants with respect to their alanine dehydrogenase activity. The significance of the genetical enzymatic and complementation characteristics of the partial revertants will be discussed.

4.43. Structural Gene for Ornithine Transcarbamylase in Neurospora. R. H. DAVIS and W. M. THWAITES (Ann Arbor, U.S.A.).

Previous workers have been unable to isolate mutants of *Neurospora* lacking the enzyme ornithine transcarbamylase (OTC), which catalyzes the conversion of ornithine to citrulline in the metabolic pathway leading to arginine. The mutation *s*, recognizable by its effect upon pyrimidine- and prolinere-quiring phenotypes but not by a nutritional requirement, has been shown to contain 3 percent of normal OTC activity [1]. Using conidia of the *s* strain, many OTC-less mutants were isolated after u.v. irradiation. They were non-complementing, and segregated as single gene mutations when mated with wild type; *s* was not recovered in such crosses. Extracts of an OTC-less mutant did not interfere with OTC activity of wild or *s* extracts. The locus represented by *s* and OTC-less mutants is denoted *arg-12* and is concluded to be the single structural gene for OTC. The locus is genetically distinct from mutations at the *arg-2* and *arg-3* loci which, like *arg-12* mutants, are stimulated by citrulline and arginine but not ornithine. A similar search for OTC-less mutants starting with wild type conidia yielded only one OTC-less mutant, which was allelic with *s*. The difficulty in isolating mutants of this class from wild type may be due to a high OTC content of conidia formed by wild type.

Research supported by U.S. National Science Foundation.

1. DAVIS, *Genetics* **47**, 351-360, 1962.

4.44. Ornithine Transcarbamylaseless Mutants of Neurospora. V. W. WOODWARD (Houston, U.S.A.).

The reaction coupling carbamyl phosphate with ornithine to yield citrulline is catalyzed by the transferase, ornithine transcarbamylase (OTC). The genetic control of this enzyme has been obscured by the finding that mutants *arg-2* and *arg-3*, mutants heretofore assumed to be "blocked" between ornithine and citrulline, contain wild-type amounts of OTC, and by the discovery that the suppressor mutation, *s*, which relieves certain *pyr-3* mutants of their pyrimidine dependence, contains no more than 3 per cent of wild-type OTC activity. Attempts to isolate additional mutants lacking OTC have taken two courses: (1) since *s* (reduced OTC) suppresses certain *pyr-3* mutants, a search was made for further suppressors. Many OTC-less mutants were found, all of which require arginine, or citrulline, but none of which suppress *pyr-3*, despite the fact that the *pyr-3* OTC-less double mutants were isolated on minimal medium. The OTC-less mutants are not allelic with *arg-2* or *arg-3*. (2) Using *s* as the conidial source, the filtration and selective plating technique was used to isolate arginine-*s* double mutants. From such double mutants six of the known arginine-requiring mutants and two OTC-less mutants were recovered. Physiological and genetical properties of these mutants will be discussed.

Research supported by grant number RG-8653, National Institutes of Health.

4.45. Quantitatively and Qualitatively Altered Phenoloxydases in Podospora anserina, due to Mutations at Non-linked Loci. KARL ESSER (Cologne, Germany).

Wild strains of the ascomycete *Podospora anserina* produce melanin pigments on suitable media. The early steps in the formation of these pigments are catalysed by phenoloxydases. Two different phenoloxydases have been found in the wild strain s1-: a diphenoloxydase (laccase) and a monophenoloxydase (tyrosinase). The latter is formed in only very small amounts compared to laccase. Both enzymes can be distinguished by their substrate specifities.

During the past years we have obtained about 40 more or less pigmentless mutants, most of which have occurred spontanously. By 5-6 subsequent backcrosses with wild strain s1-, these mutants have been made highly isogenic. Some of the mutants are allelic. They all differ from the wild strain by a single gene and could be localized in 5 of the 7 linkage groups. With these deficiences in pigment formation concomitant defects in fertility are always observed. The normal cycle of development of the mutants is blocked at different stages.

Since all of the mutants exhibit different degrees of phenoloxydase activity, we wanted to know whether this is due to quantitative or to qualitative alterations of the enzymes. In order to distinguish between the two alternatives, we have performed a preliminary serological test: enzyme extracts of the mutants were assayed for cross reaction with antibody against partially purified wild type enzymes (CRM test). Some of the mutants showed considerable deviations of the Enzyme/CRM ratio which is 1 for the wild strain. Therefore, the concerned enzymes seem

to differ qualitatively from the wild enzymes.

The phenoloxydases of three of these mutants, belonging to different linkage groups and having different morphogenetic blocks, have been further investigated biochemically. Methods applied were: behavior in ammonium sulfate fractionations, substrate specificity and heat inactivation. The experiments have established both quantitative and qualitative alterations of the two enzymes.

The implications of these findings will be shortly discussed with respect to gene-enzyme relations and to the genetic basis of morphogenesis.

4.46. Purification and Properties of Tryptophan Synthetase from Neurospora crassa. M. CARSIOTIS, E. APPELLA and S. R. SUSKIND (Baltimore, U.S.A.).

A purification procedure has been developed employing Sephadex G-100 and G-200 which permits routine preparation of tryptophan synthetase of *N. crassa*. The enzyme is about 95 per cent pure by physical criteria. It has an S_{20} of 5.9 and a diffusion coefficient of $4.92 \cdot 10^{-7}$ cm^2 sec^{-1}. The Svedberg molecular weight of about 122,000 is in good agreement with the value obtained by Archibald equilibrium sedimentation. These protein preparations have been used for physical and chemical studies in an effort to provide a structural basis for previous genetic, enzymological and immunochemical observations. The results from amino acid composition studies, fingerprint analysis of tryptic peptides, and from ultracentrifugation in 5M guanidine—HCl are consistent with the existence of a quaternary structure for tryptophan synthetase, comprised of two or more subunits. Such a structure could explain the phenomenon of interallelic complementation in this system, as well as the occurrence of CRM-negative mutants.

This research was supported by Grant C-03080 of the National Institutes of Health.

4.47. Genetic Control of Tryptophan Accumulation in Neurospora. DAVID R. STADLER (Washington, U.S.A.).

Wild-type *Neurospora* fails to grow on medium supplemented with 4-methyltryptophan (4MT) due to the inhibition of one or more enzymes required for the synthesis of tryptophan. Resistant mutants have been selected after u.v. or nitrous acid treatment, and the twenty-two mutants examined to date are alike in the characteristics which follow. Resistance results not from any direct change in the regulation of tryptophan synthesis, but from the loss of the ability to accumulate 4MT (or tryptophan) from the medium. Under conditions in which wild-type accumulates tryptophan to about 15 times the external concentration, the mutants only approximate the external concentration. The wild-type accumulation is inhibited by sodium azide. The mutants are all in the same genetic region of linkage group IV, less than one map unit from *co-4*. The mutants are recessive; heterocaryons between sensitive and resistant strains are sensitive. Preliminary studies of one mutant indicate that the same gene is responsible for uptake of several other amino acids.

4.48. A Modifier Mutation affecting Tryptophan utilization by Tryptophan Auxotrophs of Neurospora crassa. B. MALING, P. ST. LAWRENCE, L. ALTWERGER and M. RACHMELER (Berkeley, U.S.A.).

A mutation (*mod-5*) which allows growth of tryptophan auxotrophs of *Neurospora crassa* on media containing yeast extract (YE) or Bactopeptone (BP) without added tryptophan segregates independently of the *td* locus and is neither locus nor allele specific. *td; mod-5* cultures require tryptophan and show no detectable restoration of tryptophan synthetase activity. *mod-5* activity is not specific for tryptophan auxotrophs; it affects the growth of a lysine and of an aromatic mutant.

The amount of free tryptophan in YE and BP is sufficient for greater growth of *td* stocks than is observed; *mod-5* appears to permit utilization of this tryptophan. Acid hydrolysis of BP and YE removes growth activity for modified strains. Sephadex chromatography of BP and YE yields fractions with activity for both *td* and *td; mod-5* cultures. The active substance in these fractions has been tentatively identified as tryptophan.

Differential growth on enriched media may be due to greater sensitivity of unmodified strains to inhibitors. Some BP sephadex fractions appear to have a differential inhibitory effect on *td* and *td; mod-5* cultures. The nature of the inhibitor and the mechanism of the increased resistance of *td; mod-5* strains to it is not known. However, differential responses of modified and unmodified strains to tryptic digests of β-lactoglobulin indicate that the inhibitor may be a protein constituent. Equivalent growth of both strains is obtained at low concentrations of digest but

growth of unmodified mutants is inhibited severely at high concentrations.

4.49. Genetic and Biochemical Studies of Cytochrome C deficient Mutants of Yeast. FRED SHERMAN and HARRY TABER (Rochester, U.S.A.).

In a previous investigation, a single-gene mutant of yeast was found which had a decreased concentration of cytochrome *c*. The cytochrome *c* isolated from this mutant (cy_{1-1}) was different from wild type (cy_{1-1}) cytochrome *c* by a number of criteria.[1]

A rapid method was developed for examining the cytochrome spectrum of yeast strains at −190°C in order to obtain other mutants controlling cytochrome *c*. After examining over 14,000 strains that were treated with either ultraviolet light or nitrous acid, ten mutants were isolated that had a decreased amount of cytochrome *c* but normal or near normal amounts of cytochromes *a* and *b* (defined as cy). All ten mutants were crossed to wild type (CY) and the original cy_{1-1} mutant, and the sexual progeny analyzed. Also, diploids were constructed from all pairwise combinations of the eleven cy mutants. The results clearly indicated that six loci were involved.

In addition to the above strains, two mutants were found having a mutation of a cy gene and a "loss" of the cytoplasmic factor which controls the formation of cytochromes *a* and *b*.

The cytochrome *c* from some of the cy mutants has been purified and compared to wild type cytochrome *c*. These mutants have approximately 20 per cent of the normal amounts of cytochrome *c*, but little or no change in respiration. Electrophoresis and chromatography studies of the cytochromes *c* are now in progress.

---

1. SLONIMSKI and SHERMAN, in preparation.

4.50. Nature of a Suppressor Mutation affecting Adenylosuccinase. JOSEPH S. GOTS and EDITH G. GOLLUB (Philadelphia, U.S.A.).

Mutations occurring in the *ade-B* locus of *Salmonella typhimurium* lead to the loss of activity of adenylosuccinase, a bifunctional desuccinylating enzyme required for two sequential reactions in the biosynthesis of adenylic acid. A revertant of one of these mutants was found to be, by phage-mediated transduction, the result of a nonallelic suppressor mutation. We previously reported that the adenylosuccinase activity of the suppressor mutant differed from that of the wild type by a number of physicochemical properties and that the differences indicated that the *ade-B* mutation created an altered protein whose enzymic inactivity could be partially activated by the suppressor mutation. More recent findings make this explanation unlikely. Seven new *ade-B* mutants have been isolated and identified by recombination analysis as distinctly different single-site alleles of the *ade-B* locus. These fall into four complementation groups as determined by abortive transduction. Introduction of the suppressor gene into any of these mutants leads to a suppressor phenotype. This suggests that the suppressor mutation allows an unrelated enzyme to take over the function of adenylosuccinase. Support for this thesis was obtained by showing distinct immunological specificities between the wild type and suppressor enzymes serving as antigens. The suppressor enzyme is apparently not related to other known desuccinylating enzymes (e.g. argininosuccinase). Attempts to identify the normal function of the wild type allele of the suppressor locus are being made by searching for (a) natural repressors of the suppressor enzyme and (b) secondary mutations in the suppressor locus which could create a complete loss of function.

4.51. Linkage Groups of Genes controlling Isoleucine, Valine, and Leucine Biosynthesis in Bacillus subtilis. C. ANAGNOSTOPOULOS and M. BARAT (Gif-sur-Yvette, France).

Genetic analysis was carried out by transformation on a certain number of auxotrophic mutants of *B. subtilis* obtained by u.v. irradiation and requiring either isoleucine (*il*$^-$), both isoleucine and valine (*il-val*$^-$) or leucine (*leu*$^-$). The *il*$^-$ strains were shown to bear mutations on the structural gene for the enzyme threonine deaminase. The *il-val* markers are the result of single-step mutations affecting the genes of the enzymes common to both isoleucine and valine pathways. The *leu*$^-$ mutants are blocked in the last steps of leucine biosynthesis.

All *il* markers were found to be linked to each other but unlinked to the *il-val* or *leu* markers. The *il-val* and *leu* markers on the other hand are linked and can all be carried on the same piece of transforming DNA as shown by reciprocal crosses and experiments involving double mutants

It seems therefore that in *B. subtilis* the biosynthesis of isoleucine valine and leucine follows the same pathways as determined in other microorganisms. Some of the genes controlling the last

steps of these pathways are clustered; the threonine deaminase gene lies outside that cluster. A map of this segment of the *B. subtilis* genome which bears the *il-val* and *leu* markers is being constructed.

Results of biochemical and genetic studies on the regulation of these pathways will also be presented.

4.52. Non-genetic Control of Amino-acid Substitutions in the Biosynthesis of the Antibiotic Polypeptide Tyrocidin. B. MACH (New York, U.S.A.).

Tyrocidin is a decapeptide antibiotic known to exist in three forms, A, B, and C, differing by known single amino-acid substitutions involving phenylalanine and tryptophan. The occurrence of these forms might be under direct genetic control, possibly as the result of mutations. However, unlike the amino-acid replacements observed in proteins, they might result from environmental effects.

The relative amount of the three forms of tyrocidin synthesized by *Bacillus brevis* under a variety of conditions, either by single bacterial clones or by large volumes of culture, was determined after incorporation of C^{14} amino-acids and purification of the polypeptides. It was found that the molecular forms of tyrocidin synthesized, and therefore their specific amino-acid sequence, depend on the availability of the amino-acids involved in these substitutions. Variations in the amounts of these amino-acids in the culture medium result in drastic changes in the ratio of tyrocidin A, B and C synthesized, and even in the induced production of a new polypeptide, tyrocidin D.

Some aspects of this environmental control over amino-acid substitution and its possible application to the biosynthesis of other types of polypeptides will be discussed.

4.53. The Colour and Fragrance in Plants and Their Inheritance. JOHN POLITIS (Athens, Greece).

In 1911 we commenced a study of the formation of anthocyanin in plant cells, which up to that time was unknown.

In that paper we had shown that the anthocyanin in certain flowers first appear in the cytoplasm inside special corpuscles, which we had named cyanoplasts. In geminating seedlings of *Raphanus sativus* and other Cruciferae, anthocyanin is formed principally in the cells of the subepidermal layer of the cotyledons and hypocotyl axis. Each of these cells in its early stages contains a nucleus, chloroplasts and a spherical cyanoplast developing a red colour due to anthocyanin.

The fact that the cyanoplasts often appear near the nucleus and the fact that the colours of the flowers as well as other substances (tannins, chlorogenic acid, etc.) are inheritable characters, led us to suggest that a cyanoplast is a gene, escaped from the nucleus, and able to multiply by budding and to produce anthocyanin.

The fragrance of flowers and of other plant parts is also an inheritable character. Researches made by us on the glandular hairs of different Labiatae and Compositae proved the concomittant secretion of a bitter substance and an essential oil.

In order to explain the relation between the above two substances as well as the transmission of this inheritable character to the progeny, we believe that a gene which comes out from the nucleus is the cause of the production of a bitter heteroside, from which through enzyme action, an essential oil is produced.

SECTION 5

MUTAGENESIS

5.1. Application of Radiation Denaturation of DNA in Combination with Chemical Mutagens to Affect the Mutational Process. R. I. SALGANIK (Novosibirsk, U.S.S.R.).

It has been established that some mutagens (hydroxylamine, hydrazine, dimethylsulfate and others) react much more intensively with heat denaturated DNA than with the native one; but applying these mutagens alone, excepting heat denaturation of DNA, under the conditions of our experiment, does not result in significant breaking of hydrogen bonds in DNA. The hydrogen bonds in double-strand structure of DNA can also be broken by means of radiation energy (ultraviolet radiation). This effect can be realized not only *in vitro* but *in vivo* as well and besides that it can be dosed. The raising of the dose of the ultraviolet radiation increases the number of denaturated regions in DNA. It is reasonable to suppose that those regions of DNA in which are prevailing weaker A-T bonds and which are less saturated with stronger G-C bonds are broken in the first place.

It has been shown that radiation denaturation also increases the ability of DNA to react with investigated chemical mutagens.

The selectivity of biological effect of applied mutagens may be raised in connection with their ability to react in denaturated regions of DNA with certain nitrous bases (hydroxylamine → cytosine, hydrazine → thymine, dimethylsulfate → guanine).

Stepped irradiation with denaturating doses of ultraviolet rays in combination with certain chemical mutagens has been applied for affecting the frequency and the spectrum of mutations in bacteria.

5.2. Chemical and Biological Effects of the Reaction of Deoxyribonucleic Acid with Mutagenic Alkylating Agents. B. S. STRAUSS, R. WAHL, M. MAILLIS, J. COSTON and N. SCHWARTZ (Chicago, U.S.A.).

Transforming deoxyribonucleic acid (DNA) obtained from *Bacillus subtilis* undergoes at least two reactions when treated with the mutagenic alkylating agent methyl methanesulfonate (MMS). The first is a local "denaturation" which occurs upon treatment of DNA with either MMS, ultraviolet light, X-rays, or when DNA is denatured by heat and then annealed. This local reaction sensitizes the DNA to inactivation by an enzyme found in *B. subtilis* and in *Micrococcus lysodeikticus*. DNA inactivation is apparently due to strand breakage.

The second reaction results in alkylation of the DNA. DNA carrying alkyl group(s) is active for transformation and can replicate *in vivo*. Alkylated DNA is exponentially inactivated by heating well below the thermal denaturation temperature. This inactivation is probably due to loss of alkylated purines since enzymatic, ultracentrifuge and thermal denaturation studies indicate that alkylated and heated (50° C) DNA need not contain single strand breaks. Apurinic DNA is, therefore, inactive for transformation and (we presume) for replication *in vivo*.

Mutation induced by alkylating agents must be due to mispairing while the alkyl group is still part of the DNA molecule rather than at the apurinic stage since apurinic DNA does not replicate. This hypothesis is supported by the results of studies on the reversion specificity of *lac*$^-$(*i*$^-$*z*$^-$) strains of *Escherichia coli* obtained by one of us (N.S.); *lac*$^-$ mutations induced by the alkylating agent ethyl methanesulfonate behave as though they were transitions rather than the mixture of transitions and transversions expected if mutation occurred at the stage of apurinic DNA.

This work was supported by grants from the National Institutes of Health (U.S.A.) and from the National Science Foundation.

5.3. Chemical Mutagenesis on Separated DNA. S. E. BRESLER (U.S.S.R.).

1. By means of bacterial transformation we are able to make a quantitative study of chemical mutagenesis on separated and purified DNA.

To know the conditions of quantitative assay for transforming activity of DNA the homogeneity

of a population of competent *Bacillus subtilis* bacteria was studied. These experiments were performed by means of genetic measurements (double transformation with two unlinked markers) and by means of registration of radioactive DNA uptake by competent bacteria (the track autoradiographs method of Levinthal was applied to study the homogeneity of DNA uptake). The results showed a great inhomogeneity of a *B. subtilis* population in transformation.

The knowledge of these details permitted us to choose the conditions for quantitative measurements.

2. Chemical mutagenesis on purified DNA by means of nitrous acid and u.v.-irradiation was studied. Genetically altered DNA molecules were registered by means of transformation of a recipient strain. Kinetics of mutagenesis and inactivation of DNA were measured and principles and equations of physical chemistry were applied to both processes. Both reactions are one hit processes, requiring one effective collision with the mutagen.

3. The efficiency of mutagenesis *in vitro* depends on the ratio of two kinetic constants — rate of mutagenesis versus rate of inactivation. The reaction of inactivation was studied in detail. It was shown that many genetic loci are inactivated simultaneously.

Possibility for reactivation of chemically modified DNA was studied. Reactivation can be performed by means of fusion and annealing of DNA in solution (Doty—Marmur's method) together with homologous but genetically unmarked DNA, or by a slight fragmentation of damaged DNA with ultrasound.

These facts give some new points to the mechanism of chemical attack on DNA macromolecules.

5.4. The Mutagenic Effect Produced by Radiation and Chemical Agents on Extracellular Phages. A. S. KRIVISKY (Moscow, U.S.S.R.).

The phage s_d *Escherichia coli* SK, isolated by the author, is endowed with a unique capacity to show hereditary variation when irradiated in extracellular state. Below are exposed the results of experiments serving to induce plaque mutations of phage s_d acted upon by u.v.-rays, ionizing radiation and nitric acid. The appearance of mutations should not be attributed to selection, multiplicity reactivation or host-reactivation. When the mutagen is applied to the host cell only, no phage mutations arise. The capacity to produce mutations in response to the *in vitro* action of mutagenes is characteristic of various strains of phage s_d. The mutants induced appear in equal numbers when plated on different strains of host cells. The initial plaque of the mutant induced contains only phages of this mutant type. It may therefore be concluded that the capacity to produce hereditary variation when exposed to mutagenes *in vitro* is inherent in phage particles; these mutations arise as a result of a direct action produced by the mutagen on the DNA of a resting phage, and are phenotypically realized directly after the first intracellular replication of the phage DNA.

The mutations do not revert after the action of the mutagen by which they were induced; such reversions may, however, arise spontaneously.

The examination of lethal and mutagenic dose response curves shows that the relation between these two types of damages is typical of each mutagen applied. The molecular mechanism of damages seems to be identical in both cases, since radioprotectors are equally efficient in the case of lethal or mutagenic effect.

The highest mutation ratio per surviving phage ($\sim$2-3 per cent) is caused by u.v.-rays. The effect is somewhat smaller in the case of nitric acid, and still smaller in response to various sources of ionizing radiation. The mutagenic effect due to u.v.-rays is characterized by the presence of a maximum corresponding to the viability of phage$\sim 10^{-2}$–10^{-3}; a further increase of the dose leads to a sharp drop of mutation ratio per survivor. No such effect could be observed in our experiments with a temperate phage kappa of *Serratia marcescens* in which an increase of mutation ratio is observed after u.v. irradiation up to a viability $\sim 10^{-6}$. The causes responsible for these differences in the behaviour of different phages are being analysed.

In contrast to other phages, mutations failed to arise after u.v.-irradiation of the vegetative intracellular phage s_d.

The possible molecular mechanism of mutagenesis of phage s_d and the prospects of its application as a model for radiogenetic investigation are discussed.

5.5. Induced Mutations in Extracellular Actinophages. S. I. ALIKHANIAN and N. M. MKRTUMIAN (Moscow, U.S.S.R.).

Induced mutations in bacteriophages described in literature include mutations obtained by exposing to various mutagens: (a) phage-infected cells, (b) phages and cells simultaneously, (c) host cells only. Phage mutations obtained after single reproduction of preliminarily mu-

tagen-treated phages on the bacterial cell were recently reported. Thus, these were mutations found in the descendants of the phages treated. Induced phage mutations arising directly after the treatment of bacteriophages were also reported. There are no reports, however, on such cases with actinophages, nor are there reports on obtaining induced mutations in actinophages in general.

The authors of the present work tried to obtain mutations in extracellular actinophages by exposing them to mutagens and to reveal mutations immediately after the treatment of phages with mutagens. For this purpose the authors used an actinophage belonging to the phage group that affects streptomycin producing cultures. H-mutations were studied. As the host cultures sensitive strain A. streptomycini B-6 and resistant strain A. streptomycini No. 675 were used. Spontaneous frequency of H-type mutations in our phage was 1 per 10^3 of all inoculated corpuscles. As mutagens u.v.-light, nitrous acid and sarcolysine hydrochloride were used.

Repeated tests have shown that sarcolysine hydrochloride pronouncedly increases the frequency of H-type mutations which exceeded in some experiments the frequency of spontaneously occurring mutations byor der 2 or 3, whereas u.v.-light and nitrous acid did not produce any notable increase of the mutation frequency.

Thus, it was the first case of obtaining induced mutations in actinophages and, what is more important—in extracellular actinophages.

Further studies of sarcolysine as a mutagen were carried out with several derivatives of this preparation which differed from sarcolysine hydrochloride in an additional amino acid chain. A number of polyfunctional chlorethylamines were also studied.

The fact that all the mutagens used cause mutations in extracellular actinophages may serve as a ground for a new interpretation of the mode of phage mutation.

5.6. The Action of Some Mutagenic Agents on Bacteriophage ØX-174. J. H. VAN DE POL and G. A. VAN ARKEL (Utrecht, The Netherlands)

The inactivation of bacteriophage ØX-174 with nitrous acid (Tessman, 1959) and ultraviolet rays (Setlow and Boyce, 1960) follows a single-hit kinetics. It has also been found for the inactivation of T4 with hydroxylamine by Freese and coworkers (1961). We likewise found for ØX-174, when treated with nitrous acid and ultraviolet rays, an inactivation curve that was single-hit. Upon treatment of the phage by hydroxylamine, however, an inactivation curve was obtained that extrapolated at zero time to approximately 2. In contrast to expectations the inactivation of ØX-174 by hydroxylamine thus appears to be a double-hit event. Results will be presented of experiments performed in order to test possible explanations of the curves.

Another notable finding was that the inactivation constant depended on the way the phage was grown, presumably on the medium used for propagation. The nature of this difference in sensitivity will be discussed.

The mutagenic action of hydroxylamine was studied by determining the number of host range mutants appearing on C/ØS, a phage-resistant mutant of *E. coli* C (Van de Pol, Vendrig and Van Arkel, 1963; cf. Van Arkel and Van de Pol, these abstracts). A method has been developed for the detection of the induced mutants. The number of mutants was measured as a function of the dose, and was compared to the values calculated assuming either single- or double-hit kinetics of mutagenesis.

The effect of mutagenic agents on isolated ØX-DNA and on double-stranded phage DNA is under investigation.

5.7. Synergistic Mutational Effects Between Radiations and Chemicals. DELBERT M. SHANKEL (Lawrence, U.S.A.).

When cells of *Escherichia coli* are impinged on membrane filters, subjected to nonlethal dosages of ultraviolet light and subsequently incubated in media containing certain chemicals, e.g. caffeine, theophylline, theobromine, a great increase in mutant numbers occurs. This increase is much greater than the total obtained from the two treatments employed separately. These "synergistic" effects of the chemicals can be prevented partially by the inclusion of very small amounts of other chemicals such as cysteine or 2-animo,4-sulfonamido-phenyl-disulfide in the post-irradiation phenotypic expression medium. The synergistic activity of the caffeine-type compounds appears to be related to certain features of their chemical structure. The amount of ultraviolet dosage plays a crucial role in the synergistic effect; nonlethal dosages providing for the maximal synergistic effect, while increasing dosages result in the gradual disappearance of the synergistic activity. Radioactive tracer experiments indicate that the synergistic chemicals are not incorporated into the nucleic acids of the cells. No synergistic relationship

between X-irradiations and chemicals has, so far, been observed. Possible mechanisms for this unusual type of biological activity are discussed in view of the available evidence.

5.8. Mutation during and after Synthesis of DNA. HERBERT E. KUBITSCHEK (Argonne, U.S.A.).

In tryptophan-limited continuous cultures of *Escherichia coli* B/1, try⁻, the rate of caffeine-induced mutation to resistance to bacteriophage T5 is constant, independent of growth rate. However, mutation rate is proportional to growth rate for cultures limited with glucose, lactate, succinate or phosphorus. These different responses do not appear to be due to different cellular concentrations of caffeine nor to death or reversion of mutants before expression of the mutant phenotype. The simplest interpretation of the two kinds of response is that the mutagen affects DNA either during or after synthesis. If the mutagen acts only during the formation of DNA then, as predicted by the error hypothesis, mutation rate should be proportional to the rate of replication of DNA, and hence to growth rate. Alternatively, if the mutagen acts after formation of DNA the rate of mutation should be constant since DNA is present in relatively constant amount. The primary evidence supporting this interpretation is that responses to two other mutagens are in agreement with expectation. There is evidence that the first, ultraviolet light, acts upon DNA rather than its precursors. If so, mutation rate should be independent of growth rate for cultures limited either with glucose or with tryptophan. This result is found. For the second, 2-aminopurine, mutation is expected to result from the incorporation of this base analog into DNA. Hence mutation rate is expected to be proportional to growth rate for either limiting factor. This proportionality is also observed.

Work supported by the U.S. Atomic Energy Commission.

5.9. Elective (Specific Directed) Mutation in Bacteria. FRANCIS J. RYAN and STEPHEN D. CETRULO (New York, U.S.A.).

The demonstration by Nagata that the chromosome in synchronously dividing *E. coli* Hfr replicates synchronously and with a polarity from the hind (F containing) end allows the following experiment. Similarly synchronized bacteria are exposed to short pulses of a base analogue which is mutagenic only upon incorporation. Only those genes which are replicating during the pulse are expected to undergo induced mutation. Preliminary experiments bear out this expectation. Applications of this technique to genetic problems, such as mapping, will be discussed.

5.10. New Derivatives of β-chloro- ethyl- amine as Active Mutagenic Factors. S. I. ALIKHANIAN and M. OGANESIAN (Moscow, U.S.S.R.).

It is known, that a number of β-chloro-ethyl-amine derivatives, such as embichin, chlorambucil and sarcolysine are highly mutagenic.

We have studied for genetic purposes a large series of previously not investigated amino acid derivatives of β-chloro-ethyl-amine, synthesized in the laboratory of I. L. Knuniantz. Thirty-four new compounds included two derivatives of *p*-di (2-chlorpropyl) amine, one derivative of *p*-chloro-ethyl-sulfide and thirty-one derivative of n-di (2-chloro-ethyl)amine. These agents belong to monofunctional, bifunctional, trifunctional and tetrafunctional compounds. This means that all derivatives had an "attacking" β-chloro-ethyl group with the exception of some derivatives having as an "attacking" 2-chlorpropyl group instead of 2-chloro-ethyl group.

From 37 compounds tested we selected seven most active compounds which were studied more thoroughly. Among them methyl-bis-(β-chloro-ethyl)-amine (embichin), *p*-propyl-(bis-β-chloro-ethyl)amine (chlorambucil), and bis-(β-chloro-ethyl)-amino-phenyl-alanine (sarcolysine) were studied previously with respect to their mutagenic action on *Neurospora*, *Penicillium chrysogenum*, *D. melanogaster*, phage T4 of *E. coli* and other organisms.

Compounds Nos. 6 (Ethyl ether of S-β-chloro-ethyl-dl-cysteine hydrochloride), 31 (Bis-/chlorpropyl/phenylacetic acid), 32(N-formulsarcolysine), and 33 (N-acetylsarcolysine) were the most active among the new compounds studied by us.

For comparative purposes highly active mutagenic factors, such as ultraviolet light, diethylsulfate, and ethylenimine were used. Strain H-6 of *Actinomyces olivaceus* served an as object in our experiments. The mutations were esti mated according to three characteristics, that is methionine deficiency (M^-), streptomycin sensitivity (S^s), and vitamin B_{12} synthesis (B_{12}). According to the first two characteristics the frequency of back mutations

to M^+ and that of direct mutations to S^r per 10^7 colonies was estimated. According to the third characteristic variations with respect to the level of vitamin formation were determined.

The results of the study showed that compound No. 6 by its effect with respect to the frequency of mutations in loci M and S is equal to embichin, chlorambucyl, sarcolysin and ultraviolet light; 10-20 times superior to diethylsulfate with respect to the mutation frequency and much more superior to these mutagens by the action on induced variation with respect to vitamin formation. Compounds Nos. 31, 32 and 33 by their effect on methionine deficiency and vitamin formation are 5-6 times superior to all these mutagens, including ultraviolet light.

It should be noted that these compounds have a different effect on the two loci. Compound No. 32 increases the mutation frequency by 37,000 times with respect to methionine deficiency as compared to the frequency of spontaneous mutations, thus being 70 times superior to ultraviolet light by its mutagenic effect on the same locus, whereas with respect to streptomycin sensitivity this compound increases the frequency of mutations only by 150 times as compared to the frequency of spontaneous mutations, thus being 7 times inferior to the ultraviolet light with respect to the mutation frequency induced by this mutagen. No such markedly differentiated effect on the two loci was observed in compounds Nos. 31 and 33.

On the basis of these results it is possible to: (1) discuss some problems of the mechanism of chemical mutagenesis; (2) consider the possibility of differentiated mutability of various genes under the action of the same mutagenic factor and (3) assume that new compounds with a strong mutagenic and specific effect are found.

5.11. Comparative Studies on the Mutagenic Action of Carcinogenic Substances, Nitrous Acid and Diethylsulfate in Saccharomyces cerevisiae. F. K. Zimmermann, H. Marquardt and R. Schwaier (Freiburg, Germany).

Certain carcinogenic substances have been described that are supposed to act by producing diazoalkanes which alkylate cellular structures. Since mutations can be induced by alkylation also, we tried to compare the possible mutagenic action of carcinogens to mutagens of known mutational pathways: Nitrous acid as a transitional and diethylsulfate as a transversional agent. The carcinogenic substances were: N-nitroso-N-ethylurethan (ethylating), N-nitroso-N-Methylurethan (methylating).

The mutational activities were checked in a backmutation test with four haploid, adenine requiring strains of *Saccharomyces cerevisiae* all mutated spontaneously at the ad_6-locus. The mutagenic activities are compared on a modified mutational/lethal hit base.

All carcinogenic substances are potent mutagens, the methylating compound being more efficient than the ethylating one. There is a striking difference between diethylsulfate and N-nitroso-N-ethylurethane. In two strains only the latter is active, in one strain diethysulfate alone and in one both are active together. The ethyl and the methyl nitroso compound, however, parallel each other qualitatively in all strains. Excepting for one strain there is a fair coincidence in activity with nitrous acid.

Mutational differences between diethylsulfate and N-nitroso-N-ethylure then may be due to differences in chemical reactivity. But the similarity of the nitroso compounds among each other and to some extent to nitrous acid might indicate that the mutagenic action resides in a mechanism different from alkylation.

5.12. Mutagenesis in Corynebacterium. J. Nečásek (Prague, Czechoslovakia).

The possibility of demonstration of the relative specificity of mutagens was studied by inducing biochemical mutants in the strain *Corynebacterium* VUA 9366. This strain is biotin dependent, produces large amounts of glutamic acid and has a very low spontaneous and induced mutation rate. Nitrogen mustard, ethyl methanesulphonate and ultraviolet light were used as mutagens. Biochemical mutants were isolated by the penicillin method. Mutants with identical dependences isolated in a single experiment were classifie das caused by a single mutation. On the whole 25,000 isolates werein vestigated after application of the three mutagens mentioned above. Nearly 1 per cent nutritionally deficient mutants was obtained, in which approximately one half required specific amino acids for growth.

The results indicate that there exists a qualitative difference between the mutagenic action of alkylating agents and ultraviolet light. With respect to arginine biosynthesis the alkylating agents induce mutants requiring arginine (step 3) and mutants requiring either arginine or citrulline (step 2). On the other hand the ultraviolet light induces mutants requiring also arginine and mutants requiring either arginine or citrulline or ornithine (step 1). The difference in frequencies of mutations blocking step 1 and step 2 was statistically significant. It is concluded that the

relative specificity of mutagens may exist not only on the allele level, but also on the intergenic one, and that different mutagens may act with different intensity on individual whole loci.

5.13. Comparison of Ultraviolet and Ethyl Methane Sulphonate induced Mutations of Adenine Loci in Saccharomyces cerevisiae. W. P. COSTELLO, E. A. BEVAN and M. W. MILLER (Oxford, Great Britain).

Mutations at the adenine-2 locus in *S. cerevisiae* cause the accumulation of a red pigment. If an adenine-2 strain is treated with mutagenic agents a small percentage of white colonies is observed among the survivors. These are due to either reversion to wild-type at the adenine-2 locus, or to a mutation at a locus prior to adenine-2 in the pathway of adenine synthesis, which prevents the accumulation of the red pigment. By reference to their complementation reactions with standard strains, these double mutants can be classified into five adenine loci.

Two of these loci, adenine-5 and adenine-7, as designated by Roman, are very closely linked. Our data suggest that the adenine-5/7 complex may be interpreted as a single locus showing complementing sub-units.

A total of 27 u.v. and 69 EMS-induced mutants of the general adenine-5-7 type were isolated and classified further on their complementation interreactions. Of the u.v.-induced mutants 4 were ad-5, 21 ad-7, and 2 ad-5/7, the double mutant: of the EMS-induced mutants 27 were ad-5, 39 ad-7, and 3 ad-5/7, indicating a degree of specificity for the ad-5 type mutation by EMS.

Survival-mutation studies for buffered and unbuffered EMS treatment indicate that while the overall mutation rates of the two are comparable with respect to survivors, EMS buffered at pH 5.8 kills only 40 per cent over a period of 2 hr whereas unbuffered EMS in saline, in which the pH falls from 7.0 to 2.8 over 2 hr, kills 80 per cent.

5.14. The Pattern of Some Biochemical Mutants of E. coli after X-rays and Cancerogenic Substances. A. RÖSENER and H. A. KÜNKEL (Hamburg, Germany).

By means of methods published by Lederberg and by Holliday it is possible to identify various biochemical mutants of bacteria produced by mutagenic agents and to analyse the distribution of the different types. In our investigations on the radioresistant strain *E. coli* B/r most of the spontaneous mutations to auxotrophy are serine/glycine-dependent. Other biochemical mutants occur just sporadically. Nearly the same distribution is observed after X-irradiation. Further experiments were carried out with several nitrogen-compounds. These substances which belong to the group of nitrosamines or nitrosamides were proved to be highly cancerogenic by Druckrey *et al.* Furthermore they are organotropic, too, and produce malignant tumors in different organs according to their chemical structure. After application of these agents a specific pattern of biochemical mutants is observed which differs from the "spectrum" obtained by X-irradiation or spontaneously in a typical way. These results are discussed and compared with the findings of similar experiments which were carried out on *Drosophila* in our laboratory, simultaneously.

5.15. Reversion Patterns among Genetic Sites in Salmonella typhimurium on Treatment with Chemical Mutagens. A. EISENSTARK and RUTH VANSICKLE (Manhattan, U.S.A.).

Initially, auxotrophic mutants of *Salmonella typhimurium* LT2 were obtained by induction of prototrophic cells with ultraviolet, X-rays, neutrons, nitrous acid (NA), 2-aminopurine (AP) and diethylsulfate (DES). The collection also included a number of spontaneous mutants. Approximately 400 of the single-site auxotrophic mutants were examined for reversion to prototrophy following treatment with a series of chemical mutagens. Among the observations were: (1) the method of induction of forward mutation influenced the reversion pattern in a distinct manner; (2) base analogues failed to revert over half of the NA mutants, indicating that NA was not restricted to transition capability; (3) N-Methyl-N^1-nitro-N-nitrosoguanidine reverted the same sites as DES, but the two showed distinct quantitative differences for certain specific sites; (4) 4,5,6 triaminopyrimidine-sulfate-hydrate was highly mutagenic, but reverted a smaller number of sites than did AP; (5) deletions were not observed when either AP or DES were used to induce mutation in the prototrophic strain; (6) reversion patterns were independent of either lysogeny or the presence of a second mutation within the strain tested; (7) a large fraction of apparent reversions did not involve restoration of true wild-type, indicating possible mutation at a suppressor locus. These could be easily scored as stable small-colony-formers when plated on minimal medium.

A number of these suspected suppressor mutants were tested, using transduction experiments, to determine whether each was site-specific, locus-specific, or non-specific.

5.16. Genetic Analysis of Adenine—1 Revertants in the Fission Yeast Schizosaccharomyces pombe. C. H. CLARKE (Edinburgh, Great Britain)

Ultraviolet induced adenine—1 auxotrophs isolated by Prof. U. Leupold have been used in reverse mutation studies with *S. pombe*. Adenine-independent revertants of spontaneous, HNO_2 and u.v.-induced origin have been obtained and tested for the presence of suppressor mutations. Revertants giving no adenine-requiring recombinants among 300-500 progeny ascospores, after backcrossing to wild-type, are classified as "reversions".

One adenine-1 mutant, number 40, gives spontaneous revertants which are mainly of a suppressor type, while most HNO_2 and u.v.-induced revertants of this same mutant are "reversions". Another temperature-sensitive mutant, adn.-1, 199, gives revertants spontaneously and with HNO_2 treatment which are mostly "reversions". u.v.-induced revertants of mutant 199 are however predominantly of the suppressor type. Furthermore the appearance of HNO_2-induced "reversions" of mutant 199 is only weakly, that of u.v.- induced suppressor revertants strongly, inhibited by the addition of L-methionine to the minimal medium upon which adenine-independent colonies are scored. These two differences between HNO_2 and u.v.-induced revertants of the same mutant may be related. The results indicate an unusual example of mutagen specificity for individual alleles.

Full details and results will be submitted for publication to *Genetical Research* in due course.

5.17. The Use of the Suppressors of Methionine$_1$ for the Study of Mutation in Aspergillus nidulans. LORNA J. LILLY (London, Great Britain).

During a survey of spontaneous reversion rates from nutritional requirement to protrophy in *Aspergillus*, it was found that the change from methionine requirement to prototrophy occurred with exceptionally high frequency (approx. 10^{-5}).

These reversions from methionine requirement can be shown to be due to suppressor genes, all those so far tested being unlinked to the methionine$_1$ locus. The suppressors have various visible (morphological) phenotypes ("A", "B", "C", etc.). If each phenotype is determined by a single cistron, these suppressors could be used for the simultaneous visible scoring of forward mutation in several cistrons.

The genetics of some of the phenotypes which occur with highest frequency are being investigated. The results of crossing one strain of type "A" with one of type "B" indicate that each strain carries a single suppressor and that these suppressors are unlinked. Crosses between strains of type "A" show that at least two unlinked loci can independently determine this phenotype. Crosses between the other phenotypes are in progress and the suppressors are being located.

If two unlinked loci control the "A" phenotype this phenotype is not suitable for the visible scoring of mutation in a single cistron. This result may affect the interpretation of results obtained by workers who are using this system to study chemically induced mutation.

5.18. Kinetic Studies of Chemically Induced Reverse Mutations in Neurospora crassa. H. G. KOLMARK and B. J. KILBEY (Edinburgh, Great Britain) and S. KONDO (Misima, Japan).

The experiments were designed to follow the effects of chemical concentration and time of treatment on mutation induction. Conidia from the strain K 3/17 ad-3A, 387ol, were treated with diepoxybutane (DEB), or ethylenoxide (EO) in aqueous solution.[1] The temperature was kept at 21° or 22 C during treatment. Treatments with DEB were carried out for varied periods with concentrations varying from 1.0×10^{-2} to 3.16×10^{-1} M. At the higher concentrations of DEB it was found that the frequency of adenine reverse mutations among survivors was determined by the product of time and concentration (the "dose"). After a short initial period the frequency is approximately a linear function of (conc. time)^2. This result suggests that the induction of adenine reversions by DEB has a two-event mechanism. It seemed possible that this mechanism was due to the existence of the two reactive epoxygroups in the molecule of DEB. However, similar experiments with EO, which has only one epoxygroup, gave the same kinetics. It therefore seems more likely that the basis for a two-event mechanism resides in the induced genetic change. The monofunctional compound, EO, is by far the more efficient when similar doses of the two compounds are compared. Mutation frequency

is independent of time ("dose rate") at the higher concentrations. At the lowest concentrations used, there is a decreased response to a given dose.

1. Consult: KOLMARK and KILBEY, *Z.f. Vererbungslehre* **93**, 356-365, 1962, regarding techniques.

5.19. Mutation and Crossing-over Induced by Mono- and Bifunctional Alkylating Agents. G. MORPURGO (Rome, Italy).

The ability of methyl bis (β-chloroethylamine, 2-chlorotriethylamine hydrochloride, diepoxybutane and ethylene oxide) in inducing mutation and mitotic crossing-over in a diploid strain of *Aspergillus nidulans* has been tested.

Mono- and bifunctional compounds are equally active in inducing mutation. Only bifunctional compounds are able to induce high frequency of somatic crossing-over. These effects on crossing-over can be attributed to the cross-linkage of non sister chromatids.

5.20. Genetic Analyses of Photo-reactivation, Dark-reactivation and Resistance to Ultraviolet Light in Escherichia coli. P. VAN DER PUTTE and A. RÖRSCH (Rijswijk The Netherlands).

The genetic constitution of mutations in *Escherichia coli* which affect the radiation sensitivity of the strain has been studied. One mutation leads to loss of the tendency to form long filaments after u.v. irradiation, a second to loss of the ability to repair u.v. lesions in the DNA of the cell in the dark (dark-reactivation) and a third mutation to loss of the ability to repair u.v. lesions by subsequent irradiation of the cell with visible light (photo-reactivation).

By conjugation experiments with auxotrophic mutants it is proved that all three properties are chromosomally determined and may be located as distinct markers on the bacterial genome. Methods to screen large numbers of recombinants for the markers which affect the radiation sensitvity of a strain will be described. The results will be published in full in the *Biochim. Biophys. Acta.*

5.21. Reactivation of U.V. Irradiated Escherichia coli K-12 (λ) by Treatment with EDTA. RENANA BEN-GURION (Ness-Ziona, Israel).

When lysogenic bacteria are irradiated, the prophage genome passes from a state of repression to an active state, phages are being produced, and the lysogenic bacteria are killed in this process. It appears, therefore, that induction (a process as yet not fully understood) renders the lysogenic bacteria more sensitive to irradiation than their nonlysogenic derivates. Because of the higher sensitivity of lysogenic cells to irradiation, these organisms could perhaps be suitable tools for the study of irradiation effects.

When the lysogenic bacterium *Escherichia coli* K-12 (λ) (strain 169) was irradiated with ultraviolet light and then transferred to a medium containing EDTA, this resulted in a partial reactivation of the lysogenic bacteria. The number of survivals was increased and the production of phage decreased, as compared with the irradiated controls. The composition of the bacterial growth medium, as well as that of the medium to which the bacteria were transferred after irradiation, had a marked effect both on the number of surviving cells and on the reactivation resulting from the treatment with EDTA.

5.22. X-ray and Ultraviolet Light Mutagenesis in a Threonine Requiring Escherichia coli K12 studied by Recombination Techniques. T. KADA and H. MARCOVICH (Paris, France).

During the mating process in *E. coli* K12, the chromosome of the male cell (Hfr) is transferred into the female (F^-) without any detectable amount of cytoplasmic material. This system, therefore, is a workable one for determining whether the initial step in X-ray or u.v. mutagenesis is a cytoplasmic or nuclear one. This work deals with crosses between *E. coli* Hfr $thr^-{}_{33}leu^+$ sensitive to T6 phage and *E.coli* $F^-thr^-{}_{33}leu^-$ resistant to T6 phage. Having irradiated the male before mating with the female, we scored the reversion to threonine independence among the recombinants possessing the resistance to T6 and having received in addition the leu^+ character. The thr and leu determinants are closely linked, and leu serves here as a scoring factor which assures that the thr region of the male chromosome has been injected into the female. When the male is irradiated with X-rays, the mutant character can be immediately transferred to the zygote. With u.v. irradiation, no such immediate transfer is seen. This transfer takes place only afterwards, increasing with time up to 100 per cent in 24 hr culture in broth. In reciprocal irradiation involving the female, it appears that the male genetic material is mutated if injected in a u.v. irradiated female, but not if X-ray is used. These

results suggest that X-ray mutagenesis, in the example studied here, is exerted directly on the chromosome, and that u.v. mutagenesis involves a rather long-lasting cytoplasmic step before the mutant character is integrated in the chromosome.

5.23. The Effect of U.V.-Light and X-rays on the Variability of Bacillus licheniformis. F. PALEČKOVÁ (Prague, Czechoslovakia).

The relationship between irradiation dose, killing effect and production variability of a bacitracin-producing strain of *B. licheniformis* was studied. The organism was exposed to u.v.-light for 4, 8, 16, 32 sec; the survival curve was sigmoidal. In experiments with X-ray treatment doses of 200,000 r, 400,000 r, 600,000 r were used. The survival curve was linear. The effect of these mutagenic factors on the variability of the antibiotic-producing ability of the strain was studied and submitted to statistical analysis.

5.24. A Region of the Chromosome of Escherichia coli K12, abnormally Resistant to Ultraviolet. F. JOSET and H. MARCOVICH (Paris, France).

Studies dealing with the effects of radiations on genetic recombination in *E. coli* K 12 have shown that a region of the male chromosome is abnormally resistant to ultraviolet and α radiations. This region, located between the determinants for Tryptophane and Histidine, represents about one-fourth of the whole chromosome.

No genetic markers have yet been found in this region, as compared to about 100 for the rest of the chromosome; assuming a random distribution of the markers this fact might have a biological signifiance.

Two suggestions can be made which could explain the resistance to u.v. and the absence of genetic markers:

1. This region might be almost completely empty of DNA. This hypothesis seems to be ruled out by ^{32}P labeling experiments, which show a normal sensitivity to radio-phosphorus per unit length. These experiments have shown the existence of a second series of lesions induced by ^{32}P. These lesions can be transferred to the female during a cross, and prevent the integration of the labelled material in the zygote chromosome.

2. If not absent, the DNA of this region might have an abnormal base composition. The molecular basis of u.v. damage is thought to be mostly a dimerization of thymine. As this region is relatively insensitive to u.v., one may think that thymine dimers are less likely to be formed, either because of a low thymine content, or because of the absence of neighboring thymine-molecules.

Experiments using base analogues such as 5-bromouracil, are being done to test this possibility.

5.25. The Effect of Temperature on the Response to Ultraviolet of Neurospora crassa. B. J. KILBEY (Currie, Great Britain).

The temperature of the conidial suspension during ultraviolet irradiation has been found to influence the mutagenic response of the strain *K 3/17 ad-3A 38701, inos 37401.*[1] A dose of ultraviolet administered at 3-4 C may produce two to three times the number of adenine reversions compared with a similar dose administered at temperatures between 25 C and 30 C. Survival and inositol reversions do not appear to be greatly influenced by this difference in temperature.

The evidence at present available suggests that a temperature sensitive recovery process is acting to repair adenine reverse mutations. Interest is centred on the specific nature of this process and it is hoped that this may be clarified by the study of other *ad-3A* mutants and *inos* mutants which respond to ultraviolet light, and by an examination of the effect of varying the experimental conditions.

1. A full description of the characteristics of this strain may be found in *Zeit. für Vererbungslehre* **93**, 356-365, 1962.

5.26. Genetic Studies of a Mutation to Radioresistance induced by ^{32}P decay in Saccharomyces cerevisiae. E. MOUSTACCHI, H. HOTTINGUER-DE MARGERIE, and H. MARCOVICH (Paris, France).

Different agents (^{32}P decay, ultraviolet light, mustard-gas, ionizing radiations) induce in haploid *Saccharomyces cerevisiae* a mutation towards resistance to the lethal action of ionizing radiations. DNA content per cell of these resistant strains show that they are still haploid. This is confirmed by the fact after that sporulation of diploid strains from a cross between a radiosensitive and a radioresistant strain the viability

of the spores and the segregation of the mating type are normal.

The tetrad analysis of these strains shows that the resistant character segregates from the radiosensitive one with a ratio of 2: 2. In other words, one genetic determinant is responsible for this character.

Radiosensitivity (S^+), which is the wild type, is dominant over radioresistance (S^-). The diploid cells (S^+ S^-) are less resistant than the resistant haploid parent (S^-). Their survival curve is superimposed on that of the diploid wild type (S^+ S^+).

Different sites (three at least) are implicated in this character; the survival curves of some S^-S^- diploids are identical with that of the S^+S^+ and the others are identical with that of the S^-; the latter diploid cells are homoallelic and the former are heteroallelic.

The size of the target is about 1/20th of the whole genome. This estimation is made through a dose-effect relationship for the induction of this mutation by ^{32}P decay or by X-rays.

When the S^- character is introduced into a cross, deviation from the 2: 2 segregation ratio for different biochemical markers is observed: there is a large excess of prototrophs. These deviations may be accounted for by a ^{32}P induced mutation of a suppressor gene.

5.27. Determination of Radiation-induced Mutation Rates of Recessive Lethal Alleles in Saccharomyces. Wolfgang Laskowski and Klaus Haefner (Berlin, Germany).

After irradiation with small doses of u.v. and X-rays diploid *Saccharomyces*-strains were isolated, which proved by tetrad analysis to be heterozygous for a single recessive lethal allele (+/1). The radiation sensitivity of some of these strains was compared with the sensitivity of the diploid parent strains homozygous for the corresponding wild type alleles (+/+). From these data the u.v. and X-ray induced rates for the mutation of wild type alleles to the recessive lethal condition (+→1) were calculated and found to be several orders of magnitude larger than mutation rates from prototrophic to auxotrophic condition.

Detailed data will be published in *Z. für Naturforschung*.

5.28. A Hereditary Propensity to Untimely Death in Yeast and its Temporary Reversal by Radiation. Allen P. James (Chalk River, Canada).

A respiratory-deficient strain of yeast is unstable when cultured on a medium usually considered optimal for growth. The instability of these "vegetative petites" is characterized by the production of moribund cells. Analyses involving the study of individual cell lines have demonstrated that clones derived from a single cell frequently differ in their degree of instability. In general, the rate at which moribund cells are produced increases in successive generations; extinction of the strain is prevented only by occasional and temporary reversals of this trend. Complete stability can be obtained either by mutation or by culturing the strain on a minimal medium. The tendency to extinction can also be reversed temporarily by exposure of the cells to ultraviolet light but not to X-rays. The result of experiments designed to uncover the underlying mechanism and influence of radiation are discussed.

5.29. The Mutagenic Activity of Ethyleneimino Derivatives with Different Numbers of Reactive Groups. H. Lüers and G. Röhrborn (Berlin-Dahlem, Germany).

We compared the mutagenic activity of tri-, bi-, and monofunctional ethyleneimines for studying the relation between the number of reactive groups and the degree of mutagenic action in this class. We also tested three of their basal compounds without any groups of alkylating ability.

The tested compounds were as follows: trifunctional: (A) tris-ethyleneimino-benzoquinone-1,4, (B) tris-ethylene-thio-phosphor-amide; bifunctional: (C) 2,5-di-n-propoxi-3,6-bis-ethyleneimino-benzoquinone-1,4, (D) 2,5-bis-ethyleneimino-benzoquinone-1,4; monofunctional: (E) dipiperino-phosphoric-acid-ethyleneimine, (F) 4-ethyleneimino-naphtoquinone-1,2, (G) 2-ethyleneimino-5, 6, 7, 8-tetrahydro-naphtoquinone-1,4; without ethyleneimino groups: (H) p-quinone, (I) 1, 3, 5-triazine, (K) 2,4,6-triamino-1, 3, 5-triazine.

Using the Berlin wild stock of *Drosophila melanogaster* the rates of sex-linked recessive lethals have been determined by the Basc method in broods of three days after having fed or injected the Berlin wild males with aqueous solutions of the test substances. With respect to the mutagenic effects on the postmeiotic stages of the spermatogenesis there are striking differences between the different compounds. Trifunctional ethyleneimines proved to be more mutagenic than bifunctional ones, and these had a higher efficiency than the monofunctional ethyleneimines.

The differences between these three groups are significant. The three basal compounds did not show any distinct mutagenic effect. Moreover, we got differences between the mutation rates of the postmeiotic stages within single groups. So A proved to be more mutagenic than B, and E more than F and G. All ethyleneimines also induced mutations in the meiotic stages of the spermatogenesis. In premeiotic stages a mutagenic efficacy could be established for A, C, and D, but not for E, H, and K.

5.30. Somatic and Gonadal Mosaicism in D. melanogaster. E. A. CARLSON and J. L. SOUTHIN (Los Angeles, U.S.A.).

Simultaneous experiments in chemical mutagenesis using the specific visible dumpy (2, 13.0) and using sex-linked lethals have demonstrated a considerable degree of mosaicism for both approaches. The two methods are not directly comparable because only gonadal tissue is assayed in the sex-linked lethal test, while both somatic and gonadal tissue may be assayed for the specific visible test. The use of brood analysis (postmeiotic and premeiotic sperm) for both assay systems indicates a shift from postmeiotic mosaically occurring mutant individudals to premeiotic "complete" (non-fractional) mutant individuals. This is expressed by modification of the premeiotic F_2:F_3 sex-linked lethal frequency and by the presence of both somatic and gonadal complete mutation in most of the premeiotic dumpy mutants. These studies have enabled us to calculate the fraction of a gonad carrying the mutant tissue, the number of individuals carrying mosaic sex-linked lethals, and the total mutation frequency for agents inducing mosaicism. A theory which predicts the ratio of F_2:F_3 sex-linked lethals from the analysis of the mutant classes in a specific visible test has also been inferred from the data presently available.

Sex-linked lethal experiments have demonstrated an F_2 (gonadal complete) mutation frequency of 11.3 per cent and an F_3 (from F_2 gonadal mosaic) mutation frequency of 3.8 per cent. The number of F_2 gonadal mosaics in the total F_2 females sampled is 9.21 per cent. The portion of gonad containing sex-linked lethals is 44 per cent. It can be shown from our data that the cells forming the polar cap are non-randomly selected and probably do not consist of more than four cells in most instances. The F_2:F_3 mutation frequency for 1-4 day sperm is 1.8; for 5-6 days 1.1; for 7-9 days 4.1; for 10-11 days 7.9; for 12-14 days 24.8 and for 15-18 days 13.8. The mean F_2:F_3 sex-linked lethal frequency for all broods is 3.0. These results are corrected for spontaneous sex-linked lethal frequencies. The mutagen used for this study is monofunctional quinacrine mustard (I.C.R.-100).

5.31. The Mutagenic Activity of Formaldehyde-treated Ribonucleic Acid in Drosophila melanogaster. THOMAS ALDERSON (Cambridge, Great Britain).

In experiments using a chemically-defined axenic treatment medium, it has been shown that formaldehyde exhibits no mutagenic activity towards *Drosophila melanogaster* larvae unless ribonucleid acid is present in the treatment medium. Further analysis of this phenomenon has shown that adenylic acid is the active component of ribonucleic acid uniquely concerned. The presence of adenylate in the treatment medium is apparently necessary for the mediation of the mutagenic activity of formaldehyde, whether adenylate is present as the free mononucleotide or bound in ribonucleic acid polynucleotide. An *in vitro* reaction product of formaldehyde with adenylate appears to be the effective mutagenic alkylation.

Since formaldehyde-induced mutagenesis has not previously been attained in the absence of free formaldehyde, experiments have been carried out to determine whether the necessary formation of alkylated-adenylate is itself a sufficient condition for the mediation of the mutagenic activity of formaldehyde. Ribonucleic acid treated with formaldehyde under conditions used in mutation tests and dialysed free of unbound formaldehyde, was found to be decidedly mutagenic towards *Drosophila* larvae. Ribonucleic acid similarly handled, but not treated with formaldehyde, showed no mutagenic activity. No difference is observed between the two series in the developmental time of the larvae or in the emergence of imagines. It would thus appear that the *in vitro* alkylation of adenylic acid by formaldehyde is both a necessary and sufficient condition for the mediation of the mutagenic activity of formaldehyde.

5.32. The Elimination of a Specific Chromosome Segment by Nucleic Acids in Drosophila melanogaster. O. G. FAHMY and MYRTLE J. FAHMY (London, Great Britain).

Macromolecules (nucleic acids, proteins and ionic polymers) were shown to be mutagenic as regards the induction of small chromosome deletions (*Minutes*). Three grades of phenotypic

expression were distinguished: *Minute - slight*, *M(s)*, with only the macrochaetes reduced in length and thickness; *Minute-intermediate*, *M(i)* with more extreme bristle effect, accompanied by some abnormality of wings, eyes or body; *Minute - extrem*, *M(e)*, highly pleiotropic with several external features grossly affected. Radiation induced *Minutes* showed all grades of expression with a preponderance of the *M(i)* class, whereas those of macromolecules were either *M(s)* or *M(e)*, with the *M(i)* class virtually absent.

A correlation was established between the phenotypic expression of the *Minute* and the genetic position of the chromosome deletion causing it. In a sample of 165 *M(e)* mutations induced by various macromolecules all but 3 (which werenearer the *M(i)* class) were on the IVth chromosome. The deletion involved the proximal 4/5th of the salivary gland IVth chromosome, and covered the majority of its marker genes, including the most proximal: *sparkling-poliert* (*spa*^{pol}.) and *eyeless* (*ey*). In contrast, of the 34 *M(s)* mutations studied, 33 were on the large autosomes (IInd or IIIrd). The proportion of the *M (e)* class among the *Minutes* induced by various macromolecules was different, reaching a maximum of 95 per cent with DNP and a minimum of 29 per cent with polymethacrylic acid. This indicates a differential distribution of the chromosome deletions with the various macromolecules.

The frequency of induction of IVth chromosome deletions with DNA was compared with that occuring with radiation. Among 47 fertile *Minutes* induced by DNA, 38 were on the IVth chromosome (81 per cent) and all covered *spa*^{pol}.; whereas with 46 X-ray *Minutes* recovered in a comparable experiment only 13 occurred on the IVth (28 per cent), 12 of which covered *spa*^{pol}. These two distributions are very significantly different ($\chi^2_{[1]} = 26.0$, $P < 10^{-6}$) indicating a high degree of selective gene elimination with DNA.

5.33. The Genetic Effects of Labeled DNA Precursors. W. D. KAPLAN, H. D. GUGLER and K. K. KIDD (Duarte, U.S.A.).

Sex-linked recessive lethals have been induced in *Drosophila* males by feeding tritiated thymidine to larvae. In three experiments feeding was restricted to 8 hr to limit the incorporation of the isotope. The most heavily labeled germ cells appeared in the brood sperm utilized for the first of three 3-days broods tested for induced lethals. In one experiment, during which larvae were permitted to feed on labeled food throughout larval life a higher frequency of labeled sperm bundles was obtained and also heavier labeling of individual sperm.

Induced mutation rates reflected the degree of incorporation, and mutations were restricted to those broods with labeled sperm.

The physical characteristics of the beta emissions of tritium — low energy and short mean path — led us to test the distribution of the induced mutations. The pattern is nonrandom and differs significantly from those obtained by the use of X-irradiation. The region of the X-chromosome from 1 to 20 is relatively free of mutations, whereas the regions between 30-35, 50-55, and 60-65 have a higher number of induced lethals than would be expected at random.

Mutations have also been induced by tritiated deoxycytidine. These mutants will be localized and the distribution compared to the one observed with H^3 thymidine. If the pattern of induced mutations reflects the varying frequencies of the pyrimidine bases along the length of the chromosome, the pattern induced by H^3 deoxycytidine should differ from that observed with the use of H^3 thymidine.

5.34. Incidence of Mutations in Drosophila melanogaster raised from Flies fed on Irradiated Medium. S. NIRULA, M. S. SWAMINATHAN, A. T. NATARAJAN and R. P. SHARMA (New Delhi, India).

Earlier studies at the Indian Agricultural Research Institute on the effects of culture media, potato mash and fruit juices irradiated with ionizing radiations on mitosis in root meristems of barley, *Vicia faba* and onion grown on them have revealed that such irradiated products may have radio-mimetic effects. In view of the obvious bearing of these data on assessing the wholesomeness of food material sterilised through ionizing radiations, a study was initiated in *Drosophila melanogaster* to ascertain whether the mutational load is enhanced in flies fed with irradiated food.

The basic medium consisting of glucose agar and yeast is irradiated with 150 k rad of gamma-rays (sterilizing dosage) at a 160 curie Co^{60} source. Young Oregon-K flies (soon after emergence) are fed on this irradiated medium. The flies are allowed to breed and parent flies are killed about 7 days later. The male flies which emerge out of the larvae fed exclusively with irradiated food are then used for conducting M-5 tests on normal culture medium for further

F^1 and F^2 breeding. The experiments have been repeated thrice.

Sex-linked recessive lethals have been found only in the families derived from the irradiated medium-series (6.44 per cent). A wide range of phenotypic changes was a striking feature of the F_2 families from the irradiated medium cultures (their frequency ranging from 0.13-0.37 per cent against 0.018-0.095 per cent in control on population basis). Some of the mutant flies, viz. curly wings, yellow body, and another with dominant wing mutation were found to breed true. A few changes like half thorax, rotated abdomen and absence of neck belong to the non-inherited group of abnormalities of Morgan (Bridges and Sturtevant 1925) they were observed only in the irradiated medium series. The implications of these studies will be discussed.

5.35. Experiments with Chemical Compounds which Reduce or Increase Mutagenic Effects of Ionizing Radiations. H. A. KÜNKEL and A. RÖSENER (Hamburg, Germany).

This paper deals with investigations on three chemical compounds well known as radioprotecting agents with regard to somatic radiation damage: cysteine, aminoethylisothiuronium (AET) and 5-hydroxytryptamine (serotonin). Experiments were carried out on *Drosophila* and on *E. coli*. In *Drosophila* the rate of ray-induced sex-linked recessive lethals was tested after X-irradiation by means of the Muller-5-Method. In the experiments on *E. coli* the rate of back-mutations was observed of a leucine-dependent and of a streptomycine-dependent strain to prototrophy. Cysteine considerably reduced the X-ray induced back-mutations in *E. coli*. No influence, however, was observed in *Drosophila*. Serotonin reduced the rate of mutations in *Drosophila* as well as in *E. coli*. Protection, however, was only small. After AET the ray-induced rate of mutations significantly increased although this compound *per se* has no mutagenic effect. Lastly experiments are reported with bromodeoxyuridine which is incorporated into DNA instead of thymidine and provides increased radiosensitivity in some cases.

5.36. The Problem of Antimutagens in Connection with Mutagenesis and Chemical Protection. N. P. DUBININ (Moscow, U.S.S.R.).

The problem of antimutagens plays a paramount role in the control of the spontaneous mutation process and in the investigation of the essence of the natural biochemical protection of the cell.

The class of antimutagenic substances consists so far of very few compounds. The need for new antimutagens is great. For the solution of this problem we have founded our investigations on the following principles:

1. The search for new antimutagens in the group of substances of universal protection, i.e. protecting the cell from possibly many various effects. Kihlman has shown that streptomycin protects against ultraviolet and γ-rays and α-particles. Our experiments with streptomycin have shown that it is an antimutagen.

2. Searches in the group of substances which, being protective against radiation, are at the same time a natural and an active cellular metabolite. The aminoacid arginine, responding to these requirements, was also found to be an antimutagenic compound.

The antimutagenic effect of streptomycin, cysteamine and arginine has been proven on onion roots and broad beans, which had been protected by these substances from the occurrence of spontaneous chromosome rearrangements. The cytological analysis of the antimutagenic effect of the compounds studied has shown that it is connected with a decrease in the frequency of primary breaks and not with the processes of the re-union of fragments. In experiments with recessive sex-linked lethal mutations we have shown that streptomycin produces an antimutagenic effect upon the occurrence of spontaneous gene mutations.

Depending on the concentration of the antimutagenic compound it may, in optimum concentrations, produce an antimutagenic effect, in high doses it may prove to be mutagenic, in average and minimum concentrations it may be neutral.

As a rule, in certain concentrations the antimutagens acquire properties of substances which are able to protect the chromosomes against the effect of ionizing radiations. It is shown that the character of this protection is different for different phases of the cellular cycle.

Of great importance in the problem of mutagensis is the analysis of their differential effect on various genes.

5.37. Methods for Estimating Differential Radiosensitivity. I. I. OSTER and E. POOLEY (Philadelphia, U.S.A.).

Although marked differences in the sensitivity of the various stages of germ cell development

to ionizing radiation have been recognized, conflicting estimates of the relative amounts of damage have been reported by some investigators. Fortunately, it is becoming increasingly apparent that such discrepancies are in large measure, if not entirely, due to the siphoning off of cell samples representing different stages of development (and therefore different intrinsic radiosensitivities) at the time of treatment. Not only would such mixtures result in false estimates of the relative radiosensitivity of the various stages but it is known that many external and/or internal factors [1] are capable of altering the degree of heterogeneity of any particular sample of cells which is being analyzed. The extent to which this occurs is often exceedingly variable even within the same experiment.

By utilizing a combination of techniques we have been able to avoid these difficulties and have made cytogenetical analyses of cells representing homogeneous stages of development at the time of treatment. These methods include the use of our so-called multi-purpose stocks (i.e. strains which allow for the detection of several different types of genetical damage in the offspring of the same treated individual), histological examination of the germ tract of the treated individuals, and a sampling technique that involves the treatment of immature stages which contain a preponderance of germ cells in a similar stage of development. The clear-cut pattern of germ cell radiosensitivity as regards various types of genetical damage which has emerged for *Drosophila* is in general agreement with that obtained more recently for the mouse by Dr. L. B. Russell.

This parallelism suggests that the mechanism(s) underlying differential radiosensitivity may be quite widespread.

In addition to a comprehensive report of the results obtained with radiation, comparisons will be made with the data obtained by treating the reproductive cells with chemical mutagens.

This work has been supported by grant AT (30-1)-2618 from the U.S. Atomic Energy Commission.

1. E.g. C. AUERBACH, Sensitivity of the *Drosophila* testis to the mutagenic action of X-rays. *Z. indukt. Abstamm.- u. Vererb.-Lehre* **86**, 113 (1954).

5.38. The Rates of Visible Mutations in Sequential Broods of Irradiated Drosophila melanogaster Males. ALOHA HANNAH-ALAVA (Turku, Finland).

The brood pattern of recovered mutations was determined for three different types of visibles-dominants and Minutes in all chromosomes and recessives at specific third-chromosome loci — in the progeny of *D. melanogaster* males of two genotypes: wild-type, Oregon-RS, males (Exp. I) and males heterozygous for 12 third-chromosome recessive markers (Exp. II), mated singly and sequentially to females of an appropriate genotype for detecting mutations at specific loci and/or crossovers. The males in Exp. I were 2-24 hr of age and in Exp. II 24-48 hr of age at the time of treatment (3000r hard X-rays) and initial mating. The sequential broods derived from these males, mated up to 24 days, totaled 94,081 and 42,951 F_1 offspring for the irradiated and control series respectively.

Except for the first brood (1-3 days) in which there was a lower incidence of mutations in the progeny of the older males than the younger males, the curves of induced mutational response were not significantly different in the two experiments. In the broods following the period of excessive sterility (6-8 days) 113 mutations were recovered from 46,922 offspring of the irradiated males. The slopes of the curves in the premeiotic broods (11-24 days) suggest that one of the consequences of radiation of the early gonial stages is elimination of the germ cells with potential visible mutations to a greater extent than elimination of germ cells with either induced crossovers or recessive lethals with no visible phenotype.

Research supported by IAEA contract No. 31 and USAEC contract No. AT (30-1) 2690.

5.39. The Induction of XO Males, by Irradiation of Drosophila. melanogaster Males. B. LEIGH (Leyden, the Netherlands).

It has been found that, after the irradiation of adult males, there is a difference in the sperm samples which show the highest frequencies of lethals and translocations, and those which show the highest frequency of XO males. A set of experiments were carried out to investigate this discrepancy. Twenty-four-hour old males, of the genetic constitution $X^{c^2}yB$; sc^8 Y, were treated with doses from O-3000 r. A brood pattern of five 2-day broods was used, with six females per male per brood. The F_1 was scored for XO males, non-disjunction females, and

sex ratio. The highest frequency of XO males was found in sperm sampled on the 7-8th days after irradiation, presumably corresponding to young spermatocytes, and this was not accompanied by any comparable increase in the number of non-disjunction females. From the structure of the ring-X it is expected that most breaks will result in loss of the chromosome. This is supported by the fact that the same dose of irradiation produces more XO males from the ring-X than from a rod-X. The dose effect curve for the production of XO males is a straight line, one-hit event. A comparison of this dose effect curve with that of the change in sex ratio gives further evidence that XO males are mainly produced by loss of the X chromosome. A hypothesis to explain the different peaks for the two types of breakage-involving effects, XO males and translocations, will be discussed.

Sponsored by the Institute for Radiopathology and Radiation Protection.

5.40. The Brood Pattern of X-ray Induced Crossing-overs in Drosophila melanogaster Males. Jaakko Puro (Turku, Finland).

The pattern of induced crossing-overs in *Drosophila melanogaster* males was studied using a hybrid stock of 12 recessive markers in the third-chromosome. Adult males, after treatment with 3000 r X-rays, were mated singly and sequentially (at 2- or 3- day intervals for the first three broods and daily from the 7th to the 24th day) to females of an appropriate genotype for detecting crossovers. Fertility and fecundity of the treated males, after decreasing to the lowest level on day 8, had a marked recovery on day 10 followed by a small depression on days 11 and 12; no differences between the experimentals and controls were found for any of the rest of the broods. The number of tested F_1 offspring totaled 51,964 from 46 treated and 25,778 from 11 control males.

With rare exceptions, proven crossovers were first detected on the 9th day but the highest incidence was on the 10th day after treatment. Clusters of crossovers, many of which continued in several broods, began on the 11th or 12th day.

The evidence from the continuing crossover-clusters substantiates the hypothesis proposed by the author (1962) from clusters of recessive third-chromosome lethals, that the treated pre-definitive gonia — on an average five in number per male, basing the estimation upon the size of clusters — are responsible for the recovery of sperm production to the control level by the 12th day. Furthermore it appears that the high incidence of crossovers, on the 10th day, is the result of induction of crossovers in definitive (secondary) gonia.

Research supported by IAEA contract No. 31 and USAEC contract No. AT(30-1) 2690.

5.41. An Analysis of X-ray induced Non-disjunction in Drosophila. A. J. Bateman and Ann C. Chandley (Manchester, Great Britain).

It is common to distinguish between two forms of meiotic non-disjunction: at first and second division respectively. This is a gross simplification. There are many possible mechanisms resulting in non-disjunction. Some of them are recognisable by their genetic effects. An analysis of matroclinous females in *Drosophila*, produced by X-rays, has been made in an attempt to determine which modes of non-disjunction are, in fact, operating in their production. Though the results are ambiguous, some definite conclusions can be drawn.

5.42. Dose-dependence of X-ray Induced Non-disjunction in Drosophila melanogaster Females. Ann C. Chandley and A. J. Bateman (Manchester, Great Britain).

The frequency of induced non-disjunction in female germ cells has been estimated on eleven successive days following irradiation with X-rays. Doses of 1, 4, 6 and 8 k rad have been used and the results indicate a non-linear dose-dependence both for female (XXY) and male (XO) exceptions.

Over the first six days, the frequency appears to increase with the square of the dose. In spite of the frequency of male exceptions being 4-5 times as great as that of the female exceptions, the shapes of the dose-dependence curves are closely similar.

After day 6, when the female and male exceptions have equal incidences, the dose-dependence departs even further from linearity.

5.43. Induction of Mutations and Cell Killing in Irradiated Drosophila Spermatogonia. Per Oftedal (Montebello, Norway).

Treatments of *Drosophila* spermatogonia with

acute doses of 55r, 110r, 160r and 310r gave a clearly non-linear dose-effect curve for sex-linked recessive lethals. In order to explain the results, a mathematical model for the irradiated cell population has been formulated, considering cell killing, variation in sensitivity to killing and to mutation induction with cell cycle stage, and length of cell cycle. Thus, the frequency of mutations observed will be equal to the product of: dose absorbed by sensitive cells × number of sensitive cells × sensitivity of sensitive cells × survival of sensitive cells, divided by: total number of cells in population less killed sensitive cells. In this first approximation, effects on resistant cells have been disregarded. According to this model, protraction of doses in this dose range should lead to higher yields of mutants. Protraction of 144 r, 267 r, and 542 r over 8 hr (four spermatogonial cell cycles in the irradiated embryo), have given results in support of the hypothetical model, showing a linear increase in mutation rate with dose, with a slope higher than 2×10^{-5} sex-linked lethals per r, indicating a sensitivity at least as high as for mature sperm, for doses lower than a few hundred r.

The work has been supported by The Norwegian Cancer Society.

5.44. Modification of the X-ray induced Rate of Sex-linked Lethals by Nitrogen Post-treatment and Fractionation of the Dose in Drosophila melanogaster. A. D. Tates (Leyden, The Netherlands).

Male flies carrying a ring X-chromosome, were exposed to a flow of purified nitrogen for 25 min, immediately after having received a dose of 1450 r (55 r/sec). Results of five independent experiments show a significant increase of mutation frequency due to post-treatment in spermatids and late spermatocytes, 9.5 per cent lethals (3744 tested chromosomes) were found after irradiation without post-treatment and 10.8 per cent lethals (5190 chromosomes) with N_2 post-treatment; normal deviate = 2.21 and $P = 0.0139$. A possible interpretation of the results is that N_2 post-treatment blocks repair of pre-mutational damage.

In two experiments a third group of flies received N_2 post-treatment after a delay of 25 min. A comparison of the results of delayed versus direct post-treatment shows a significant decrease of the mutation rate in the group with delayed post-treatment. The percentage for the direct treatment is 9.54 per cent (2222 chrom.) and for the delayed treatment 7.15 per cent (2545 chrom.); normal deviate 2.538 and $P = 0.0055$. This result suggests that in young spermatids and late spermatocytes repair, under these experimental conditions, is completed within 25 min.

Five experiments were carried out to investigate whether dose fractionation favours repair. The mutagenic effects of a dose of 1350 r given in five equal fractions, seperated by two hour-intervals, were compared with that of unfractit onated irradiation. The results show a significandecrease of mutation frequency due to fractionation, in the same germ cell stages where N_2–post-treatment had been effective. Percentages of lethals for the fractionated and unfractionated groups are 8.63 per cent (4994 chrom.) and 9.77 per cent (4676 chrom.) respectively (normal deviate 3.01 and $P = 0.0013$).

5.45. Oxygen Effects and Dose Fractionation in the Developing Germ Cells of Drosophila virilis. Mary L. Alexander (Austin, U.S.A.).

One-day-old males of *D. virilis* were treated with 1000r of X-rays while under 74.7 lb pressure of argon gas and in a gaseous atmosphere of 45 lb argon + 19.7 lb oxygen. X-rays were given at a rate of 1000r/min as a single dose and as two doses fractionated by a period of 40 min. Additional tests were made with 2000r under 64.7 lb of argon. Postmeiotic, meiotic and premeiotic germ cells were tested for induced biological damage.

Dominant lethals in postmeiotic sperm and spermatid cells were increased by the presence of oxygen. In sperm and spermatids, there were no differences in the percentages of dominant lethals or translocations with dose rate changes in argon or when oxygen was present. These results differed from those observed for *D. melanogaster* where dose fractionation in argon, but not oxygen, increased translocations, sexlinked lethals and dominant lethals in spermatids. The premeiotic cells of *virilis*, as *melanogaster*, showed an enhancement in dominant lethals with dose fractionation in both anoxic and oxygenated gases. Enhancement with dose fractionation in *virilis* appears to be limited to later spermatogonial stages. The stem cells do not respond to dose fractionation.

Enhancement of lethals in premeiotic cells has been observed in anoxic and oxygenated atmospheres of gases in both species. Differences in response of spermatid cells in *virilis* and *melanogaster* may be due to oxygen metabolism or other metabolic differences in the two species. Spermatogenic cysts are not prevalent in young males of either species and the differences cannot

be explained by mixed populations of resistant and sensitive spermatid cells.

Work supported, in part, by Contract No. AT-(40-1)-3014 with the United States Atomic Energy Commission.

5.46. Effect of Penicillin Feeding on the Reduction of Radiation Induced Mutation Rate in Drosophila melanogaster. Toshio Shiomi (Chiba, Japan).

It has been reported by Burdette (1961) that *D. melanogaster*, grown on medium containing penicillin, exhibits a diminished X-ray induced mutation rate. The present work reports the following results: a wild type *D. melanogaster*, Canton-S, was grown in a culture medium containing penicillin G (20,000 units per ml); 24 hr old virgin males hatching from these cultures were irradiated with X-rays. The induced sex-linked recessive lethal mutations in mature sperms were tested by the Muller-5 method. X-ray doses used were 500, 1000, 2000, and 3000 r.

(a) The radiation induced mutation rates in penicillin-fed *Drosophila* are found to be about one-half of those for flies grown in normal culture conditions.

(b) The radiation dose-effect relationship remains linear.

(c) Penicillin containing medium lengthens the growth period of flies by nearly one day as compared to the normal culture, and the rate of emergence is higher than in the control.

(d) The feeding of penicillin during the first half of the larval stage is more effective in reducing the induced mutation rate than when fed during the second half.

The interpretation of this action of penicillin on the radiation induced mutations, especially its effect in the early stage of feeding, is difficult. One might assume that the metabolic patterns of the larvae are affected by penicillin or its derivatives, and the genic material itself or its surroundings are changed to more radioresistant or easily recoverable conditions. Whether the effect is caused by penicillin itself or by its degraded forms remains for future work.

5.47. X-ray Induced "Dominant Lethals" in Inseminated Eggs of Drosophila. (a) Experiments in the stage between completion of meiosis and beginning of cleavage. H. Ulrich (Zürich, Switzerland).

In insects, pre-adult mortality of the progeny of irradiated parents is thought to result from radiation induced "dominant lethals" in the genome of the maternal or paternal gametes. These "dominant lethals" can be either gene or chromosome mutations. Similar radiation effects (e.g. embryonic mortality) are found if instead of the gametes the progeny (e.g. young embryos) is irradiated. This raises the question whether in both types of experiments pre-adult mortality is the result of "dominant lethals" (i.e. alterations in the genome) and/or "physiological effects" (i.e. effects in cell components others than chromosomes).

During the last 10 years we have accumulated quite a number of experimental results on inseminated *Drosophila* eggs, X-rayed after deposition in the stage between completion of meiosis and beginning of first cleavage. Most data (e.g. differential radiosensitivity of cell parts with or without a nucleus, the one hit dose action curves for mortality and recessive lethals, influence of oxygen during irradiation, etc.) were consistent with the assumption that the most important radiation effects are alterations in the genome which behave like dominant lethals (probably of a one hit type predominantly). However, new results (experiments with cold post treatment and the analysis of radiosensitivity of eggs and embryos with different chromosomal constitutions) indicate that, at least some of the radiation effects have to be considered as "physiological effects".

5.48. X-ray Induced "Dominant Lethals" in Inseminated Eggs of Drosophila. (b) Experiments with different stages between insemination and end of second cleavage division. F. E. Würgler (Zürich, Switzerland).

The nature of X-ray induced mortality in inseminated *Drosophila* eggs has been studied by analysing the radiosensitivity in different stages of meiosis and early cleavage. The highest sensitivity was found during late anaphase/early telophase of meiosis II and each cleavage division analysed so far. Sensitivity was lowest when the nuclei were in interphase.

Dose action curves for the different stages vary greatly in shape and slope. The one hit curve found for egg samples containing all stages between completion of meiosis and beginning of cleavage (see Ulrich, preceding report) could be shown to result from superposition of different non linear dose effect curves. This result invalidates the strongest argument supporting the hypothesis that radiation induced mortality is of a one hit type.

It has been shown by various authors that insect embryos developing from irradiated gametes can die at different stages of development. In *Habrobracon* and *Drosophila* different lethal syndromes seem to result from different kinds of nuclear damage as shown by Von Borstel (1961).

Different lethal syndromes can also be distinguished after irradiation of inseminated *Drosophila* eggs. The dependence of the relative frequencies of different syndromes after irradiation in specific stages of nuclear divisions and the dose dependence of the different syndromes have been recorded. The results indicate that radiation induced genetic effects alone can hardly explain all the facts.

5.49. The Restitution of Radiation Induced Breaks in Structural Heterozygotes of Drosophila. E. GOLDSCHMIDT, E. BARAK, N. BERNSTEIN and R. FALK (Jerusalem, Israel).

Males of *Drosophila melanogaster* carrying the *Cy* (II L) inversion and their structurally homozygous brothers were given an X-ray dose of 5000 r. The salivary glands were scanned in larval offspring of both groups of males, and all breaks induced in euchromatic sections of the entire chromosome set were located. The rate of induced aberrations was higher in the F_1 produced by *Cy* fathers.

The hatchability of eggs fertilized by irradiated males of both groups was studied in order to compare the brood patterns and the overall rates of "dominant lethals" induced during the premeiotic stages up to the first meiotic anaphase.

It has been suggested that the loop configuration in the heterozygous bivalent facilitates non-homologous contact and thus promotes abnormal restitution. A more comprehensive hypothesis proposes that each of two perfectly synapsed homologues provides a "splint" for the normal restitution of its partner. Hence asynapsis *per se* will interfere with the normal repair of breaks. On this assumption the structural heterozygosity of one bivalent, promoting asynapsis in non-homologous elements, may be responsible for the increased production of abnormal restitutions in the entire chromosome set.

5.50. Recessive Lethals in the IV Chromosome of Drosophila melanogaster. H. J. BELITZ (Berlin, Germany).

After X-ray treatment of ey^2/ey^2 males of *Drosophila melanogaster* with doses of 3, 4.5 and 6 kr respectively 30 chromosomes with recessive lethals and 11 with semilethals were found. In allelic tests these 41 mutated chromosomes were outcrossed with each other. Two chromosomes contained two lethals each. Among the 43 mutations no more than 25 occupy one locus; the others are small deficiencies: 12 at least comprise two loci, 4 each three loci, and 2 four and sixteen respectively. By aid of these overlapping deficiencies a part of this chromosome could be mapped over a range of about 20 loci including the locus of ci. Among the induced lethals two clusters were found: one consisted of 5 mutations induced in a spermatogonium and occupying probably one locus; the other was composed of two lethals, which gave a positive result in the allelic test, but proved to be overlapping small deficiences including the locus of ci.

5.51. Crossing-over in Males of Dorsophila Induced by Radio Frequency Treatment. GEORGE H. MICKEY (Ridgefield U.S.A.).

Although crossing over normally does not occur in the male germ cells of *Drosophila*, reports in the literature indicate that this phenomenon has been induced by treatment of male flies with high temperature, X-rays, gamma-rays, ultraviolet rays, formaldehyde and nitrogen mustard. Our experiments yielded significant numbers of recombinants in the progeny of heterozygous (3ple) male flies treated with radio frequency of 20 megacycles. Recombinants frequently occurred in clusters and usually without complementary classes. The distribution of exchange regions was nonrandom. The induced crossovers appear to have occurred in spermatogonial cells rather than in spermatocytes.

5.52. Different Types of Mortality Including Prolongation of Female Lifetime after X-raying Drosophila melanogaster Imagines. H. NÖTHEL (Berlin, Germany).

The influence of X-rays on the lifespan of *Drosophila melanogaster* imagoes has been studied by application of 17 different doses (100 KV, 0-125 kr) to 1-2 days old males and females of the Berlin wild stock.

A prolongation of female lifespan is induced by doses >4 kr, it culminates at 11 kr in 150 per cent of the mean survival time of unirradiated females. The prolongation is not accompanied by alterations in locomotion or copulation of the females concerned and is independent from

radiation effects in the males mated with them. Measurements of fecundity have proved the prolongation to be correlated with the radiation-induced sterilization of the females. This has been confirmed furthermore by investigations with unirradiated virgin females having a low fecundity, with females of a special strain showing a high radiation resistance with regard to fecundity, and with chemically (by TEM) sterilized females.

In the males mean survival-time, T_{50}, T_{100}, and the time of the highest mortality decrease linearly when plotted logarithmically against a raster of linearly increasing doses. The females show in the same raster corresponding curves, except a sharp increase caused by the prolongation of lifespan. This makes the LD_{50} of females (96 kr) twice that of males. The linearity of these curves is interpreted with an always identical type of damage. In both sexes the normal distribution of the mortality rate over the days after irradiation is altered at doses >90 kr. A second peak appears at the third day, growing with increasing doses. Corresponding to this, at 90-100 kr the gradient of the declining survival time curve is strengthened. So a new type of mortality seems to be manifested at high doses. In agreement with the radiation syndrome at these doses it is interpreted as a central-nervous-death.

5.53. Cytological Interpretations of Five Types of Induced Modification in the Oviposition-pattern of the Wasp Habrobracon. DANIEL S. GROSCH (Raleigh, U.S.A.).

In contrast to *Diptera* where ovaries may not mature for several days, at eclosion, differentiated and transitional cells are present in the sequential series provided by the polytrophic ovariole of the parasitoid wasp *Habrobracon*. Furthermore, ovariole sinvariably number only four, and quantitative modifications of the pattern of egg deposit may be traced back to the cellular state of their contents.

When eggs are plotted against days, control females quickly reach a sloping plateau maintained for about 15 days until the sharp senile decline occurs. Low and moderate radiation exposures result in (1) a two-humped curve. The interposed valley, which can dip to temporary infecundity, attests to the vulnerability of transitional cells which undergo five successive mitotic divisions. High doses of radiation destroy the oogonia as well as transitional cells. This results in (2) a rapid decline to permanent infecundity within the first week. Single-meal ingestion of metal cations and classic organic enzyme inhibitors produce a constant deficit throughout life, reflected as (3) a curve lower and parallel to that of controls. A general debility of somatic tissues ensues rather than a direct effect upon the gonads. Ingested animetabolites can also give type 3 curves, but more typical is (4) an initial lag phase of up to five days resulting from oocyte retardation and degeneration attendant interference with nurse cell function. Nuclear disturbance is involved and may be obtained with agents influencing either protein or nucleic acid synthesis. Aureomycin, methotrexate, DON, or FUDR give similar type 4 curves when used in proper concentration. Antimitotic agents provide still another type of curve (5) characterized by a compensatory deposit of temporarily arrested cells which make up for earlier deficits.

5.54. Virus-host Relationship and the Effects of X-ray Induced Mutants in Heterozygous Condition. R. C. BAUMILLER (Woodstock, U.S.A.)

The only reported phenotypic effect of the "virus" sigma has been to make its *Drosophila* host sensitive to CO_2. L'Heritier [1] has reported strains of virus carrying flies which differ according to the stability of the virus-host relationship. The experiments, presently communicated, examined the effects during developmental stages of X-ray induced mutants in heterozygous condition on several of these strains [1].

Sensitive (S) and sibling cured (R) virgin females were collected from the strain to be tested and mated to sibling cured males who had (X) or had not (U) received 3000r of X-rays. In a cured line the presence of the virus can no longer be demonstrated. After a two-day mating period the females were placed in net-enclosed cylinders and eggs were collected over one-half hour periods. Each hour, beginning with the 21st hour after oviposition, the number of larvae hatching were scored.

The effect of mutants in heterozygous condition on time of egg hatching varied according to the stability of the virus in the strain tested. The reasons for this difference in effect will be discussed.

A portion of this work was supported by a grant to Dr. I. H. Herskowitz from the Atomic Energy Commission (Contract AT (11-1)-633)

while the author was a Postdoctoral Fellow of The National Foundation.

1. *Adv. Virus Res.*, 1958.
2. For the effects of such mutants in the wild type Oregon-R strain of *Drosophila* see BAUMILLER, *Genetics*, 1963.

5.55. Effects of Irradiated Chromosomes on Viability in Heterozygotes and Hemizygotes. RAPHAEL FALK, NEHAMA BEN-ZEEV, and SHULA BAKER (Jerusalem, Israel).

Drosophila males were irradiated with an X-ray dose of 2000 r and mated according to a design that permitted the determination of viability effects in both the heterozygotes and hemizygotes carrying the irradiated X-chromosome. Apart from the induced lethals and semi-lethals, the milder quasi-normal viability mutations could also be detected in the hemizygotes for irradiated chromosomes.

In crowded cultures, when deleterious mutations caused a decline in the number of males hemizygous for the irradiated chromosome, the remaining genotypes competed for the available space. If the efficient competitors were heterozygous for an irradiated chromosome as well, the induced viability effect was offset in the final outcome by their advantage in competition. The heterozygotes for irradiated chromosomes thus exhibited a spurious increase in viability as compared with their controls which carried unirradiated chromosomes. When intra-culture competition was kept at a minimum it could be demonstrated that the induced mutations had an adverse effect on the viability of heterozygotes.

The heterozygotes carrying semi-lethal mutations were the most seriously affected. The heterozygotes for quasi-normal chromosomes were considerably less viable than the controls, their viability being reduced at least as much as that of heterozygotes for lethals.

This work was supported by grant GM 8258 from the U.S. Public Health Service.

5.56. Further Data on Overdominance. BRUCE WALLACE (Ithaca, U.S.A.).

Studies have been completed on the average viability effects of radiation-induced mutations (second chromosome; *Drosophila melanogaster*) in heterozygous condition in flies (1) otherwise homozygous for their second chromosome, (2) heterozygous for two different chromosomes obtained from the same locality, and (3) heterozygous for chromosomes obtained from widely separated localities. Two chromosomes from each of three localities were used for these studies; radiation levels were 0 r, 250 r, 750 r, and 2250 r.

At the time this is written statistical analyses are incomplete. Nevertheless, the data suggest that the mean viability of homozygotes is improved by the new heterozygosity (confirming the report made at the Xth Int. Cong. of Genetics) while that of heterozygous individuals is lowered. The implications of these observations will be discussed.

5.57. Transitory Increase in Genetic Load in Irradiated Laboratory Populations of Drosophila melanogaster. H. L. CARSON (St. Louis, U.S.A.).

Four replicate populations (two control and two experimental) of se ss e^s k ro stock were maintained for three years in vial populations in which food, space and change schedule were rigidly controlled. Populations produced by this design are small (not more than 200 adults) and are equilibrated under strong natural selection. Population size and production were measured weekly. The experimental populations received radiation treatment; the dose was given over a period of two years and totalled 65,000 r units of X-ray. Rate of administration was 1000 r/week with three periods of 10, 10, and 20 successive weeks during which radiation was suspended. No radiation was given in the final year. Genetic loads carried by each population were measured three times during the final year. Random lots of eggs from each population were collected, counted and then adults were reared from these eggs under ideal conditions. Yields of adults from the irradiated populations were significantly below those of the controls at the first measurement. This indicates that these populations were carrying genetic loads due to the history of radiation. These differences, however, were absent after one year, indicating that the loads due to radiation had disappeared. This effect is ascribed to the efficiency with which natural selection removes new deleterious mutants from small populations. At the termination of the experiment, population sizes of the experimentals did not differ significantly from the controls.

5.58. Fitness of Irradiated Populations of Drosophila melanogaster. K. SANKARANARAYANAN (New York, U.S.A.).

Males in experimental populations of *D. melanogaster* were treated with acute doses of X-rays (2000 r, 4000 r, 6000 r and 7000 r) and the effects of irradiation on population fitness were studied under conditions of relaxed selection, the relaxation being obtained mainly, by minimizing larval competition. In one population, males received an initial dose of 7000r units and no further irradiation in subsequent generations. At other dose levels, there were populations where the males received X-irradiation (1) at generation O only and (2) at generation O and in every subsequent generation. From the latter, sub-populations were derived where irradiation was stopped after 5, 10, and 15 generations. The components of fitness investigated are (1) hatchability, (2) viability from the larval to the adult stage, and (3) viability from the egg to the adult stage.

An important finding that confirms the results of earlier investigators is the observation that the radiation induced mortality (dominant lethal effect) is mainly (but not exclusively) at the egg stage. A second finding is the surprisingly rapid recovery to the control level in hatchability and viability rate following the cessation of irradiation.

A detailed analysis of the second chromosomes is in progress to get an estimate of the radiation-induced genetic load and to gain an insight as to how much of the genetic load is adaptively incorporated into the gene pool of the population.

Work done during the tenure of a Columbia University Pre-Doctoral Fellowship (1961-62) and Boese Fellowship (1962-'63).

5.59. Alterations of Viability Characters in an Irradiated Population of Drosophila. V. BOCHNIG (Berlin, Germany).

Alterations of viability of *Drosophila melanogaster* have been studied after X-raying a population in its consecutive generations. The population used had been derived from the Berlin wild stock. In each generation 500 males and 500 females (1-5 days old) have been irradiated with an acute dose of 2100 r. Up to more than thirty generations of irradiatian viability characters have been studied in intervals of about five generations by testing lifespan, fecundity, and fertility after application of different X-ray doses ranging from 0-100 kr. The results obtained show that in each of the three characters studied the irradiated population reached a significantly higher degree of viability than the Berlin wild stock under all experimental conditions. The higher fitness of the irradiated population is explained by an effect of heterosis. A great number of mutations must have been induced by the irradiations and accumulated in the population. As a consequence a higher degree of heterozygosity must have been reached.

5.60. Mutational Recurrence or Genetic Compensation of Lethals in Drosophila Natural and Irradiated Populations? M. L. REGULY and A. R. CORDEIRO (Porto Alegre, Brazil).

The total frequency of 30.3 per cent lethal II chromosomes of *D. willistoni* from an irradiated "A" population do not differ from the control "E" population, 30.5 per cent ($x^2 = 2.17$) (714 strains). This analysis was made 77.5 generations after radiation. The "A" population exhibited 6 out of ten retested lethals and the "E" 3 of these.

Nevertheless, three lethal loci are exclusively found in "A" irradiated population. The allelism tests we performed and the calculated average mutation rate per locus in the II chrom. of *willistoni*, 0.000008 obtained from the data of Dobzhansky *et al* [1] allows the estimation, with Wright's formulae, of the equilibrium levels for each lethal assuming complete recessivity.

According these calculations the six more frequent induced lethals, still present in population "A" and the three spontaneous alleles of "E" population are at 10 to 24.4 times above the expected values.

The lethal survival in nature, at such high frequencies, can only be explained by mutation pressures many times above the normal average observed or by overdominant alleles providing genetic compensation or both, by mutational and balanced effects. We suggest that selection of modifiers and or overdominant allelic forms, tends to buffer (balance) the recurrent lethal producing loci.

1. *Genetics* **37**, 650 1952.

5.61. Changes of Reproductive Performance of Drosophila willistoni at Two Inbreeding Levels. A. J. CENTENO, M. L. REGULY and A. R. CORDEIRO (Porto Alegre, Brazil).

From 608 wild inseminated females 123 outbred strains with inbreeding of 0.26 and 124 sibmating strains with f = 0.65 (at 6th generation) were obtained.

At the 5th generation each strain have been divided in two strains each with 15 pairs of flies.

For each strain one culture was submitted to 600 r of a Co^{60} gamma source.

The relatively outbred strains gave a total of 40,126 flies in the control (K) and 41,363 from the irradiated parallel cultures (R).

An hierarchal analysis of variance showed no significant difference between treatments (F = 0.002, F.05 (1244) = 3.89). The same non-significant result was obtained at the more inbred strains in a total of 28,158 flies in the control and 27,100 F_1 of irradiated strains.

A paired analysis of irradiated-control failed to show any significant overall increase of the irradated or the control. Considering all the paired strains in which a betterment of reproductive performance was observed after radiation, a parcel of 51.22 per cent among 123 strains and 45.16 per cent among 124 strains, contributed to a significant *t* value.

We have indications that several strains do increase performance after radiation. The average effect on the total population is certainly counterbalanced by the others that shows a significant decrease.

produced an overall enhancement of egg-adult viability; K (d) = 32.53 per cent, R (d) = 44.44 per cent for 10,650 eggs fertilized by control and irradiated spermatozoids. Nevertheless, the spermatozoids produced from irradiated spermatides and spermatocytes showed a slight decrease in a total of 11,230 eggs, K (d) = 32.5 per cent R (d) = 30.8 per cent.

The increase of egg-adult viability among the (d) level "low" class is K (d) = 7.53 per cent, R (d) = 40.85 per cent, placing its average viability over that of "high" class after radiations: K (d) = 62.95 per cent, R (d) = 33.74 per cent. The variance increased significantly among the irradiated spermatozoid block, considering that the extremes (high and low) of viability regressed toward the average more among the radiated than between the control the median class showed the greater increase in variance.

Our results partially support the Wallace [1] and Wallace and Dobzhansky [2] results. Considering that the more commonly induced mutations are the ones that recur most in natural conditions also, and consequently, are more likely to be co-adapted in balanced genic systems of a race gene pool, these are probably the same that improved the "low" class strains.

1. *Evolution* **12,** 532.
2. *Genetics* **47,** 1027.

5.62. Restoration, Without Selection, of Balanced Genetic Load by Radiation of Drosophila Inbred Strains. A. R. Cordeiro, M. L. Reguly and A. J. Centeno (Porto Alegre, Brazil).

Starting with wild inseminated *D. willistoni* from natural populations, four levels of inbreeding were obtained: (a) outcross, about "zero level" (150 strains); (b) outcross with sib mating last (5th) generation, level: 0.39 (101 strains from 608); (c) sib mating 5 generations, level: 0.69 (116 strains from 608), and sib mating 41 generations, near level 1.0 (1 strain from 50). Significantly different egg-adult viabilities were observed among these series: (a): 68.5 per cent (b): 46.9 per cent (c): 29.7 per cent = (d): 32.5 per cent.

Levels (b), (c) and (d) strains were sub-cultured in two simultaneous and parallel series of replications, one (R) received 600 r from a Co^{60} source, the other (K) was submitted to the same conditions, except radiation.

A total of 127,010 eggs have been counted in these experiments. The most pertinent results are: at the (b) and (c) levels the (K) did not differ from (R). Nevertheless at the (d) level radiation

5.63. Persistence of Lethals in Irradiated Natural Populations of Drosophila willistoni. E. K. Marques, H. Winge, M. Napp and C. M. P. Maciel (Porto Alegre, Brazil).

An isolated wood in the grassland region of R. G. Sul (Brasil) received during one year six releases of about 71,000 individuals F_1 of irradiated samples from a laboratory population originated from the same wood. A total of 45,000 r from a Co^{60} gamma source was delivered to these six samples. The F_1 of the last irradiated sample, $6R_1$, as well as the samples collected in the isolated wood, 5 generations ($N6R_1$) and 15 generations ($3N6R_1$) after the release of $6R_1$ have been genetically analysed for the II and III chromosomes.

The $6R_1$ exhibited a significant overload of lethals (II chromosome: 69.75 per cent; III chromosome: 30.87 per cent in regard to its control values from another unirradiated isolated wood (II: 26.34 per cent; III: 19.02 per cent). The allelism increased for each sample: II: $6R_1$ = 6.39 per cent and Control = 1.02 per cent; III: $6R_1$ = 4.00 per cent and Control = 0.29 per cent. This

increase persisted five generations in nature: $N6R_1$: II = 3.82 per cent; III = 3.22 per cent. This high allelic frequency was maintained in spite of the return to control values of the III chromosome lethals. The II chromosome lethal frequency was still significantly higher than the control (44.44 per cent). The allelism tests intersamples ($6R_1$ $N6R_1$: II = 5.18 per cent; III = 3.03 per cent and $6R_1$ Control: II = 0.22 per cent; III = 0.07 per cent allowed us to estimate the radiation induced lethal *persistence* at the $N6R_1$ to be 75.82 per cent for the II and 89.51 per cent for the III chromosome.

The same type of calculations made for the $3N6R_1$ gave 11.48 per cent for the II and 5.30 per cent for the III chromosome, as values of *lethal persistence* from the $6R_1$, 15 generations after under natural selection. There are indications that several components of the adaptive value were depressed (1) and that chromosomal inversions might "protect" some lethals (2). The persistence of *some* lethals suggests their complete recessivity as even some balanced effects.

1. Marques and Maciel, *Experientia* **17**, 404, 1961.
2. Cordeiro, *Experientia* **17**, 405, 1961.

6.64. Genetic Effects of γ-Irradiation on the Percent Adult Emergence of Drosophila melanogaster. J. C. DeFries and R. W. Touchberry (Urbana, U.S.A.).

Newly emerged males and females from two populations (randombred and inbred) derived from Luce's wild type strain were subjected to various dosages of γ-irradiation (0, 500, 1000 or 1500r) and mated in all possible combinations within populations, resulting in a four X four factorial arrangement of treatments. Three mating pairs were included in each subclass of two replicate experiments, requiring a total of 96 mating pairs per generation in each experiment. Eggs were collected over a 10-day period following treatment and the percent of the offspring to emerge as adults was determined. In addition to these data of generation one (offspring of treated flies), similar data were obtained for generations two and three, resulting from full-sib matings of generations one and two, respectively. Somatic and genetic effects would be expressed in generation one, whereas only genetic effects would be expressed in subsequent generations.

Highly significant linear depressing effects resulting from treatment of both males and females were observed in the data of generation one, with the magnitude of the effect being greater for males than females. Highly significant effects resulting from treatment of the original males, although smaller in magnitude than in the first generation, were again found in the data of generations two and three; however, no effect was evident from treatment of the original females in these later generations.

This investigation was supported in part by Research grant GM-07951-03 from the Public Health Service.

5.65. The Sex Ratio in the Offspring of Irradiated Avian Males. M. Vojtíšková, V. Matoušek and A. Lengerová (Prague, Czechoslovakia).

Birds proved to be advantageous material for studying the radiation effect on the sex ratioin the offspring of irradiated homogametic and non-irradiated heterogametic parents, since

(a) they can be bred in large numbers under standard conditions;

(b) in contrast to mammals, the homogametic sex in avian species is male; this circumstance makes it possible to extend the followup of the dose-effect relationship to a higher dose range, since the sterilizing dose for males is much higher than for females. In addition, each irradiated individual has a large number of offspring, which is very useful from the aspect of the requirements of statistical analysis.

In the present experiment a total number of 11,874 birds was used to estimate the shift in the sex ratio after the X-ray irradiation of males. Within the dose range 0-600 r the shift was found to lie between the limits of $1 \cdot 10^{-5}$ to $8 \cdot 10^{-5}$ per 1 r of irradiation with a confidence coefficient of 0.95.

5.66. Influence of Fast Neutrons on Frequency of Lethal Mutations in the Reproductive Cells of Male Mice. M. D. Pomerantzeva (Moscow, U.S.S.R.).

The males of white mice were totally irradiated by fast neutrons (with average energy $\sim$ 1MeV) in the horizontal canal of a nuclear reactor. The average dose rate of fast neutrons was 350 rad/hr, the concomitant dose of γ-rays was 85 rad/hr. The following doses of fast neutrons were used: 17, 34, 57, 114, 171 and 228 rad. Immediately after irradiation and on the 18th day after irradiation each male was kept for 3 days with 2 to

4 non-irradiated females. The females (328) were dissected on the 13th to 16th day of pregnancy for counts of corpora lutea, implantations, and post-implantation losses. The results obtained show that the treatment both of the spermatozoon and of spermatids led to the exponential dependence of the dominant lethals frequency upon the dose. The average probability of one dominant lethal appearance per dose unit (mutation rate) was $6.0 \cdot 10^{-3}$ for the spermatozoon irradiation and $11.0 \cdot 10^{-3}$ for the spermatid irradiation. The dose which leads to the death of 50 per cent of embryos, calculated by probit-method, was 106 ± 10 rad for the spermatozoon irradiation and 64 ± 9 rad for the spermatid irradiation. Thus the spermatids appeared to be ~ 1.7 times more genetically radiosensitive than the spermatozoon.

The probability of dominant lethal appearance which leads to the death of embryos before implantation was ~ 0.3 both for the spermatozoon and spermatid irradiation.

The comparison of the results obtained with the data in the literature leads us to the conclusion that in the case of the mature spermatozoon irradiation the genetical effect of fast neutrons is about 4 times higher than that of X-rays. In the case of the spermatid irradiation the RBE of fast neutrons is somewhat lower.

5.67. A Dose-rate Effect in Mice with Chromosomal Mutations. A. G. Searle and R. J. S. Phillips (Harwell, Great Britain).

Lyon, Phillips and Searle have shown that 1200 r acute X-irradiation of mouse spermatogonia delivered in two equal fractions 8 weeks apart, leads to a decrease of 15 per cent in litter-size of offspring at birth. This is due to the induction of dominant lethals acting around embryonic implantation. Translocations, leading to semi-sterility (or complete sterility in the male if sex-linked), were induced in about 4 per cent of survivors. The dominant effects of 1200 r chromic gamma-irradiation of spermatogonia, spread over 12 weeks at 100 r/week, have now been investigated, using the same mouse stocks. Results so far show that the dominant lethal effect is much reduced, with a decrease in litter-size of only about 2 per cent in the irradiated series. In addition, the rate of induction of translocations is decreased to about 1 per cent.

Two different phenomena are probably responsible for the lower yield of chromosomal mutations: (i) a reduction in the magnitude of the non-linear component of the dose response when the dose-rate is low, and (ii) a greater capacity for "repair" with low than with high dose-rate irradiation, as found with specific locus mutations.

5.68. The Influence of Radiation on the Genetic Control of Neonatal Mortality in Swine. D. F. Cox (Ames, U.S.A.).

Duroc and Hampshire swine are used in a continuing experiment designed to measure the genetic effects of radiation. The first generation offspring produced by normal males and females are compared with offspring from males given a single exposure of 300 r X-irradiation at least six months prior to matings with untreated females. Present data include 951 litters representing 9640 pigs.

Survival from birth to 42 days was 72 per cent in the control group compared with 68 per cent in the group sired by irradiated males. No significant changes in the number born, the sex ratio or the number of late fetal deaths have been found. The rate of mortality was determined in three intervals of postnatal life; day one including those born dead, the period between day one and day six, and the period from day six through day 42. The rations of the mortality rates in the irradiated group to those in the control group for the three intervals studied were 1.090, 1.529 and 1.020, respectively. The mortality rates in the offspring from irradiated males exceeded the controls in each interval but the period between the first and sixth day after birth appears particularly sensitive to the genetic effects of radiation. Interpretation of this pattern of mortality resulting from paternal irradiation will be presented on the basis of information concerning the breed, sex, and individual genetic factors contributing to variation in the probability of survival.

This work is supported by Contract AT(11-1) 707 from the U.S. Atomic Energy Commission.

5.69. Heterosis in the Response of Mouse Embryos to Irradiation. Donald J. Nash and John W. Gowen (New Brunswick and Ames, U.S.A.).

Mice of three inbred strains, BALB/Gw, K, and S, and the hybrids derived from them were exposed *in utero* on day $6\frac{1}{2}$, $10\frac{1}{2}$, $14\frac{1}{2}$ or $17\frac{1}{2}$ of gestation to single whole-body doses of X-rays of from 20 to 320 r. Data were obtained on the incidence of malformations at birth, postnatal growth from birth to maturity, life span, and

lifetime reproductive function when treated mice were mated to untreated mice. Comparison of the two general types of progeny, inbreds and hybrids, revealed significant differences among these genotypes. Following treatment with 160r at $10\frac{1}{2}$ days gestation 64 per cent of the inbred embryos were born with external malformations compared to 29 per cent of the hybrid progeny. In addition, the incidence of stillborn births among inbred progeny after 160r at $10\frac{1}{2}$ days was greater than that of the hybrids (100 per cent vs. 64 per cent). The general response of the inbreds and hybrids also was observed in those treatments which produced the most noticeable effects on growth and reproductive performance. For example, after 320 r at $17\frac{1}{2}$ days gestation irradiated mice later produced a mean number of 24 progeny in a one year period compared to 63 progeny in the controls. Of particular significance is the fact that all of the inbred embryos that had received this treatment later were sterile. Half of the hybrid progeny produced litters however. Results indicate that heterotic effects may exist during prenatal stages of development.

This work has received assistance from Contract No. AT (11-1) 107 from the United States Atomic Energy Commission.

5.70. An Attempt at Genetic Extinction of a Small Hybrid Mouse Population by Gonadal Irradiation. EARL L. GREEN (Bar Harbor, U.S.A.).

Genetic extinction of a population may be claimed only if a non-irradiated generation, one or more generations after the last irradiated generation, fails to reproduce. This experiment was an attempt to extinguish a small population of hybrid mice (8 mated pairs in each generation) by exposure of the gonads of the male parents, when six weeks old, to 900 r of X-rays in each generation. The gonadal dose of 900 r was selected because it neither killed nor permanently sterilized these hybrid mice. Seven weeks after exposure, each male was mated to a randomly selected non-sister female of the same generation. All mice of this population (DX-GE) were descended from a four-way cross of strains C3HeB/FeJ, C57BL/6J, DBA/2J, and BALB/cJ.

After six generations of irradiation, genetic extinction had not been achieved. Furthermore, reproductive performance (fertility, number of litters, number of offspring born and weaned, average litter size) had not been greatly affected.

5.71. Influence of Cobalt-60 continuing Irradiation on Fertilities and Life Spans of Different Strains of Mice [1]. JANICE STADLER and JOHN W. GOWEN (Ames, U.S.A.).

Continuous 22 hour a day irradiations have now been received by up to 18 successive generations of different strains of our mice. Full lifetime fertilities, life spans and other characteristics of the successive generations of progenies are being taken. Five generations now have completed life spans. Dosages varying from 0.03 r to 0.10 r per hour allow reproduction to continue. Both parental sexes as well as in uterine development are under irradiation. Irradiation to the progenitors prior to conception, ancestral irradiation, may be separated from that received by the mouse itself, direct irradiation. The results showed that within these dosage ranges mice maintain numbers in first litters directly comparable to those observed for unirradiated mice of like strains at corresponding periods in the colony. Sex ratios were comparable within the different strains. Strain differences in reproductive performance under irradiation were evident. The factors most important to reproductive worth of both irradiated and unirradiated mice were similar: time when fertility was initiated, well balanced reproductive sequence and litters adjusted to physiological capacities of the parents. Search for visible gene mutations showed none in the untreated mice and one recessive, brachypod, in the irradiated group. This mutation appearing in the 9th B X S generation is allelic and phenotypically comparable to a similar mutation from mice receiving high dosage acute irradiation some years earlier. Cumulative irradiation dosages over 10 generations exceeded 1500 r or 27 times the 50 per cent sterilizing acute dose for the females, 4 times that for the males and more than 2 times the acute lethal dose for either sex.

---

1. Journal Paper of the Iowa Agricultural and Home Economics Experiment Station, Ames. Project Nos. 1180 and 1187. Assistance has been received from Contract AT(11-1) 107 Atomic Energy Commission, U.S.A.

5.72. X-irradiation Effects on Lifetime Reproductivities of Different Strains of Mice. JOHN W. GOWEN and JANICE STADLER[1] (Ames, U.S.A.).

Effects of single dose X-ray irradiations to

young adult mice on total lifetime fertilities and progenies, as distributed by litter sequences and strains, have shown profound differences between the sexes. Males of 8 inbred strains fertilized up to 25 litters in 2390, whereas females had up to 15 successive litters in 707 conceived. Acute irradiations from 0 to 320r scarcely affected male fertility or the size of litters obtained at each successive gestation. After 320r, male fertilities, the numbers of their litters and of their progenies within litters, decreased orderly on an irradiation dose-dependent basis. Irradiated female fertilities, the numbers of their litters and the numbers of their progenies, all decreased regularly even with the lowest X-ray dosages of 20r. Sex differences may seem surprising for each sex has nearly equal genic materials for ion pair absorption. However, sperm potential of the males exceeds egg potential of females by a large factor. A large number of sperm may be inactivated, yet sperm capable of fertilization are still available for full fertility. After this point is reached dose dependence of fertility for both sexes becomes comparable. Sex ratios for the progenies of either irradiated males or females are similar. Dose dependence of the traits is affected by strain. These data are striking contrast to those of low daily dosages, 0.1 to 0.03r per hour, of 22 hour per day Cobalt-60 irradiation to be presented at this Congress.

1. Journal Paper of the Iowa Agricultural and Home Economics Experiment Station, Ames. Project Nos. 1180 and 1187. Assistance has been received from Contract AT (11-1) 107 Atomic Energy Commission, U.S.A.

5.73. Cytogenetic Radiosensitivity of Various Phases of Nuclear Cycle of Human Cells in Tissue Cultures. N. P. Dubinin (Moscow U.S.S.R.).

The problem of the effect of ionizing radiations on the heredity of man is one of the principal ones in contemporary biology. In this respect the data concerning radiogenetics of mammals are of great importance. However, the main direction in the experimental analysis of the problem is determined by investigations of the effect of radiation on the chromosomes in the nuclei of human cells.

The study of the effect of radiation on the cellular nucleus necessarily runs the following trends: (a) analyses of the dependence of various types of nuclear changes on the chromosome structure in various phases of the cell cycle, as well as on the relative capability or inability of fragments to reunite; (b) various degrees of sensitivity of chromosomes to the primary breaks in various phases of the cell cycle; (c) the time of appearance of different types of rearrangements due to prolongation of the cell cycle as the radiation effect.

The effect of 50 r of X-rays on every phase of the cell cycle of man was studied in tissue cultures of $1\frac{1}{2}$–2-months-old embryos. It was found that the highest sensitivity relates to the early prophase and to the second half of the postsynthetic phase. The first half of the postsynthetic phase was 5 times less radiosensitive as compared with the mitotic prophase. Throughout the synthetic phase, radiosensitivity is 2-3 times higher as compared to the first half of the postsynthetic phase. During the presynthetic phase, radiosensitivity *falls again* to the level of the first half of the postsynthetic phase.

It was established that fusion and non-fusion of the distal and proximal breaks, when they occur at isoloci, proceed independently. The fusing capacity of the distal fragments is at its maximum at the early prophase and gradually falls to a minimum towards the onset of the presynthetic phase. The fusing capacity of the proximal fragments is at its maximum at the stage of synthesis of DNA and it falls both at the onset of early prophase and the presynthetic phase.

Throughout the cell cycle the chromatid rearrangements (100 per cent at the prophase and during the whole postsynthetic period) are being displaced by chromosomal rearrangements (100 per cent at the onset of the presynthetic period). The presence of a mixture of chromatid and chromosome rearrangements at the synthetic phase and for the greater part of the presynthetic phase points either to the occurrence of an asynchronous synthesis in various cells, or to the presence of a mechanism of chromatid rearrangements of chromosomal origin, and perhaps to the simultaneous action of both these mechanisms.

The investigation of a great number of chromatid rearrangements in the course of the spontaneous mutation process, revealed the occurrence of some real number of chromosome rearrangements.

The data obtained have shown that the knowledge of quantitative and qualitative chromosome changes, induced by radiation at various phases of the cellular cycle, should determine a new approach to the very methodology of the investigation of radiocytogenetics of human cells. These data also point to the necessity of studying the effects of the dose, type

and rate of radiation. The dynamics of chromosome changes, induced by radiation, and the mechanism of their occurrence at different phases of the cellular cycle, seems to be a key to new investigations of the protection of the nucleus from the hazard of radiation. In this respect, our first analyses indicate, on the one hand, to the existence of new sites in the chemical protection and, on the other hand, in the mechanism of the occurrence of changes in the nucleus of cells, induced by radiation.

5.74. On Intracellular (Chromosomal) and Organismal Mechanisms of Control in Mammalian Radiosensitivity. J. J. KERKIS (Novosibirsk, U.S.S.R.).

According to frequency of cells with chromosomal aberrations appearing for 1 r while irradiating *in vitro* embryo fibroblasts of human, guinea pigs, mice, rabbits and hamsters with the doses from 5 to 50 r all these mammals arrange according to the decreasing radiosensitivity as follows: guinea pig = man < mouse < rabbit < hamster. These organisms have the same arrangement according to increasing LD 50/30 inherent in them and according to chromosome radiosensitivity of bone marrow cells *in vivo*. It is doubtful whether these coincidences are accidental and appears to indicate the leading role of nuclear structure in the control of general radiosensitivity of organism. This point of view is also confirmed by the data obtained in our laboratory on conformity of cellular and general protection degree by using chemical radioprotectors.

It is known that adrenalectomy removes strain differences on radiosensitivity in mice. Direct quantitative dependency between the activity of adrenal metabolism and radioresistency takes place among mice. We have investigated the influence of adrenalectomy upon chromosomal radiosensitivity. It has been established that removal of the adrenal metabolism increases the chromosome radiosensitivity approximately for two times. The average level of adrenal activity is genotypically conditioned. On the other hand, the products of chemical metabolism have an influence upon the radiosensitivity of chromosomes themselves. The adrenalectomy before and after irradiation has shown that its influence appears to express by creating different conditions for realization initial radiation damages. The genetical conditionality of adrenal activity, its effect upon general radiosensitivity of an organism and upon sensitivity of chromosomes of separate cells and, at last, higher degree of dependency of adrenal activity itself upon the state of the central nervous system—all these demonstrate complication of interaction of cellular and organismal systems of the control on radiosensitivity and will complicate the estimation of genetical danger degree at different doses of irradiation.

5.75. The Effects of Protection Against Genetic Damage caused by Ionizing Radiation in Mammalian Sex Cells. N. J. NUZHDIN and G. V. NIZNIK (Moscow, U.S.S.R.).

In spite of numerous investigations on the possibility of protection against genetic damage in mammalian germ cells, the evidence available is rather contradictory. This is apparently explained by the fact that owing to physiological barriers the protective drugs used for injection into the organism do not reach sex cells, or while reaching, are incapable of displaying protective action. In this connection we studied the effects of some protective drugs used in irradiation of spermatozoa *in vitro* on the rate of dominant lethal mutations occurred in them.

The experiments were carried out on chinchilla rabbits. Spermatozoa were given γ-rays Co-60 with a dose of 800 r in the saline solution, in the solution of β-mercaptoethylamine (MEA), S, β-aminoethylisothiouronium Br. HBr (AET) in corresponding concentrations and also in an atmosphere of CO and N_2.

Females were artificially inseminated after mating with a vasectomized male and sacrificed at 18-20 days of pregnancy. The ratio of the number of live embryos to that of corpora lutea was taken as an index of the rate of dominant lethals.

The investigations have shown that the insemination of females with unirradiated spermatozoa in both the saline solution and the solutions containing protective drugs ensured a high percentage of embryo survival (83 per cent) in relation to the corpora lutea of pregnancy.

The insemination of females with spermatozoa irradiated in the absence of protective drugs led to their high mortality rate (84 per cent). The results obtained showed that such protective drugs as MEA and AET, well-known for their good action, did not protect spermatozoa from the appearance of dominant lethals, if introduced into the ejaculate prior to irradiation (15 to 30 min). The percentage of viable embryoes in females inseminated with spermatozoa which were irradiated in the presence of the above mentioned protective drugs did not differ from

that in females inseminated with unprotected spermatozoa.

The irradiation of spermatozoa in CO and N_2 after a preliminary passage of the gases through the ejaculate diluted with saline solution, revealed a higher percentage of viable embryoes as compared with irradiated controls.

The highest percentage of normally developing embryoes was observed in females inseminated with spermatozoa irradiated in nitrogen.

This level was almost four times as higher as in spermatozoa irradiated in the air and only 1.5 times lower than in unirradiated controls. The death of embryoes largely occurred in the preimplantation period of development.

5.76. The Effect of Cysteine Pretreatment on Radiation Induced Dominant Lethals in Mice. U. H. EHLING (München, Germany).

Hundred (101 C3H) F_1 males 10-12 weeks old were divided into 4 groups. Groups I and II received an intraperitoneal injection of 15 mg cysteine, Groups III and IV an equal volume of saline. Groups I and III were irradiated with 600 rads of X-rays. The interval between injection and irradiation was 25-30 min. After treatment each male was caged separately with a hybrid female for a period of 7 days. Every 7th day the females were replaced. The uterine contents of fertilized females were examined $13\frac{1}{2}$ to $16\frac{1}{2}$ days postconception. The proportion of implanted embryos in three successive matings, expressed as percent of controls (Group II+IV), was in Group I: 88 per cent, 90 per cent, 45 per cent and in Group III: 87 per cent, 84 per cent, 41 per cent. Classifying embryos as alive or dead (including resorption sites) gave the following mean numbers of two respective types per litter in the successive mating series, Group I: 3.9 and 4.0; 4.6 and 3.4; 1.0 and 3.1, and Group III: 3.8 and 4.0; 4.3 and 3.3; 1.5 and 2.2. The pooled results of both control groups are, Group I: 8.5 and 0.5, and Group IV: 8.4 and 0.5. The results clearly demonstrate differences in the effect of cysteine in different stages of spermatogenesis. More data are being collected to evaluate the response of different gametogenic stages. AET will be used in additional experiments.

5.77. Effect of AET against the Dominant Lethality Induced by X-rays in Male Mice. A. LÉONARD and J. R. MAISIN (Mol, Belgium).

C^+ male mice injected or not with AET (2-β-aminoéthylisothiureabromide-hydrobromide or chloride-hydrochloride) were given 400 r on the whole-body or on testes only and mated with virgin females immediately after treatment.

At the 17th day of their pregnancy, the females were dissected and the preimplantation loss and intrauterine death rate scored. AET does not give any protection against dominant lethality induced by 400 r on the spermatozoa: after 400 r, the number of post-implantation deaths increased in the same way in protected and non- protected groups while the preimplantation loss was the same than in the control group.

These results and the data obtained with 400 r and 1200 r on the dominant lethality induced in spermatogonia and in spermatozoa will be discussed.

5.78. Population Dynamics of Irradiated Type A Spermatogonia of the Mouse. E. F. OAKBERG (Oak Ridge, U.S.A.).

Type A spermatogonia are the stem cells of the seminiferous epithelium, and by the process of stem cell renewal, maintain a constant population while forming an unlimited number of cells which differentiate into spermatozoa. Since the duration of all other spermatogenic stages is short in relation to the total reproductive span, type A spermatogonia are the cells of primary importance in both genetic damage and fertility of irradiated male mammals. Degeneration of cells in interphase or early prophase reduces type A cells in irradiated testes to a small fraction of control numbers. With acute exposures of 300 r or higher, the surviving cells multiply before differentiation into later stages is resumed. The population dynamics of these few surviving cells, however, is not well understood. Our present experiments were stimulated by the observation[1] that two 500 r doses 24 hr apart gave a mutation rate 5 times that obtained for 1000 r given as a single exposure. Synchronization of the cell population by the first dose fraction was suggested as a possible explanation. Our quantitative histological analysis of mouse testes 24 hr after 500 r support Russell's hypothesis of a difference in the cell stages present. Type A spermatogonia were almost exclusively in interphase. A few early prophases were counted, but no later mitotic stages were observed. Experiments are now under way to determine the relative frequencies of G_1, S and G_2 phases in spermatogonia arrested in interphase by irradiation.

---

1 Russell, *Proc. Natl. Acad. Sci.* **48**, 1724-1727, 1962

5.79. A Comparative Genetic Analysis of the Radiosensitivity of Germ and Somatic Cells of Monkeys (Macaca mulatta) and Mice. M. A. Arsenieva, N. N. Orlova and E. D. Bakulina (Moscow, U.S.S.R.).

The investigation is dedicated to a comparative cytogenetic study of radiosensitivity of germ and somatic (bone marrow) cells of monkeys and mice. The animals were exposed to 10, 25, 50, 100, 200 and 400 r of X-rays.

The comparative analysis carried out at some stages of prophase of meiosis showed that after irradiation by the doses of 10–100 r the genetic radiosensitivity of germinal epithelium of monkeys was 2-25 times higher than that of mice.

The highest radiogenetic sensitivity of spermatocytes I of monkeys was found at the stage of diakinesis- metaphase I. In average, 1r. causes at the prophase of meiosis in monkeys, the incidence of 0.15 per cent of chromosome rearrangements as compared with 0.06 per cent in mice.

The cytohistological analysis of spermatogonia of monkeys, exposed to the doses of 50-100r of X-rays showed that the type B spermatogonia are especially radiosensitive. The death of type A and B spermatogonia occurs after the exposure to the doses mentioned above, at the interphase as well as in the moment, when their division begins. The irradiation of spermatogonia A leads also to the depression of their mitotic activity. There was no renewal of mitotic activity of spermatogonia A after 30 hr following the irradiation. Type A_2 spermatogonia were found to be more radiosensitive than the spermatogonia A_1.

On the whole the radiosensitivity of spermatogonia of monkeys was found considerably higher than that of mice.

Cytological investigations of the rate of chromosome aberrations in bone marrow cells of monkeys and mice after exposure to 100, 200 and 400 r of X-rays also discovered the higher genetic sensitivity of monkeys.

The higher genetic radiosensitivity of germ and bone marrow cells of monkeys ascertained by the analysis, points to the necessity of more detailed investigation of the problem of comparative genetic radiosensitivity of mammals *in vitro* and *in vivo* including the studies based on the cells of man in tissue culture.

5.80. Radiosensitivity of Different Meiotic Stages in Rabbioocytes Discovered in Blastocysts. W. Kuhlmann (Münster, Germany).

Most experiments concerning radiosensitivity of different meiotic stages have been made until now in *Habrobracon*, *Drosophila* and mice. The aims of such investigations are

(1) to radiate the different stages as precisely as possible and

(2) to detect the different categories of damage which are induced in the different meiotic stages by adequate genetic and/or cytological methods.

For several reasons the rabbit seems to be especially suitable for such experiments: (1) Since the ovulation is induced only by mating (or pituitary hormons) the stages of meiosis from dictyoten op to telophase II are precisely predictable. (2) Since the implantation is relatively late (7th day) the blastocysts with a great number of calls can be recovered by operating or by sacrificing the animal on the 5th or 6th day after mating, and after adequate treating every blastocyst gives some analysable metaphase-plates for chromosome study.

In order to detect anomalies it was necessary to analyse the normal chromosome-complement and to set up a karyogram, which revealed a great similarity to the human chromosome-complement, not only in number (44) but also in the morphology—in contrast to the mouse which has got only telocentric chromosomes. The radiation experiments yielded the greatest sensitivity for inducing dominant lethals as measured by degenerating blactocysts shortly before metaphase, that means in diakinesis, while radiation at the time of metaphase (about 6 hr after mating) with the same dosis of 100 r remained without detectable effect. The possible reasons for this difference to the results in Habrobracon—not in mice—will be discussed. Experiments for detecting the presumably second sensitive phase shortly after telephase II (see *Habrobracon*, *Drosophila*, mice) are not yet completed, likewise the chromosome-studies in blastocysts for cytological evidence of induced mutations and will be reported about at the conference.

5.81. Studies of the Mechanism of the Effect of FUdR on Chromosomes. Sandra Bell and Sheldon Wolff (Oak Ridge, U.S.A.).

Fluorodeoxyuridine (FUdR) is a specific DNA synthesis inhibitor in virus-infected *Escherichia coli* [1]. Taylor *et al.* [2] have shown that FUdR-treated lateral roots of *Vicia faba* have chromosome gaps and are shattered within 3 to 5 hr after treatment. These aberrations were inter-

preted to be an effect by FUdR on DNA synthesis.

A series of experiments was undertaken in which roots were treated 15 min with H^3-thymidine to label cells in S and then treated with a mixture of 10^{-5} M FUdR and 10^{-4} M uridine. Chromosome gaps and shattering were found in those cells treated 2 to 4 hr. FUdR markedly inhibited the mitotic index within 4 hr. These results were similar to Taylor's. Autoradiograms of these cells, however, showed that the broken chromosomes were not labeled and thus must have been broken during the stage following synthesis. Thus, controsy to what was postulated previously, FUdR appears to break chromosomes of *Vicia* independently of DNA synthesis.

When lateral roots were treated with FUdR and uridine preceding treatment in H^3-thymidine, autoradiograms showed labeled nuclei. The number of grains over the nuclei was not significantly different from that in untreated cells. In other experiments in which roots were grown in FUdR, uridine, and 10^{-5} M thymidine, there was almost as much chromosome breakage as in the roots grown in FUdR and uridine only. At concentrations of thymidine (10^{-3} M) 100 times greater than the concentration of FUdR, the level of chromosome damage was only slightly above the level of the control cells grown in the absence of FUdR.

1. S. S. Cohen *et al.*, *Proc. Natl. Acad. Sci., U.S.*, **44**, 1004-12, 1958.
2. *Proc. Natl. Acad. Sci., U.S.*, **48**, 190-198, 1962.

5.82. Cytological and Biochemical Analyses of Chemically-induced Chromosome Breakage. Norman S. Cohn (Athens, U.S.A.).

The roots of *Vicia faba*, *Allium cepa*, and *Pisum sativum* have been treated with the following agents: 5-fluorodeoxyuridine, 5-bromodeoxyurdidine, hydroxylamine, and certain amino acids. Feulgen smears were prepared for cytological analyses of chromosome breakage as determined in metaphase and anaphase of mitosis. In addition, chromatographic analyses of certain of the treatments were performed using specific methods of extraction for histone and non-histone protein from treated and untreated roots. It is our contention that breakage of chromosomes must involve protein as well as nucleic acid, and preliminary data tend to support this. While biochemical analyses of all treatments are yet incomplete, observations on *Vicia* and *Pisum* indicate quantitative as well as qualitative differences in certain amino acids, when comparing treated with untreated material. Treatment with amino acids alone cast doubt on their use as radiomimetic agents, contrary to the work of Sharma and Sharma. Using relatively short treatment times and various periods of recovery, 5-FUdR and hydroxylamine produce significant levels of chromosome breakage, but 5-BUdR has little effect. These results confirm the studies of other workers (Kihlman; Taylor, Haut, Tung; Somers and Hsu). In addition, these studies have indicated that FUdR induces breaks after DNA synthesis as well as during the synthetic phase. While reunion of broken ends is relatively low, significant reunion does occur. This is supported by the presence of anaphase bridges as well as exchanges and sister union of Isochromatid deletions observed at metaphase.

Supported in part by the U.S. Atomic Energy Commission, contract AT (11-1) 826.

5.83. The Effects of 5-fluorodeoxyuridine (FUdR), 5-chlorodeoxyuridine (CUdR), 5-bromodeoxyuridine (BUdR), and 5-iododeoxyuridine (IUdR) on the Frequency of X-ray-induced Chromatid Aberrations in the Root-tips of Vicia faba. B. A. Kihlman (Uppsala, Sweden).

Pretreatments with CUdR, BUdR, and IUdR at concentration of 100μ M increased the effect of a given dose of X-rays by factors of 1.3, 1.6, and 1.9, respectively.

IUdR increased the frequency of X-ray-induced aberrations to the same extent whether the roots were irradiated under anaerobic or aerobic conditions.

In contrast to the thymidine analogs CUdR, BUdR, and IUdR, the deoxyuridine analog FUdR produced chromosome damage in the absence of radiation.

When X-irradiation was combined with a FUdR-treatment which by itself did not produce any chromosome damage, the number of anaphase fragments was markedly increased and the number of bridges decreased, in comparison with the X-ray control. The FUdR treatment was equally effective when given after as when given before and during irradiation. These results were interpreted as an enhacnement of the chromosome-breaking effect of FUdR by X-rays. The chromosome damage produced by FUdR was reversed by thymidine and thymidine analogs; this is consistent with the idea that the effect of

FUdR on chromosome structure is a result of its inhibitory effect on the synthesis of thymidylic acid. The experiments have shown that whereas FUdR-induced thymidine deficiency produces fragmentation of chromosomes, the incorporation of CUdR, BUdR and IUdR into chromosomal DNA does not itself cause chromosomal aberrations, but makes the chromosomes more sensitive to the chromosome-breaking effect of X-rays.

5.84. Ultraviolet Action Spectra for Chromosome Aberrations of Thymidine Analogue-substituted Mammalian Cells in Vitro. ERNEST H. Y. CHU (Oak Ridge, U.S.A.).

A clonal quasidiploid line of Chinese hamster cells grown directly in quartz dishes were irradiated with monochromatic ultraviolet (u.v.) light. Previous studies (Chu, 1962) had established the average cell generation time and dose-effect relationships. The u.v.-induced chromosome aberrations were qualitatively indistinguishable from those induced by X-rays in both plant and animal materials. The u.v. action spectrum for chromosome aberrations reaches a peak frequency at 2652 Å and approximately parallels the u.v. absorption spectrum of nucleic acids.

For varying periods prior to irradiation, different concentrations of 5-bromodeoxyuridine (BUdR) or 5-iododeoxyuridine (IUdR) were included in the growth medium. A few hours' treatment with BUdR did not induce chromosome abnormalities. Prolonged exposures (24 and 48 hr) of cells to 10^{-5} M BUdR did not inhibit mitosis and induced only infrequent chromosome aberrations, although higher concentrations caused increasingly severe damages to the cell.

Cells pretreated with BUdR for 24 hr showed an increased sensitivity to u.v. in terms of induced chromosome aberrations. The most significant modification of the u.v. action spectrum was observed in cells which had been exposed to BUdR for 48 hr. Particularly noteworthy is some thirty-fold and six-fold increase of aberration rates at 2250 Å and 3130 Å, respectively. IUdR-substituted cells also had a modified action spectrum, but IUdR- or BUdR-substituted cells responded differently to u.v. at various wavelenghts.

The facts that the u.v. action spectrum is similar to nucleic acid absorption spectrum and that direct chemical alterations of the DNA molecules modify the u.v. radiosensitivity strongly suggest that the initial DNA damages probably account for the majority of u.v.-induced chromosome breakages.

5.85. Actions of 5-bromouracil deoxyriboside on Plant Chromosomes. F. K. S. KOO (Mayagüez, Puerto Rico).

The thymidine analogue 5-bromouracil deoxyriboside (BUdR) upon its incorporation into cellular DNA is known to increase the radiosensitivity of the cells. In the present experiments with chromosomes in root tip cells of *Zebrina pendula*, *Rhoeo discolor*, and *Allium capa*, the following additional aspects of BUdR effect have been observed in some or all of these species: (1) BUdR often prevents the contraction of the secondary constriction, giving an appearance of a stretched region between the chromosome arm and the satellite. (2) BUdR induces breakage more readily at the centromere than at other regions of the chromosome. Since the constituent of the centromere is presumably not the same as that of the genetic material along the chromosome arms, so the mechanism for breakage induction could be also different. (3) BUdR is capable of inducing breakages either in one of the sister chromatids or in both. Based on the semi-conservative hypothesis for DNA replication and chromosome duplication, each chromosome is supposedly composed of one old and one new chromatids and BUdR is expected to be incorporated into the newly formed chromatid. If the breakages represent the manifestation of weakness at the sites of BUdR incorporation in the chromatid, they should occur only *in one* of the sister chromatids in a given chromosome. The detection of breakages in both chromatids indicates that BUdR is also capable of disrupting chromatids by other means. (4) In the combined treatment with gamma-rays, BUdR interacts with radiation to produce a synergistic effect in the induction of chromosomal aberrations.

5.86. Experiments Utilizing Labeled Nucleic Acid Precursors and Analogues in Plant Cell Research. HAROLD H. SMITH (Upton, N.Y., U.S.A.).

Investigations on the uptake and incorporation of DNA precursors and analogues are being made to study certain cytological, genetic and morphological phenomena. Incorporation of 5-iododeoxyuridine (IUdR) and 2-aminopurine into chromosomes of *Vicia faba* was demon-

strated by use of the tritiated analogues with autoradiography. Replacement in DNA was shown utilizing I^{131} labeled IUdR and by CeCl density gradient centrifugation. Phasing of DNA synthesis in *Vicia faba* cells was accomplished by treatment with 5-aminouracil (5AU). Studies on the rate of entering and leaving DNA synthesis during 5AU treatment were made with double labeling techniques involving H^3 and C^{14} labeled thymidine. Uptake of H^3 deoxycytidine apparently forms a pool which is used slowly in DNA synthesis, thus providing a means for autoradiographic studies of various other subsequent treatments affecting rate of DNA synthesis. Incorporation of H^3 labeled arginine into chromosomes of *Vicia faba* has been successful and is of significance in investigations on nuclear proteins.

In *Arabidopsis thaliana* the normal distribution of DNA synthesizing cells in the shoot meristem, as well as the morphogenetic effects of incorporated IUdR, have been traced with tritiated thymidine. The incorporation of IUdR into gametophytic tissue, a prerequisite to mutagenic studies, is being investigated with an I^{125} labeled form of this nucleoside analogue.

Kinetin (6-furfurylaminopurine) in combination with indolacetic acid initiates tumor formation when applied to the apical meristem of genetically tumor conditioned seedlings of *Nicotiana suaveolens-langsdorffii*. Tritiated kinetin is being used to investigate the fate of this aminopurine in cells of the meristematic region.

Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

5.87. Effect of Metabolic Inhibitors on X-induced Chromosome Breakage in the Ehrlich Ascites Tumour. Carmen Bertucci de Lozzio and Juan I. Valencia (Buenos Aires, Argentina).

Increased sensitivity to X-irradiation has been found with pretreatments with chloramphenicol, 5-fluordeoxiuridine, sodium azide or oxygen, in the Ehrlich ascites tumor ELD.

Irradiation with 2000 r at 500r/min, 240 kV, 15 mA, 1 mm Al, do not inhibit tumor growth. When the same 2000 r were delivered after one hour treatment with either chloramohenicol 500 μg/ml to 2 mg/ml, 5-fluordeoxiuridine (5-fluoruracil deoxiriboside, FUDR) 4×10^{-5}M, sodium azide 0.005 M or oxygen at 30-60 mm Hg (over the normal atmospheric tension), the result was a permanent inhibition of the tumor growth. The same pre-treatments before irradiation with 500r did not inhibit tumor growth.

The cytological analysis showed a high frequency of chromosome aberrations in the first post-irradiation division. All pretreatments increased the number of breaks per cell compared to that observed when the same dose of radiation was given alone. The qualitative and quantitative yield varied in each case. For example, FUDR produced a segmental shattering of the chromosomes while oxygen or chloramphenicol induced a high frequency of chromosome fragments and few chromosome and chromatid exchanges; sodium azide increased the frequency of all types of aberrations, including chromatid exchanges, but no chromosome shattering.

It is apparent that pretreatment with FUDR induces a very typical kind of aberration by interfering with DNA synthesis and perhaps some other processes, which seems to be related to the normal integration of the chromosome. On the other hand oxygen, sodium azide and chloramphenicol seems to act on the rejoining mechanism by preventing synthetic processes of restitution.

This research was supported by a grant of the Argentine National Research Council.

5.88. The Influence of Nucleic Acid Base Analogues on the Scales of Ephestia kühniella. Ernest W. Caspari and F. W. Muth (Rochester, U.S.A.).

Larvae of *Ephestia kühniella* strain NCR were injected with varying amounts of 5-bromodeoxyuridine (5 BDU), 1μg60μg per animal. The animals developed normally, and no reduced viability compared to the controls was observed. The hind wings were studied for the occurrence of aberrant scales. Abnormal scales occurred with high frequency. In the lower range of concentrations, 1-10 μg, the number of aberrant scales rises with increasing dosage, but between 10-30 μg the number of aberrant scales remains stable. At still higher dosages a further increase appears probable. The production of aberrant scales is strongly reduced or completely inhibited by equimolar amounts of thymidine. 5-bromouracil and 2-aminopurine produce few if any aberrant scales. The types of aberrant scales appear different from those induced by radiation. A characteristic type called l ("leaf") is predominant at low concentrations of BDU, but does not increase at medium levels while it decreases at higher concentration. It has not been found on irradiated wings. Other characteristic types of

aberrations have similar characteristics dose curves. Frequently, two aberrant scales are found very close together, so that they may be regarded as the derivatives of one affected cell. The two aberrant scales may be either of the same type, or may be different from each other. The former case occurs most frequently in irradiated wings, the latter type of doublets is more frequent in BDU-induced aberrations. Since BDU is an analogue of thymidine, and thymidine suppresses the described effects of BDU, it is suggested that the effects are due to incorporation of BDU into the DNA of the developing cells and represent mutational changes in the genetic material.

This investigation was supported by grant AT (30-1)-2902 from the U.S. Atomic Energy Commission.

5.89. Interactions between Cations and Chromosome Damage induced with Mesyloxy Compounds. J. MOUTSCHEN and M. MOUTSCHEN-DAHMEN (Liège, Belgium).

Chromosome aberrations induced with several mono- and difunctional mesyloxy compounds were modified by some cations in broad bean and barley. Cu^{++} and Zn^{++} considerably enhanced the rate of chromosome aberrations induced with EMS (ethyl methane sulfonate) and to a lesser degree MES (Methyl ethane sulfonate) and MMS (Methyl methane sulfonate). The optimal conditions for this interaction were investigated. The effects of these ions were observed to be lesser for difunctional compounds Myleran and dimethyl myleran. Some cations like Ca^{++} and Mg^{++} were inefficient with monofunctional compounds but reduced the rate of aberrations with difunctional. The modifications of the types of aberrations were considered. One cation: Al^{+++} not only modified the efficiency of Myleran but also the distribution of aberrations. Some interpretations are given.

5.90. Effects of Substances Blocking Heavy Metals on Interphase Nuclei and Mitosis. DOLORES ANGULO and F. SILIÓ (Madrid, Spain).

In our previous publication we called attention to the action provoked on interphase nuclei by EDTA. The abnormalities induced by this agent encouraged us to realize another work on biologic activity of substances blocking heavy metals, using also CNK to verify its effect on plant species with the test of *Allium cepa*.

In these experiments we have used CNK, that does not present any chelating action on Ca and Mg, in order to compare its effects with those produced by EDTA in relation to the belief that this agent owes its action to the chelation of divalent cations Ca^{++} and Mg^{++}.

The effects obtained with either agent show similar effects in many aspects such as a remarkable swelling of the interphase nuclei that discovers fine reticulate or filamentous structures and furthermore heteropicnotic areas which may correspond to chromocentres, observing at the same time in mitosis the helicoidal structure owing to the disturbance of the chromosomic proteins. Likewise the great neatness observed in the material under treatment is remarkable, supplying favourable conditions for the study of chromosomic structures.

On the other hand, the EDTA produces a beginning of c-mitotic action, which effect we have observed, though very seldom, in material treated with CNK. However with this agent there appear the corresponding radiomimetic effects, which are not observed in material treated with EDTA.

Therefore the biologic action of EDTA, chelating heavy metals, may be identified in some aspects with that of CNK, which is not a chelating substance of Ca and Mg.

5.91. The Influence of Respiratory Inhibitors on Chromosome Breakage by Lasiocarpine and Monocrotaline. S. AVANZI (Rome, Italy).

In a previous publication ([1]) data on the radiomimetic effect of monocrotaline and lasiocarpine in *Vicia faba* were reported. To test whether the radiomimetic effect of the two alkaloids is related to respiration, lateral root tips of *Vicia faba* seedlings were subjected to the following treatments:

(1) simultaneous treatment with 5×10^{-3} M lasiocarpine and 0.5×10^{-3} M NaN_3 for 8 hr at 15°C, followed by recovery in water.
(2) simultaneous treatment with 5×10^{-3} M monocrotaline and 0.5×10^{-3} M NaN_3 for 8 hr at 15°C, followed by recovery in water;
(3) simultaneous treatment with 5×10^{-3} M monocrotaline and 98 per cent CO for 8 hr, followed by recovery in water.

As control material served lateral root tips recovering in water after an 8 hr treatment with: (i) 5×10^{-3} M lasiocarpine at 15°C; (ii) with 5×10^{-3} M monocrotaline at 15° or 21°C; (iii)

0.5 10^{-3} M NaN_3 and (iv) 98 per cent CO. Cytological analysis took place after 24, 36, 48 hr of recovery. It was found that NaN_3, by itself inactive, brought about a 4-fold increase in both the percentage of aberrant anaphases and the number of breaks per cell induced by lasiocarpine alone; with monocrotaline, a still stronger effect of NaN_3 was observed (6-fold increase over the control). Also CO influenced the radiomimetic effect of monocrotaline by doubling the percentage of induced aberrant anaphases.

Parallel experiments with 2,4-DNP 30′ pretreatment with 10^{-4} 2,4-DNP followed by a 8 hr treatment with 5×10^{-3} M lasiocarpine and subsequent recovery in water indicated that this inhibitor only slightly enhanced the chromosome breaking effect of lasiocarpine.

These results seem to indicate that the radiomimetic effect of monocrotaline and lasiocarpine is not positively correlated with either respiration or phosphorilation.

---

1. Avanzi, *Caryologia*, **15**, 351-356, 1962.

5.92. Interaction of Chemically Induced Chromatid Lesions in Vicia faba. Rigomar Rieger, (Berlin, Germany).

After treatment of primary roots with ethylmethane-sulfonate, myleran and ethylalcohol in all possible combinations of the agents two by two and the second treatment following the first one immediately, the number of isolocus breaks was found to be approximately additive, whereas the number of chromatid translocations was in accordance with the values theoretically expected for full interaction of the breaks induced by each of the agents. Thus it might be concluded that all agents tested are breaking the same kinds of chemical bonds in the chromosomes.

Two treatments with triethylenemelamine or with triethylenmelamine and nitrogen mustard one following the other immediately or seperated by intervals in water up to 8 hr showed that even after the longest interval used in both cases the number of exchanges fitted the expectation for full interaction of the breaks induced by the first with those induced by the second treatment. On the basis of the breakage-reunion-hypothesis and on the supposition that the fractination effect is really an expression of the time of rejoining, this result might be interpreted as meaning that breaks induced by the first treatment are staying open at least for 8 hr, suggesting the bonds broken to be of the covalent type. Full interaction was found to be confined to recovery times giving the aberration peak.

Because of the complexity of events leading to aberration production, a lot of which are unknown, interpretations of this type should be accepted with due reserve and cannot be more than working hypotheses for further experimental tests.

5.93. Chromosome Breakage by Triethylenmelamine in Vicia faba in Relation to the Mitotic Cycle. M. Buiatti and Vittoria Nuti Ronchi (Pisa, Italy).

Experiments were carried out on the induction of chromosomal aberrations by triethylenmelamine (TEM) in relation to the mitotic cycle. Seedlings of *Vicia faba* with lateral roots 5-10mm in length, were treated for one hour with 0.5 10^{-4} TEM at 19°C, after which they were transferred either into water or into 0.5 per cent colchicine.

Anaphase and metaphase scores at 2 hr intervals after TEM treatment showed an increase in aberration frequency from the 6th to the 45th hour of recovery with a slight drop at 16-18 hr.

As revealed by the relative proportions of diploid and tetraploid cells in TEM-treated and control roots subjected to continuous colchicine action, a mitotic delay by TEM of 8 to 10 hr was calculated. This might indicate that cells carrying aberrations at the first recovery hours were under treatment at the end of G_2, possibly suggesting some mechanism of aberration induction, in absence of DNA synthesis.

A strong decrease in mitotic index from the 4th to the 26th hour of recovery (mitotis index: 5,61 and 3,47 respectively) was observed; at 30 hr, no significant difference was found between treated and control root tips (mitotic index: 10, 63 and 9,98 respectively).

Roots treated with colchicine for 40 hr after TEM treatment were scored for diploid(X_1) and tetraploid (X_2) cells showing aberrations: apart from the twofold increase in aberrations frequency per cell in tetraploids, no differences in aberration types between X_1 and X_2 were noticed.

Achromatic lesions, so called "gaps", showed a continuous increase, both at anaphase and metaphase, up to 45 hr recovery, a time when most cells were in the second division. This could support the idea of "gaps" being partial breaks. Scoring of gaps at anaphase gave, in all cases, higher values than metaphase scoring; this might result from the stronger contraction of metaphase chromosomes, leading to underestimation of "gaps".

5.94. Radiosensitivity of the Chromosomes. N. TANAKA (Tokyo, Japan).

Freshly rooted plantlets of *Tradescantia paludosa* were divided into four groups: the first group was grown in a culture solution, aerated and supplemented with tritiated thymidine (2μC/ml) for 8 to 12 hr (internal beta-ray irradiation); the second group was treated identically as the first group but exposed simultaneously to gamma-irradiation at a distance 40 cm from Co^{60} source (1.19 rad/hr) (both internal and external irradiation); the third group was exposed only to the gamma-irradiation at the same intensity as the second group (Co^{60} external irradiation); and the fourth group served as a control.

The root tips were fixed with acetic alcohol at relevant time intervals. Radiosensitivity of the chromosomes was measured in four ways by observing frequency of the total aberrations, fraction of sister chromatid reunion distal (SUD), fraction of sister chromatid reunion proximal (SUP), and fraction of erosion proximal (EP) in the mitotic anaphases.

The treatment was carried out at room temperature 26°C and the mitotic cycle was estimated to stand around 16 hr, being $G_1 = 2.8$ hr, $S = 8.4$ hr, $G_2 = 2.8$ hr, and $M = 2.7$ hr, where G_1, S, G_2, and M designated first growth period, DNA synthetic period, second growth period, and mitosis, respectively.

Radiosensitive period as measured by frequency of total reunion (SUD+SUP+EP) was found at S and G_2 in the first group and at S in the second group. Frequency distributions of each SUD, SUP, and EP in the second group contrasted with each other, suggesting the combined irradiation enhanced SUD in S and G_2 while SUP in G_1 and S, and for EP in G_2.

5.95. Chromosome Aberrations and the Mitotic Cycle in Trillium. C. J. GRANT (Oxford, Great Britain).

The mitotic parameters of root tips of *Trillium grandiflorum* were measured by the flash labeling technique, and confirmed by the binucleate cell method. At 25°C the period before DNA synthesis G_1, the synthetic period S, and the post-synthetic period G_2, were of two, one and two days duration respectively.

It was found that the X_1, X_2 and X_3 divisions were labelled in such a way as to confirm the view that chromosomes duplicate before normal cell division by a semi-conservative method.

After X-irradiation of root tips, cells containing the so-called sub-chromatid, chromatid and chromosome type aberrations are found. Overlap of these types may occur within a single cell, and in species with a short mitotic cycle this makes it difficult to correlate the different aberration types with the state of the cell at the time of their induction. However the relatively long cycle in *Trillium* results in little overlap and chromosome type aberrations can be definitely correlated with induction during G_1, Chromatid types with G_2, and sub-chromatid types with prophase. Chemical agents induce chromatid types during S.

5.96. Cytological Effects of Tritiated Water on Root-tip Meristems of Allium cepa L. G. WITTMER (Rome, Italy).

Series of onion bulbs were grown, after germination, in tritiated water having specific activity of 2.5 mc/ml. Cytological examinations performed at different times from exposure to THO have shown:

- progressive mitoinhibition; after 42-48 hr, the treatment is lethal;
- no physiological effect on the chromosomal matrix: phenomena of stickiness were only seldom observed;
- strong direct effect on mitotic chromosomes, i.e. very high fragmentation in anaphase (as far as 90 per cent of anaphases with fragments); the fragmentation is highest after 24-32 hr, and at this time also the metaphases show, if even in a low grade, fragments; the fragments are nearly always acentric and free, rarely attached;
- these mitosis with aberrant anaphases (or metaphases) may end their cycle and yeld cells with 1-2 micronuclei near the nucleus;
- "restitution" of these breakages induced by β-rays occurs quite frequently, since root-tips transferred in tap-water after the THO treatment show progressive normalization of the mitotoc activity, with only rare chromosomal aberrations.

The treatments were performed in the Laboratories of the Istituto di Chimica Farmaceutica dell'Università di Roma.

5.97. Effect of X-rays on Chiasma Frequency in Coix aquatica Roxb. K. C. SUBBAIAH (Bombay, India).

The present study relates to the effect of radiation on chiasma frequency in *Coix aquatica*

(bead coix). The chromosomes of *Coix aquatica* ($n = 5$) are long (110-75μ) with terminal knobs and are differentiated into eu- and heterochromatic regions, the latter flanking the centromere on both sides. The chiasmata have been found confined only to euchromatic segments. All the five bivalents, two of which are nucleolar, are mostly of the ring type with a terminalization coefficient of 0.753.

The inflorescences were irradiated with two series of 250 kVp X-ray doses (i) 2.5 r, 5 r, 10 r, 20 r, 40 r and 80 r and (ii) 250 r, 500 r, 750 r and 1000 r. The chiasma frequency at diakinesis following irradiation was determined 24 and 48 hr after irradiation. Translocations, both ring and chain types, involving (i) two nucleolar bivalents (ii) nucleolar and non-nucleolar bivalents and (iii) two non-nucleolar bivalents were scored.

While the doses from 2.5 r to 20 r failed to produce any observable translocations, those from 40 r and 80 r were only 2 and 4 per cent respectively. The frequency of interchanges observed in the high dose series showed that they were proportionately the highest within nucleolar; lowest among non-nucleolar and intermediate between nucleolar and non-nucleolar chromosomes. A linear relationship was found to exist between the interchanges and the radiation dose.

Bivalents without any visible aberrations, such as translocations, had nearly the same frequency of chiasmata as in the control, for the entire dosage range. However, there was a slight decrease in chiasma frequency in bivalents involved in translocations. Terminalization coefficient was found to be higher with the higher doses.

5.98. The Influence of Mitotic Cycle Duration on Chromosome Damage and Cell Survival in Chronically Irradiated Pisum Root Meristems. J. VAN 'T HOF and A. H. SPARROW (Upton, New York, U.S.A.).

An hypothesis has been advanced that for chronic exposures the duration of the mitotic cycle is an important factor contributing to cellular radiosensitivity (1). To test his hypothesis, experiments were performed with root meristem cells of seedlings of *Pisum sativum* in which duration of the mitotic cycle was controlled by temperature. Seedlings were exposed to Co^{60} gamma-rays at exposure rates of 185, 250, 500 and 1000 r per day for 3 days. Following exposure, measurements were made to determine the mitotic cycle duration, the number of cells having bridges or fragments at anaphase, and the number of cells per meristem. These measurements revealed (1) that the minimum mitotic cycle duration in irradiated meristems was essentially the same as the unirradiated control, (2) that at a given daily exposure rate the percentage of anaphase cells with visibly damaged chromosomes increased with increased cycle duration, and (3) that the irradiation reduced the number of cells per meristem below that of controls. The decrease in the number of cells per meristem can probably be attributed to reproductive cell death due to genetic loss or unbalance since reduction in cell number varied directly with the percentage of cells showing chromosome damage.

The results with *Pisum* suggest that (1) for chronic exposures (up to 1000 r/day) the probability of a chromosome being damaged increased with increasing duration of the mitotic cycle, (3) the accumulated exposure per cycle required to produce a measurable decrease in the number of cells per meristem approaches a constant value, and (3) the accumulated exposure per cycle required to produce an equivalent number of cells with damaged chromosomes in meristems having different minimum mitotic cycle durations also approaches a constant value. Thus in quantitating the factors responsible for cellular radiosensitivity or cell survival the duration of the mitotic cycle must be considered, at least for chronic exposures.

Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

1. SPARROW and EVANS, *Brookhaven Symposia in Biology*, No. 14, 76-100, 1962.

5.99. Mutagenic Action of Contrast Media for Roentgen Diagnosis. A. BARTHELMESS and M. BAUCHINGER (München, Germany).

In the course of investigations on the cytogenetic action of substances which come in contact with gonadal tissues or gametes in the human body we tested 3,5-dijodid-4-pyridone-N-acetic acid-diethanolamin (I) and N,N′-adipine-di-3-amino-2,4, 6-trijodoben-zoic acid-methylglukamin (II). Both substances are (or have been) used as contrast media in urography, arteriography and venography (I and II) and hystero-salpingography (II).

Root tips of *Allium cepa* have been immersed 4 and 24 hr in the solution and fixed at intervals of 0, 24 and 48 hr after the end of treatment. Both substances caused numerous breaks and structural rearrangementsof chromosomes, mi-

cronuclei, release of droplets of DNA in the cytoplasm, and disturbance or inhibition of the regular mitotic distribution (stathmokinesis, merokinesis, non-congression). The maximal effect was obtained with both substances at a concentration between 1 and 2 per cent, which allows normal growth of the roots. However the number of cells showing cytogenetic effects often varied considerably between the different roots of the same bulb.

A dose of 60-80 r of roentgen rays gives about the same number of structural effects as 1.5 per cent of I, but nearly no abnormalities of mitotic distribution. No clearcut results as to a synergistic or antagonistic action of simultaneous chemical and radiation treatment were obtained because of the high numerical variability of all the cytogenetic effects mentioned above.

As the substance I is administered also in salpingography (in concentrations from 30-70 per cent) one has to take in account dysgenic effects in ovaries and follicles.

The results will be published in full in the journal *Strahlentherapie*.

5.100. Cytogenetic Analysis of the Sensitivity of Plants to Different Kinds of Radiation. V. V. Khvostova and S. A. Valeva (Moscow, U.S.S.R.).

1. After irradiation of seeds of resistant varieties of plants, belonging to the same species, with the same dose of ionizing radiations a lower per cent of ana- and telophases with chromosome rearrangements are produced in first mitoses of seedling roots, than in radiosensitive forms.

2. A comparison of the action on seeds of related varieties of sparcely and densely ionizing radiations has revealed the existence of two kinds of chromosome protection against radiations: (a) two varieties of peas, markedly different as to sensitivity to gamma-rays, showed equal sensitivity to fast neutrons; (b) whereas one of two varieties of maize manifested a parallel resistance to both gamma-rays and fast neutrons. These sensitivity differences were manifested at various stages of the development of the plant: after irradiation of dry seeds and seedlings as well.

3. Experiments on the storage of pea seeds during 6 months after irradiation with gamma-rays showed, that the sensitive variety exhibits after storage twice as many per cent of anaphases with chromosome rearrangements. The supposition is made that potential chromosome damages occurring under the action of gamma-rays, transforms into real breaks under the influence of the biochemical peculiarties of the cellular medium of the sensitive variety during storage.

4. Studies on the action of 2-4, dinitriphenol on germinating seeds of two varieties of maize clearly showed that 2-4, dinitriphenol has no effect on the number of rearrangements after neutron irradiation; after irradiation with gamma-rays, dinitriphenol increases the number of rearrangements, especially in the radiation-sensitive variety. These experiments show that the damage of chromosomes by the action of neutrons is not subject to modifications by the energy processes in the cell after irradiation; (2) and that, apparently, the potential damages, occurring under the action of gamma-rays, are not transformed into real ones under the conditions of the high energetic level of the cell, and are transformed only when the energetic level of the cell becomes lowered.

5.101. Selection for Radioresistance and Resistance to Chemical Mutagenes in Diploid and Tetraploid Forms of Buckwheat (Fagopyrum esculentum). R. N. Platonova and V. V. Sacharov (Moscow, U.S.S.R.).

1. Experiments on λ-irradiation of four seed progenies and on the selection of the most radioresistant plants, grown from these seeds, produced resistant forms of diploid and tetraploid buckweat populations.

In the usual ($2n=16$) buckhweat form one first selection resulted in an increase of survival of the irradiated plants. In the autotetraploids ($4n=32$) an increase in survival after irradiation was produced only after two or more selections.

2. Our $2n$ and $4n$ lines, resistant to λ-rays, were checked for two years following for resistance to densely ionizing radiations (fast neutrons). The results obtained were positive for all characters (survival, power of development and seed bearing). This was proven by comparative cytological investigations. In the gamma-resistant forms there were less chromosome damages, caused by fast neutrons, than in the usual populational seeds, receiving the same radiation dose.

3. Selection for radioresistance caused the occurrence of forms resistant also to the chemical mutagene—diethylsulphate.

4. In order to make clear the character of inheritance in the radioresistant diploid plants, resistant (selected) plants were crossed with plants from usual, non-irradiated populations. The same kind of experiment was carried out on tetraploid plants. The hybrid plants show a dominant inheritance, and radioresistance in $2n$ hybrids appeared even to be higher, than in

their radioresistant parents. This increase we explain as the result of an additional effect of heterosis. In the 4 · forms, dominant inheritance of radioresistance cannot be doubted, but it is somewhat more feebly expressed than in the diploids. The radioresistance of the 4 hybrids is somewhat lower than that of their radioresistant parents, but it is markedly higher than the radioresistance of the initial forms (non-selected).

5.102. On the Possibility of the Modification of the Ionizing Irradiation Genetical Effect in Barley. S. I. YANUSHKEVICH (Moscow, U.S.S.R.).

The possibility of the modification of the λ-rays (Co^{60}) effect on barley (*Hordeum sativum* L. ssp. *distichum*) seeds in the dormancy state under influence of condition preceding and following the irradiation is discussed. In the experiments the influence of the agro-climatic conditions on the growing plants and of the modification of nutrition conditions of the barley seed embryos (by means of homo- and heterogenous transplantation of embryo on the endosperm) upon the radiosensibility, chromosome aberrations frequency and chlorophyll mutations in M_2 was under study.

It was shown that plant growing in different agro-climatic areas led to the radiosensibility change of the seeds in the dormancy state. The differences in radiosensibility could vary in large limits and their manifestation was correlated to the moisture level of irradiated seeds. The additional data were obtained on the periodical change of the radiosensibility of the seeds in the state of dormancy kept under the conditions of laboratory.

The growing of M_1 plants from irradiated seeds in sharply different agro-climatic areas led to definite variations of chlorophyll mutations frequency in the progeny.

The influence of nutrition conditions of developing embryos of barley seeds on the irradiation effect was noted. The nutrition of the irradiated embryo by the non-irradiated endosperm substances led to the change of the chromosome aberrations total amount. The irradiated endosperm, in its turn, induced chromosome aberrations in the cells of growth zone of non-irradiated embryos rootlets.

The results of these experiments are discussed in the light of the final effect dependence upon the irradiated organism physiological state and metabolism in the periods before and after irradiation.

5.103. Variable Influence of Humidity on Radiosensitivity of Seeds from Eight Botanical Families. THOMAS S. OSBORNE, MILTON J. CONSTANTIN, and ALLYN O. LUNDEN (Oak Ridge, U.S.A.).

Classical studies with the barley grain (*Hordeum*) showed that superdry embryos are extremely radiosensitive, becoming more resistant by a factor of 4 or 5 as preirradiation relative humidity (RH) approaches 50 per cent and staying on a plateau of resistance even when RH exceeds 90 per cent. We equilibrated various dormant embryos at RH's of 10 to 85 per cent then exposed them to gamma rays at doses of 0 to 400 kr. Dry weight of seedlings in controlled environment rooms was measured. Families studied were *Compositae*, *Cruciferae*, *Cucurbitaceae*, *Gramineae*, *Leguminosae*, *Linaceae*, *Solanaceae*, and *Umbelliferae*.

Embryos of the *Gramineae* (*Hordeum* and *Festuca*) proved to be exceptional in that they showed only trivial responses to variations in preirradiation humidity. All other families gave immense changes in sensitivity, even more than 200-fold, with changes in RH. There was a peak RH for maximum resistance, with sensitivity increasing at RH's both above and below; the optimum RH differed with species. The inadequacy of current free radical-water hypotheses to explain increased sensitivity above the optimum RH is obvious.

Computer techniques with quadratic and cubic formulae were used and discussed. Parts of the data have been accepted for publication by *Radiation Botany*.

Operated by the University of Tennessee College of Agriculture for the U.S. Atomic Energy Commission under Contract No. AT-40-1-GEN-242.

5.104. Cysteamine and Sensitivity to X-rays in Barley. A. MOËS (Gembloux, Belgium).

In 1951, Bacq, Herve *et al.*[1] discovered that cysteamine and cystamine are remarkable protectors against radiations in mice.

The protective action of cysteamine has been confirmed in our experiments conducted since 1954 on barley.[2] Our tests are made with Piroline spring barley; the X-ray apparatus works at 30 kV. The inhibition of the development of the first leaf issuing from the irradiated seeds is determined by a growth test made when the control reaches approximatively 75 mm.[3]

In the first trials, the seeds were soaked in cysteamine at 1, 2, 4 per cent during 24 hr at 20 C before irradiation. We observed a marked protective action in the growth test, confirmed by the percentage of plants attaining maturity.

In 1957, we reduced the time of soaking to 1 hr and used only cysteamine 1 per cent; the higher number of surviving plants observed was significant.

In 1958 and 1961, the seeds were soaked during 1 hr in cysteamine 1 per cent, and then dessicated; the irradiation took place on seeds with normal water content (13-14 per cent). The growth test and the percentage of mature plants showed a marked protective action from 20000 r and above. The latest experiments proved that presence of cysteamine is sufficient to account for its action.

It seems that the effect of cysteamine decreases when the water content is low (4-5 per cent).

In a number of experiments, ([2]) the cysteamine applied before or after irradiation increased the number of mutants but in other trials made in 1958 and 1959, the protection afforded by cysteamine against the mutagenic effects of X-rays is of the same order of magnitude as the protection against lethality.

1. Z. M. Bacq, *et al.;* Protection contre le rayonnement X par la β mercaptoethylamine. *Arch. Internat. Physiol.* **59,** 442, 1951.
2. A. Moës; L'action de la cysteamine chez l'orge. *Bulletin de l'Institut Agronomique et des Stations de Recherches de Gembloux.* XXV, 98-107, 1957.
3. A. Moës. Water content, wave-length and sensitivity to X-rays in barley. *Effects of Ionizing Radiations on Seeds,* I.A.E.A., Vienna, 631-640., 1961.

5.105. Some Factors Affecting the Action of Chemical Mutagens. C. F. Konzak, R. A. Nilan, R. E. Heiner, and Edith E. Froese-Gertzen (Pullman, U.S.A.).

Diethyl sulfate and ethyl methanesulfonate induce a remarkably high frequency of chlorophyll mutations and a negligible frequency of gross chromosome aberrations in barley. This high mutation frequency is induced at a high rate of plant survival and low levels of seedling injury. Recent studies provide a basis from which an understanding of the relationship between different kinds of biological damage can be sought. It has become apparent that physical and chemical factors play a determining role on the action of mutagens in biological systems. Hydrolysis of diethyl sulfate in aqueous solution is 26 times faster than that of ethyl methanesulfonate. The Q_{10} for the *in vitro* hydrolysis of the mutagen varies from 3.1 for the 0-10 C range to 4.5 for the 30-40 C range, whereas the Q_{10} for the *in vivo* seedling injury response is about 2.7. Our data indicate that the rate-controlling factor in the *in vivo* system is duffusion. The hydrolysis products tend to decrease seedling growth and plant survival but do not induce mutations. These injury effects are apparently due to low pH. Highly concentrated mutagen solutions reduce mutagenic effectiveness by increasing undesirable side-effects through mass action. The mass action is accentuated with fast-reacting agents. Results from these studies offer some likely explanations for differences in the relative mutagenic efficiency of chemical agents.

Research supported in part by funds from the U.S. Public Health Service GM 10838-05 and from the U.S. Atomic Energy Commission AT (45-1)-353 and conducted under Washington State Experiment Stations Projects 1435 and 4068.

5.106. The Effects of Some Combinations of Mutagens on Mutation Frequency in Barley. T. J. Arnason, J. L. Minocha and Laila Mohammed El-Sadek (Saskatoon, Canada).

Two mutagens acting in sequence may produce more than additive mutation frequencies if, for example, one of these exposes previously protected mutation sites to the second mutagen. If two mutagens merely compete for the same sites or if the effects are completely independent the results should be additive or less than additive. Combinations of mutagens that have been tried on barley (*Hordeum vulgare* L.), are ethyl methanesulfonate (EMS) and ethylene imine (EI); EMS and nucleotides and EI and nucleotides. The frequencies of chlorophyll mutations in M_1 spikes and M_2 seedlings were recorded.

The treatment with 0.5 per cent EMS solution for 4 hr yielded 17 per cent M_1 spike mutations. A 4-hr treatment with 0.1 per cent EI solution yielded 13.8 per cent of mutant spikes. When grains were treated with both mutagens in sequence the results were more than additive. With EI applied first the mutation frequency was 43.8 per cent and when EMS was applied first the mutation frequency was 36.7 per cent. On an

additive basis the expected frequency was 30.8 per cent.

Solutions of nucleotides induced very few mutations. The highest rate (0.54 per cent) was produced by adenosine-5-monophosphate. When adenosine-5-monophosphate was applied before treatment with 2000 r of Co^{60} λ-radiation a slightly more than additive result was obtained. Experiments with EI and nucleotides are not yet completed. Three combinations of nucleotides and EMS resulted in notable increases in the mutation rate. Here, it is possible that guanine in DNA degraded by EMS was sometimes replaced by the supplied base.

5.107. Mutations Induced by the Action of Metal Ions in Pisum. GÖSTA VON ROSEN (Landskrona, Sweden).

Radiomimetic effects and gene mutations are induced by a deficiency or surplus of for a vital organism important micro elements. The frequency of mutations induced by the action of metal ions is lower than the frequency induced by ionizing radiation or some intense active chemical substances. The mutation-spectrum in chlorophyll mechanism induced by treatment with surplus doses does not differ from the spectrum at hard ionizing radiations. Treatment with a simultaneous combination of several micro metals is more effective than treatment with single metal ions. Individual metal ions do not show any trend to specific chlorophyllmutations spectra at present, but they induce some other specific types of mutations indeed.

By adding treatments with metal ions and X-ray the mitoses and cell disturbances are arrested, and the mutagene activity of the ionizing particles is somewhat reduced. The activity of the metal ions seems to be additative to the effect of the X-rays.

The micro metal elements in a living cell have functions in specific and very important processes, usually in complex protein structures. Their concentrations and activities in the cell are regulated by chelating with protein radicals in co-valent and semi-polar bonds in the protein molecule, a balance which is very sensitive and limited. It is that force of the metal ions to form complexes with protein radicals that causes their radiomimetic and mutagenic activity. By oscillating of the dissociation balance destructions in the cell metabolism occur, which may induce chromosomal disturbances and gene mutations. It is suggested that the complex forming metal ions in the evolution of the plant and animal kingdom have played a limited role, partly through their own activities, partly through an indirect effect on the active radicals from the natural radiation.

5.108. The Magnitude of the Oxygen Effect in Irradiated Barley Seeds. R. A. NILAN, C. F. KONZAK, J. R. HARLE, and R. R. LEGAULT (Pullman, U.S.A.).

The extent to which radiation-induced damage in barley seeds can be amplified by oxygen posttreatment depends on the radiation energy (LET), the tissue moisture content, and the temperature and time of storage from the initiation of irradiation to the initiation of germination. In very dry seeds ($\sim$ 2 per cent moisture) oxygen-effect factors as high as 10 were demonstrated for high intensity Co^{60} gamma-rays, and as high as 7 for 300 kVp X-rays. These dose-increase factors were obtained for radiation-induced damage measured as M_1 seedling injury, chromosome aberrations in mitotic cells of M_1 seeds, and chlorophyll-deficient mutations in M_2 seedlings. With seeds differing in moisture from 2.5 to 13 per cent, the oxygen-effect factors measured by seedling injury ranged from 9 to 1, respectively. Very dry irradiated seeds stored at room temperature appeared to maintain indefinitely their high sensitivity response to oxygen. Seeds stored at higher temperatures exhibited a reduced oxygen sensitivity but developed greater radiation-induced seedling injury. These results are consistent with established evidence from electron spin resonance analyses of the influence of temperature, moisture, and LET on the interaction of oxygen and radiation products in seed embryos.

Research supported by U.S.A.E.C. Contract AT (45-1)-353, U.S.P.H.S. Grant GM 10838-05 and funds provided for Medical and Biological Research by State of Washington Initiative Measure 171.

5.109. Electron Spin Resonance Studies on Plant Seeds of Differential Radiosensitivity. S. BHASKARAN (Karlsruhe, Germany).

Electron spin resonance studies on plant seeds of differential radiosensitivity were conducted with a view to evaluate the relationship between the production and decay of radicals and the observed differences in the radiation sensitivity. Dry seeds (moisture content 5 and 2 per cent

equilibrated over drying agents) of mustard, tobacco and *Agrostis stolonifera* were irradiated with different doses of X-rays and the number of long lived radicals produced was determined employing ESR technique. A linear dose effect relationship was obtained in all the three cases at different moisture levels. The yield of radicals in super dry seeds was much higher than in moist seeds. At 5 per cent level, the yield of radicals was least in mustard for a given dose as compared to tobacco and *Agrostis*. *Agrostis* yielded a greater number of radicals than tobacco. However, at 2 per cent moisture content, this relationship was altered in so far as mustard yielded the maximum number of radicals per gram for a given dose and no difference was found between tobacco and *Agrostis*.

The part played by oils and melanin in mustard will be discussed in explaining the radical yield at different moisture levels. The results on the effect of NO, pretreatment with heat and irradiation at low temperature will be discussed in relation to the observed biological effects.

5.110. Naturally Occurring and Artificially Induced Mutations in Maize. ANGELO BIANCHI (Milano, Italy).

Analysis of the genetic variability detectable in open-pollinated populations of maize reveals that mutations occurring in nature are almost exclusively point events. On the contrary the greatest majority of the artificially induced mutations turn out to be of chromosome type. So far this picture has not been changed greatly with physical and chemical treatments performed under different experimental conditions, even in experiment designs of high resolving power. In maize such a requirement has been met, with increasing reliability, using: (a) endosperm markers; (b) heterozygotes for suitable plant characters; (c) pollinator stocks possessing translocations between A and B chromosomes; and (d) the *waxy* locus affecting pollen grain composition. In the first and in the last case, the treatments are performed preferably on the pollen mother cells and/or on the mature pollen, in the other cases the target is usually the seed. In all cases, several factors (as temperature, moisture content, storage time, type of X-rays, dose fractionation) have been varied with the consequence of changing at times significantly the total effect, but hardly the type of response. In the chemical mutagenesis some compound (ethylmethansulphonate) turned out to be highly efficient when administered to seed, much less when applied to pollen, while the contrary is true of diepoxibutane. For the latter, moreover, the isomeric forms show significantly different mutagenic activity. While chemical mutagenesis is affecting the hereditary material of maize in some fundamental aspects similar to those of physical agents, some difference has also been detected.

X-ray treatment of very sensitive microsporogenesis stages, such as to induce more than 90 per cent of pollen abortion, have caused an appreciable increase of *wx* → *Wx* events, on the basis of pollen grains phenotypes. Since this appears the first case artificially obtained back mutation in maize, confirmatory data are necessary.

5.111. Radiation Induced Modification of Paramutation Expression. DUANE B. LINDEN (Mayaguez, Puerto Rico).

Paramutation as it is expressed at the R locus in maize involves an interaction between the gene undergoing a change (R^r) and a source capable of inducing this alteration (R^{st} or R^{mb}). The components of this system were subjected to radiation before being brought in contact to determine their radiosensitivity and to obtain additional information regarding the nature of the paramutation phenomenon.

When the site for paramutation change (R^r) was irradiated before crossing to R^{st} or R^{mb} the resulting progeny fall into three categories: one group in which there is no apparent effect of the radiation; a second group in which there is apparent complete inactivation of the paramutation process; a third group with reduced paramutation alteration. The frequency of the second and third categories suggest that radiation results in an inactivation type event rather than inducing a gene mutation alteration at the R^r locus.

Irradiation of the regulator stocks revealed differences between the two sources. When the R^r source was irradiated prior to crossing with R^r and then testcrossed there was a very low incidence of complete inactivation of paramutation but the partial alteration to a less complete change occurred very frequently. The alteration was always in one direction toward a less efficient paramutation alteration. However when the R^{mb} source was tested in this manner the results differed. There was appearent complete inactivation in some crosses. In other crosses there was an increase in efficiency of inducing paramutation alterations, as the degree of change was greater than the controls.

5.112. Effect of Gamma-radiation on Cytology of Microsporogenesis in Pinus rigida. FRANÇOIS MERGEN (New Haven, U.S.A.).

A cesium-137 (9500 curies) source was placed in a natural pitch pine-oak forest at the Brookhaven National Laboratory. Beginning on November 22, 1961, the forest was exposed to gamma-rays 20 hr each day. Pitch pine trees were exposed to radiation levels ranging from a high of 960 r/day to a level of normal background radiation. Microsporangiate strobili were collected at regular intervals to follow the effects of gamma radiation on the cytology of microsporogenesis.

Trees receiving 82 r/day and higher were unable to resume cell division and differentiation during the spring of 1962, while microspore mother cells were formed on trees receiving 75 r/day. At 56 r/day meiosis with subsequent formation of tetrads took place, and the highest level at which mature microspores were collected was 14 r/day. A slight reduction in the length of the strobili was observed at 0.4 r/day, and at 3.7 r/day they were significantly shorter than the controls. The percentage of cells with visible chromosome aberrations increased with an increase in exposure, e.g. 0.36 r/day = 1 per cent, 7 r/day = 2 per cent, 11 r/day = 12 per cent, 56 r/day = 76 per cent. Illustrations of these chromosome aberrations are given. The data will be published in full in *Radiation Botany*.

5.113. Dependence of the Effect of Dose Rate on the Physiological State of Irradiated Objects. N. I. NUZHDIN, R. L. DOZORTSEVA and N. S. SAMOKHVALOVA (Moscow, U.S.S.R.).

Sax (1939) made an attempt to explain the difference in the frequency of exchange-type chromosome aberrations when using the same dose of different intensity by his hypothesis that this difference is determined by not-identic possibility of the broken chromosome ends recombination. The results obtained by Lane (1951) and Koller (1956) led to physiological explanation of nature of the different effect of irradiation intensity.

The hypothesis of Sax can be applied only in the case of irradiation of dividing cells which have active metabolism. In view of this peculiarity we irradiated air-dry seeds of the Odessky-17 and Wintering Moskovsky strains of barley by γ-rays Co^{60} with doses of 12 and 10 kr. In the 1st case the dose rate was 423 and 55 r/min, in the 2nd one 301 and 43 r/min. The irradiated seeds were in two physiologically different states of forced and organic dormancy. The frequency of cells with chromosome aberrations (bridges and fragments) in the first mitoses of rootlet tips was under study. The rootlets of non-irradiated seeds of the same generation served as a control.

The cytological study of the rootlets of seeds irradiated in the state of forced dormancy showed a definite difference in the frequency of cells with chromosome aberrations. The irradiation with high intensity led to the appearance of more chromosome aberrations than the irradiation with low intensity (41.46 ± 2.38 per cent and 30.79 ± 1.43 per cent for one strain and 20.16 ± 1.20 per cent and 10.38 ± 0.82 per cent for the other, respectively).

The results of the irradiation of seeds in the state of organic dormancy were not the same. In the cells of the rootlets of seeds germinated immediately after they were brought out from the state of organic dormancy the chromosome aberrations percentage was in fact the same for the irradiation both with high and low intensity (9.45 ± 1.56 per cent and 6.21 ± 1.5 per cent for one strain and 11.97 ± 1.12 per cent and 10.51 ± 0.97 per cent for the other, respectively). In the seeds which were brought out from the state of organic dormancy at the same time, but grown after 10 days' storage in air-dry state the influence of irradiation intensity is distinctly seen. The high dose rate produces a higher yield of cells with chromosome aberrations than the same dose but of lower intensity (23.39 ± 1.36 per cent and 14.39 ± 1.09 per cent, respectively).

The data obtained indicate that irradiation-induced chromosome damage cannot be considered as a mechanical effect or a direct result of separate ionization events. They mainly depend upon the physiological processes in the irradiated cells.

5.114. Genetic Advance in the Height of Plants Induced by Irradiation in Wheat. KATARINA BOROJEVIĆ (Novi Sad, Yugoslavia).

The height of plant as a quantitative character is controlled by polygenic system and it may be changed by irradiation. For this reason the dry seed of wheat was treated with the different dosages of X rays and thermal neutrons.

In the R_1 both irradiations reduced the height of plants from 1 to 32 per cent depending on the dosage. The variability of this character was increased only in the group treated with high dosages.

In the R_2 and R_3 the height of plants remain reduced in all treated groups. Compared with

the untreated material, the heritability has increased in treated material and was higher in the R_3 than in R_4.

The genetic advance in the height of plants is calculated for all lines which were expected to be 10 per cent shorter than the control.

5.115. Segregation Ratios of Induced Mutations in Durum Wheat. G. T. Scarascia Mugnozza, A. Bozzini and F. D'Amato (Pisa, Italy).

Since 1956 experiments were carried out on the induction of mutations in *durum* wheat by X-rays, fast and thermal neutrons, ethylmethanesulfonate, diethylsulfate and ethilenimine. So far more than four hundred independently obtained cases of viable mutations have been isolated; moreover several hundreds of lethals are being maintained as heterozygotes. This material is the object of genetic and cytogenetic analyses. Data are presented on segregation ratios in the progeny of M_2 and M_3 individuals heterozygous for induced mutations and of M_2 progenies of the cross of viable mutants to the mother line (total mutations analyzed: 51). In a total of 28 M_2 individuals heterozygous for pigment mutations (2 albina, 2 xanthalba, 3 chloroalbina, 7 xantha, 2 virido-albina, 7 tigrina, 1 virescens, 2 chlorina, 2 anthocyanic) 19 gave a M_3 progeny with a 3: 1 segregation ratio (mutant condition recessive). In the remaining 9, the proportion of mutant individuals was much less; but in M_4 already four out of them attained the 3: 1 ratio. This result seems to indicate that the segregation deficit shown by induced pigment mutations in the first generations following treatment may be reduced or eliminated, possibly through progressive elimination of deleterious genetic changes accompanying the mutation. F_2 analysis of the crosses between pigment mutants and the mother line showed that 6 of them behaved as monogenic recessive (2 tigrina, 1 virescens, 1 viridis, 2 chlorina), whilst 2 tigrina mutations were inherited as semidominant. As to morphological mutations, only the progenies of 6 M_2 heterozygotes and the F_2 of 9 crosses "mutant × mother line" have been analyzed so far. Out of these 15 mutations, 10 behaved as monogenic recessive (2 with supernumerary spikelets, 2 semisterile, 3 waxless, 1 with defective endosperm, 1 with anthocyanic glumes, 1 with solid stem); in the remaining 5 (1 dwarf, 2 waxless, 1 anthocyanic glumes, 1 brachytic) a strong deficit of individuals with mutant phenotype was noticed, in connection with a high degree of sterility.

5.116. Chemical Mutagenesis in Triticum. H. K. Shama Rao (Bombay, India).

Ethyl-methane sulfonate (EMS), unlike radiations, has been shown to induce chlorophyll deficient sectors with or without notch or punch formation and necrotic patches in leaves as well as speltoid, sub-compactoid, compactoid and compactum-spikes, during the treated generation, in the monosomics 2B and 6B of the hexaploid Chinese-Spring wheat [1].

In both mono-2B and 6B, phenotypic changes during M_2 appeared in almost equal numbers out of a total of 37 affected families amongst 124 progenies raised from all the M_1 sectorial and non-sectorial plants. However, the mutation spectrum differred. The chlorophyll defectives, viz. lutescent, chlorina and freckled, were noticed only in mono-2B. In addition, 2 other families were with grass-clump types and another with a rudimentary-spike character.

In mono-6B, lethal dwarfs having short, thick and dark-green leaves were observed in 2 families. Another family was with a distinctive type of spike mutation, viz. "Missing-Spikelets", a character not observed in any of the nulli-, or tetrasomics; nor ever reported as appearing following irradiation.

The types of mutation common to both mono-2B and 6B were those with increased or decreased awn development and without hooded character which could all be accounted for by simple deficiency of known genes. Two other spike mutations observed were of semi-speltoid and base-compact types.

The chlorina, a number of lethals and the mutants with missing-spikelets and rudimentary-spikes segregated in approximately 1: 3 ratio showing that some of these, at least, involved single genes. The modes of their inheritance are under investigation.

---

1. Shama Rao and Sears, *Records of the Genetics Society of America*, 1962.

5.117. Different Mutation Spectra among Related Strains of Antirrhinum majus, obtained after Irradiation. Gertrud Linnert (Berlin, Germany).

Two subsequent radiation experiments were undertaken on normal and mutant strains of the pure line designated "Sippe 50" by Erwin Baur. At first pollen was treated with 3000 r; the progenies of 14 plants resulting from this experiment (= X_0 of the following experiment) were once

more irradiated as seedlings with 300 r. From every plant 59-158 progenies were grown totalling 1488. 237 mutations appeared among them. The mutation spectra of the 14 X^0-plants were quite different, because 19 phenotypes repeatedly emerging were not distributed at random, but accumulated within closely related strains. 6 of the phenotypes appeared 2-4 times within the progenies of a single X_0-plant, 9 others were detected among kindred plants. In addition mutations appeared in mendelian proportions of the progenies as a result of the first irradiation. These mutations of course were not included in these dates. The first radiation experiment yielded similar results with respect to the mutation spectra as the second one did. Coincidences so frequently occurring cannot be due to mere chance; therefore the conclusion must be drawn that some of the loci in different strains excelled above the others in their particular disposition for mutations.

5.118. Effects of Ionizing Radiations on Cucumis sativus L. and Momordica charantia L. FILOMENA, F. CAMPOS and WALDERICO M. GENEROSO (Laguna, Philippines).

Results obtained from subjecting seeds of *Momordica charantia* L. and *Cucumis sativus* L. to various levels of X-rays and r-rays showed that germination was relatively unaffected. However, differences in the relative sensitivity of cucumber seeds to X-rays and r-rays were observed. X_1 and R_1 seedlings of both species beyond 15 kr exhibited chlorophyll and morphological abnormalities. Significant differences between dosages were observed when analysis was based on growth of the plants in terms of length of the main vine. Regardless of treatment, days to floweringwas relatively unaffected by irradiations.

In *Momordica* there was an increase in the proportions of male to female flowers in the X-ray series but none in the Co^{60} series. On the other hand, sex ratio in cucumber was not altered by irradiation although reductions in the number of male and female flowers were noted. Sex reversion was observed only in *Momordica* plants on the treated and segregating generations. Two types of sex-reversed flower were observed.

Seedling mutations observed in the X_2 and R_2 generations were mostly chlorophyll abnormalities. However, a few clear-cut morphological abnormalities were detected. A few mutations having no conspicuous effect in the seedling stage but causing distinct variations in the later development are also included.

5.119. New Mutations Obtained in Vicia faba L. through the Injection of Chemicals. PIERRE BRYSSINE (Rabat, Morocco).

Sundry chemicals were injected by means of a hypodermic syringe into the hollow inside the stem of *V. faba* L.

The chemicals, though extremely toxic and injected at considerable rates (10-30 ml per stem), are rapidly absorbed by the tissues. The consecutive depressive effect was evaluated from the variation of the production of pods (number) and seeds (weight and number).

Considerable changes were observed on the treated plants: sterility, growth disorders, fasciation, chlorosis and variegation of the foliage, dichotomy of branches, anarchic proliferation of medullary tissues, disorders of the vascular system, etc.

From C_1 onward several types of mutants could be observed concerning:

(a) The vegetative system (growth habit, branching type, shape of leaf, chlorophyll deficiency, etc.)
(b) The pods (shape, size, pubescence)
(c) The seeds (size, shape, colour of seed coat and hilum)

Attention is drawn to the rare or hitherto unknown forms among these mutants (from C_2 and C_3 onward):

1. *Geant* (stems of more than 2 m with long internodes)
2. *Unifoliolata* (with a single leaflet)
3. *With tendrils* (lanceolate leaflets, branched tendrils)
4. *Begonia* (prostrate plants with very thick leaves)
5. *Wild* (dwarfed erect plants with small seeds)

As to the experienced chemicals, the best results were obtained with mitoclasic materials (at high concentrations: colchicin, acenaphthene, gamma-hexachlorocyclohexane) as well as with extracts of old seed (tobacco, soya, chick-pea). Methane-sulfonate-ethyl and acetone equally proved very interesting.

The advantage of the injection method is discussed.

5.120. Radiation-induced Reversions to Prototrophy in Tryptophan-requiring Escherichia coli WP2 B. A. BRIDGES and R. J. MUNSON (Berkshire, Great Britain).

We have attempted to follow gene duplication in populations of *Escherichia coli* B/r (strain WP2, tryptophan-requiring) by irradiating at intervals during synchronous nuclear replication. A late

log. phase culture, grown at 25° in glucose-tryptophan-salts medium, was centrifuged and the cells resuspended in salts medium (1-2 10^8/ml) and incubated for 48 hr at 25° with aeration. This resulted in a population of largely uninucleate cells.

Upon addition of glucose and tryptophan, DNA synthesis began and the amount in the cells had doubled by about 150 min. At this time the nuclei were seen to divide with a reasonably high degree of synchrony. No cell division occurred during this time. Aliquots of the suspension were irradiated (2000 rad 250 kV X-rays) at intervals during the period of 150 min and estimates of the number of induced mutants (revertants to prototrophy) were made immediately after irradiation.

If the genes for tryptophan requirement replicate in synchrony, a rapid change in the number and type of induced mutants might be expected to result. It is hoped to present results obtained using this technique with WP2 and other strains of *E. coli*.

5.121. (D.) Somatic Mutations in Ephestia kühniella: Their Dose-relationships in the Low and High Dose-range of X-irradiation and the Influence of Pretreatment on the Mutational Spectrum. ILSE MÜLLER (Köln-Lindenthal, Germany).

After irradiation of young pupae different types of mutant scales are to be observed on the hind wings of adult moths, their absolute and their relative number depending on the dose of X-rays. From 20 r up to 1000 r the frequencies of the mutants are proportional to the square of the dose, approximately. The spontaneous and the 10 r-frequencies are lower than expected from the curve in the higher dose-range.

If pupae are kept at different temperatures for some hours prior to irradiation, the mutational spectrum at certain doses differs significantly between the groups which were subjected to low resp. high temperature treatment. This difference is brought about by a change in the exponents of the dose-effect curves, which is alike for all mutant types, and a change in the sensitivity to irradiation, the amount of which is larger in the types with a relative low frequency than in the more frequent ones.

Graphs and formulas, showing the quantitative relationships, will be presented. The different mutant scales will be demonstrated under the microscope.

5.122. Contribution to the Determination of the Rules of Radioresistance of Agrarian Plants. E. SĂNDULEAC (Bucharest, Rumania).

The Rumanian research works from 1959-1962, with wet and dry irradiation of the seeds, in order to determine the critical dose for the main agrarian plants irradiated with μ, x and β emitents, have shown that the most radioresistant plants are the following: *Linum ussitatissimum*, *Brassica rapa*, *Sinapis* sp., *Lallemantia iberica*, which have the critical dose = 100,000 r; after that *Ricinus communis*, *Sesanum indicum* and *Camelina sativa* have more than 50,000 r. A few textile plants like *Abutilon avicenae* with 100,000 r and *Cannabis sativa* with 30,000 r are also radioresistant. *Nicotiana* sp. has the critical dose over 50,000 r too. From Gramineae the following are radioresistant: *Oriza sativa* > 80,000 r, *Sorgum* sp. > 30,000 r, *Panicum miliaceum* > 25,000 r, *Avena sativa* 25,000 r, *Hordeum* sp. 20,000 r; relatively resistant are *Triticum* sp. 15,000 r and *Secale cereale* 15,000 r. Radiosensitive are *Zea mays* < 10,000 r, and from other families, *Helianthus annuus* < 8000 r, *Vicia faba* > 5000 r, *Solanum tuberosum* > 5000 r, *Beta vulgaris* 5000 r. The annual Leguminosae have in a majority the critical dose 15,000 r, *Trifolium pratensa* 20,000 r, *Medicago sativa* 25,000 r, *Melilotus albus* 15,000 r, *Pisum* sp. 10,000 r.

We have established the following: (a) the amplitude of the doses is narrow for cereals about the dose 15,000 r, but the Leguminosae have a variable radioresistance; (b) the oily plants have the bigest radioresistance; (c) the plant roots are radiosensitive; (d) the polyploid sorts are more radioresistant than those with 2 *n*. The elaboration of a precise method for using the radiations as action factors which condition the change of plant heredity must have as a scientific base the rules of the radioresistance.

SECTION 6

CYTOLOGY

6.1. Heteropycnosis and Sex Chromosomes in Mosses. Lewis E. Anderson (Durham, U.S.A.).

Heterochromosomes have been studied and figured in a large number of species of mosses. Invariably these heterochromosomes are heteropycnotic and are represented in interphase nuclei as conspicuous darkstaining condensed chromosomes that remain in a tightly coiled condition. Heterochromosomes have been postulated to represent sex chromosomes in mosses, although there has been no direct evidence that this is the case. The present paper presents evidence that the heterochromosomes of mosses are sex-associated. The heterochromosomes are represented in interphase nuclei by heteropycnotic bodies of unequal size. In sporophytic tissue, a large and a small heteropycnotic body is distinguishable; in gametophytic tissue of the moss plant, archegonial plants possess nuclei with the large heteropycnotic body while antheridial plants possess nuclei with the smaller heteropycnotic body. It is probable, therefore, that the heterochromosomes represent X and Y chromosomes. Much of the work reported upon was carried out using several species of the genus *Anomodon*.

6.2. A Genetic Test of the Chromosome Replication Pattern in Maize. G. M. Greenblatt (Shorewood, U.S.A.).

Previous studies of twinned red-light variegated mutations in the pericarp of maize (Greenblatt and Brink, 1962, 1963) have shown that Modulator (*Mp*) can transpose from the *P* locus (centrally located on the short arm of chromosome one) at the time *P* and its conjoined *Mp* replicate, and that the receptor sites of *Mp* may be either replicated or unreplicated. In the latter case *Mp* replicates a second time. Each of these two possible events appear as distinguishable twin mutations. An analysis of the sites to which *Mp* transposes from the *P* locus provides a test of the pattern of replication of this chromosome arm. By means of a three-point linkage test the sites to which *Mp* transposes were measured in the light variegated sector in both classes of twin mutations. The results show that the receptor sites of *Mp* located on the short arm of chromosome one, at which a second replication takes place, are not randomly distributed between proximal and distal locations; nine cases of extra replication were at distal sites while only one was at a proximal site. In those cases where *Mp* did not replicate a second time, four were at proximal sites and one was at a distal site (the very end of the chromosome arm). This data strongly supports the hypothesis that the replication pattern on the short arm of chromosome one is highly, but not completely, polarized; the proximal regions replicate prior to the distal regions.

6.3. Bimitosis. G. Gimenez-Martin, A. Gonzalez-Fernandez and J. F. Lopez-Saez (Madrid, Spain).

In this work the mitosis within binucleate cells of onion root-tip is studied. For this synchronous mitosis the denomination of *bimitosis* is proposed. The binucleate cells, an uncommon condition, are a result of a treatment with HCCH (γ Hexa Chloro Cyclo Hexano). The two nuclei can be either both diploid or one hypoploid and the other hyperploid. At the same time, they can be completely independent or united by chromosomic bridges.

Many times, these binucleate cells lack completely the middle lamella between the nuclei. Other times, the cytoplasmic continuity is maintained only through chromosome bridges, existing in incomplete middle lamella.

Always when a connection existed between the two nuclei the two mitoses were synchronous. In this bimitosis both mitoses had either the same axis or perpendicular directions. The two mitoses were synchronous even when one nucleus was hyperploid and the other hypoploid.

The mitotic apparatus showed the same characteristics as in the normal mitosis. The separation between the poles is similar to the normal, although the cytoplasmic space was sometimes wider.

At last, the interactions between both nuclei are discussed.

6.4. Effects of Digitonin on Cellular Division Part I. L. V. Olah (Carbondale, U.S.A.).

A unique, complex effect of digitonin on *Allium sativum*, previously reported by the author, [1] was analyzed in detail. Digitonin disorganizes the spindle, and during nuclear restitution a highly refractive, viscous, hyaline substance appears. This is always closely associated with the restituting nucleus. The chromatin surrounds this substance, entirely trapping it at times. Simultaneously, synthesis of cell wall material (PAS and Iodine-H_2SO_4 positive) originates within the hyaline body, increasing its size and refringency.

Thus, the shape of the restituting nucleus is determined by the location, shape, size, and viscosity of the hyaline body. This mutual association results in indented, crescent-shaped or "hollow" nuclei.

Once polysaccharide synthesis begins, it spreads into the cytoplasm in a haphazard, ramified manner.

In some cases, semi-recovered cells are able to form a normal spindle. However, the hyaline body appears in the same cell, at ana-telophase, associated with the restituting group of chromatids. PAS testing indicated polysaccharide synthesis in this body, which results in a ramified cell wall located near the polar region.

The hypothesis is accepted that the phragmoplast consists of two categories of elements; elements of the interzonal area of the spindle and elements which originate from the polar-nuclear area. These latter elements were observed in the area of the restituting nuclei, and according to Porter *et al.*, [3] ought to migrate to the equator. Association of both these elements at the equator, changes the structure of the spindle, resulting in the appearance of the barrel-shaped phragmoplast.

It appears that digitonin hampers the migration of the "nuclear" elements from the nuclear area. If a spindle is not present, they appear in the area of the restituting tetraploid nucleus, and remain associated with it. In cells able to form spindles, digitonin blocks the migration of the polar-nuclear elements to the equator, and they remain at the polar region, forming a hyaline body associated with the chromatids of the daughter nuclei.

Rosette-crystals, formed *in vivo*, similar to digitonide crystals, were detected in the treated cells. The role of sterols and the effect digitonin may have as an anionic detergent is discussed.

1. Olah, L. V. II. *Reuniao Latino-Americana de Fitogeneticistas e Fitoparasitologistas*, p. 10, 1952. Brazil.
2. Olah, L. V. Proceedings of the IX International Congress of Genetics. Part II. *Caryologia* **VI**, Suppl., pp. 836-838, 1954.
3. Porter, K. R. and Machado, R. D. *J. Biophysical Biochem. Cytology*. **7**, 167-180, 1960.

6.5. Effects of Digitonin on Cellular Division Part II. Electron Microscope Studies. A. G. Underbrink and L. V. Olah (Carbondale, U.S.A.).

Allium sativum root tips were treated with digitonin to study the complex effects described by L. V. Olah.

These root tips were fixed 2 hr in aqueous 2 per cent $KMnO_4$ and treated with 1 per cent uranium acetate for 1 hr.

The process of the copious and ramifying cell wall formation and the development of the hyaline body induced by digitonin were analyzed. However, using this fixative the spindle substance was not observable.

Densely packed circular aggregates of profiles and vesicular-like elements were found which had the size and location of the hyaline bodies seen with the light microscope. In some cells these aggregates were morphologically identical to the elements previously termed "phragmoplast" by Porter *et al.* [1] Some reconstructing nuclei had completely enclosed these aggregates and in these enclosures polysaccharide synthesis was observed.

Most reconstructed nuclei were indented on one side having one or more branches of the ramified cell wall system almost always located in the nuclear indention. Thick and profusely ramifying cell walls, anastomizing haphazardly throughout the cytoplasm were observed with high frequency. Portions of cytoplasm assumed to be completely encased by cell wall and lacking chromatin material appeared to be disintegrating. In other cells the cell wall encircled the nucleus with a portion of cytoplasm, giving the appearance of formation of a cell within the "mother" cell.

Many cells were found in which large quantities of polysaccharide materials occupied large portions of the volume of the cell.

The identity of the aggregated profiles and vesicular-like elements with the digitonin-induced hyaline body described by L. V. Olah and the relationship of these aggregates to the synthesis of the unusually large amounts of polysaccharides is discussed.

1. Porter, K. R. and Machado, R. D. *J. Biophysical Biochem. Cytology*. **7**, 167-180, 1960.

6.6. In Vivo Studies on Mitosis in Neurospora crassa. J. WEIJER, A. KOOPMANS, and D. L. WEIJER (Edmonton, Canada).

In vivo observation of karyokinesis of the somatic nucleus of *Neurospora crassa* reveals three distinct phases of nuclear division. In the DNA Replication Phase the continuous genetic material is oriented along the edge of a disc-like nuclear structure and replicated as a ring. During Division Phase the double stranded filamentous nucleus divides longitudinally.

During Chromosome Phase the divided nucleus is visible as a structure consisting of seven chromosomes attached to one another by thread-like filaments, together with a nucleolus and a centriole.

6.7. The Communicating Pore between the Nucleus and the Vacuole in Saccharomyces cerevisiae. AKIRA YUASA (Tokyo, Japan).

By electron-microscopy it has been confirmed by several investigators that the nucleus of *Saccharomyces cereviseae* has a definite membrane which is composed of two-layers (Yuasa, 1958, 1960; Hashimoto and Gerhardt, 1960; Conti and Naylor, 1960) and also that the chromatin-threads exist in the nucleus (Yuasa, 1958, 1960; Yotsuyanagi, 1959, 1960).

When fixed with 1 per cent aqueous solution of osmic acid and stained with aceto-carmine solution the pore is seen at the contacting place between the nucleus and the vacuole at the end of prophase by light microscopy.

By electron-microscopy the ultrathin section of the cell of *Saccharomyces cereviseae* also often shows a figure in which the communicating pore is seen between the nucleus and the vacuole.

Lindegren (1947, 1947, etc.) already advocated that the nucleus contacted with the vacuole and the chromosome appeared in the latter. The above-mentioned pore is thought to be the communicating site of the nucleus with the vacuole at the end of prophase.

6.8. Observational and Experimental Approaches to Problems of Chromosome Fine Structure. HELEN GAY (Ann Arbor, U.S.A.).

The basic structural component of both the polytenic giant chromosome and the smaller mitotic-type chromosome appears to be a fiber about 100 Å in diameter. According to our interpretation, based on observational and experimental methods, these nucleoprotein microfibrils constitute a multistranded chromosome. The mitotic chromosome is composed of a relatively small number of cytologically separable units, whereas the polytene chromosome is composed of many hundreds. Treatment of living, unfixed, and fixed chromosomes with low concentrations of pronase, trypsin, or chymotrypsin induces a separation of the chromosomal strands so that the subsidiary units can be seen at both the light and electronmicroscopical levels of resolution. Analysis, by cytological and cytogenetical methods, of the effect of deoxyribonuclease and of 5-bromodeoxyuridine in producing chromosomal aberrations reveals that the chromosome responds as a nucleoprotein complex, the breaks in some cases extending across the whole chromosome. This response is similar to that evoked by ionizing radiations. Autoradiographic determinations of the pattern of incorporation of tritiated nucleosides and amino acids, along polytene and mitotictype chromosomes supports the interpretation that DNA in itself does not constitute the main structural component of the chromosomes in higher plants and animals.

This research was supported in part by grants GM-10499 from the Public Health Service, and GB-290 from the National Science Foundation.

6.9. The Karyotype of the Domestic Fowl. E. H. NEWCOMER (Storrs, U.S.A.).

There is now general agreement that the fowl carries twelve well-defined chromosomes in the male and eleven in the female. The female is therefore heterogametic for sex with an XO type of sex determination, the fifth largest chromosome being unpaired.

In addition to these so-called macro-chromosomes, there also occurs a variable number (forty to ninety) of so-called micro-chromosomes which not only vary in number but range in size from macro-chromosomal to the limits of microscopic resolution.

Previous studies from our laboratory have shown the alleged micro-chromosomes to be acentric, variably heterochromatic, prone to fusion and fragmentation and that they disappear during the second meiotic division of spermatogenesis. For these reasons they were removed from the category of chromosomes and named chromosomoids.

Recent studies by an improved technique of blood leucocyte culture and autoradiography

have revealed asynchrony in DNA synthesis between the chromosomes and chromosomoids, thus enhancing the grounds for their separation.

Photographs of intact leucocytes at metaphase show a range in number of the chromosomoids from sixty to ninety. Karyotype preparations of these cells confirm previous observations that the chromosomoids are acentric, variably heterochromatic, morphologically inconstant and as such do not fulfil any definition of a chromosome. These observations together with their DNA asynchrony indicate that they constitute an extension to the spectrum of known chromatic entities in the nucleus. Their genetic properties can only be surmised at present.

Full details will be published in the *Int. Rev. Cyt.*

6.10. Cytophotometric Studies on the Spermatogonia of Nepa cinerea (Hemiptera). OLLI HALKKA (Helsinki, Finland).

In the water scorpion, *Nepa cinerea*, the goniomitotic divisions proceed strictly synchronously in each cyst. The cyst is the smallest nutritional unit of the testis. At the 8-cell stage, the mean nuclear volume is about 430 μ^3 but only about 130 μ^3 at the 128-cell (last gonial) stage. The corresponding cellular volumes are about 1070 μ^3 and 340 μ^3, respectively. At the 8-cell stage, each cell has about 250 μ^3 of cyst surface area for its metabolism to use while at the 128-cell stage this area has decreased to about 45 μ^3. The nutritional conditions of cells in a 8-cell cyst may be quite different from those of cells in a cyst approaching meiosis.

The concentrations of the main chemical components of the cell were determined photometrically at different stages of testis development. The amount of DNA (Feulgen dye —DNAcomplex) was found to be the same in 8-cell ($\bar{x} = 10.35$ arbitrary units at metaphase measured with the two-wavelength method) and 64-cell ($\bar{x} = 9.87$) cyst nuclei as well as at first maturation division metaphase ($\bar{x} = 9.30$). Thus, the smaller size of the chromosomes at later gonial generations is due to increased "condensation" and not to a change in the degree of polyteny.

During gonial development, no statistically significant change in the concentration of arginine (the McLeish modification of the Sakaguchi test) in the cytoplasm was found. The same is true of the tyrosine and tryptophane containing proteins (Millon reaction). The ratio DNA/cytoplasmic protein therefore was higher at the 64-cell stage than at the 8-cell stage. Highest interphase concentrations of RNA (Azure B) were observed at the 8-stage.

6.11. The Formation of Chromosomal Axis in Mitosis and the Function of Nucleolus. GONPACHIRO YASUZUMI (Kashihara, Japan).

The present communication is concerned with electron microscope studies on prophase chromosomes of Ehrlich ascites tumour cells and *Pelomyxa carolinensis*, and on nucleoli of spermatogonia and spermatocytes of *Ascaris megalocephala*. Ehrlich ascites tumour cells, amoebae, and testes of ascaris were fixed in formalin, potassium permanganate or osmium tetroxide, buffered to pH 7.4-7.8 with Michaelis veronal-acetate buffer. After fixation, they were dehydrated with increasing concentrations of ethyl alcohol, and embedded in Epon epoxy resin or a mixture of methyl- and n-butyl methacrylates. Sections were cut with the Porter-Blum microtome equipped with a glass knife. DNase, RNase and trypsin were used singly and in combination to treat formalin-fixed material in block or in section. The sections were stained with a slightly modified method of Watson's lead acetate or uranyl acetate. They were examined with an electron microscope of the Japan Electron Company, model JEM-6C, or the Akashi electron microscope, model TRS, 50E 1.

The mitotic early prophase chromosome develops from the nucleolus towards the nuclear envelope and finally comes to a close association with the nuclear envelope. An axis has been found in the early prophase chromosome, consisting of a pair of tubular elements. In more advanced stages of prophase the axis is provided with symmetrically disposed processes. In the nuclei of spermatogonia and spermatacytes in *Ascaris megalocephala* at least two nucleoli are found, one of which is composed of numerous dense granules of varying size an shape, each granule consisting of an aggregation of dense particles 110 to 150 Å in size, but the other is almost compact. The dense granules are gradually separated to the composed particles which eventually appear through the pores of nuclear envelope into the cytoplasm. Thus the nucleolus seems to have two functions: the reorganization of chromosomes in mitosis and the formation or storage organ of ribonucleoprotein.

6.12. Effect of Mono- and Divalent Ions on the Structure of Isolated Metaphase Chromosomes. CAROLYN E. SOMERS (Houston, U.S.A.).

Recently, methods have been described for the mass isolation of metaphase chromosomes. With the isolation of metaphase chromosomes,

rather than interphase chromosomes, preservation of chromosome morphology can be used as a significant criterion in the evaluation of the techniques used. Chinese hamster cells grown *in vitro* provide an excellent source of material for chromosome isolation since 30 to 50 per cent of the cells can be accumulated at metaphase with a 12-hr colcemid treatment. Treatment of metaphase cells for 3 min with 0.1 M sucrose containing 7×10^{-4}M $CaCl_2$ and 3×10^{-4}M $MgCl_2$ serves to hydrate cells sufficiently for chromosome spreading while maintaining a minimal degree of chromosomal hydration. Following this hypotonic treatment, cells can be disrupted in 0.4 M sucrose containing 5×10^{-4} M each of $CaCl_2$ and $MgCl_2$ by drawing the cell suspension into a 30 ml syringe and expelling it through a 15 gauge hypodermic needle. Divalent ions are required to preserve chromosome morphology when chromosomes are isolated in sucrose. In order to test the effects of ions on metaphase chromosomes, a drop of 0.4 M sucrose (5×10^{-4}M $CaCl_2$ and $MgCl_2$) containing isolated chromosomes was placed on a slide and covered with a cover slip. Chromosomes were perfused with 0.4 M sucrose containing desired test ions. Changes in chromosome morphology were observed with phase contrast optics. Chromosomes will swell or uncoil if divalent ions are replaced by monovalent ions, e.g. NaCl. Uncoiling can be reversed by subsequent addition of divalent ions. If extensive swelling or uncoiling has occurred, the metaphase configuration is destroyed, and the divalent ions promote a contraction to the chromonema level only.

6.13. Chromosomes of Normal and Tumour Epithelioid Derivatives (2n=22) of the Chinese Hamster. GEORGE YERGANIAN (Boston, U.S.A.).

Normal adult epithelia derived from various organs of the Chinese hamster, *Cricetulus griseus*, fail to proliferate *in vitro* beyond several passages. In contrast, "spontaneous" carcinomatous derivatives proliferate extensively and have presently exceeded the 45th passage while averaging 18 hr generation times. In addition to the retention of classic diploidy, tumor derivatives lack contact inhibition, display a metaphase: anaphase ratio of 10 : 1, and an average single cell plating efficiency of 20 per cent.

Eighty per cent or more of the metaphases are classic diploid, and some 5 per cent each are hypodiploid, hyperdiploid, and tetraploid. Equally stable are clonal derivatives (X_10 quasidiploid + trans I: III, and $2n$ + Iso LAIII). Each clonal subline exhibits varying degrees of cytopathic effect (CPE) caused by an adenovirus (type undetermined) isolated from one of five original primary sublines.

Of possible significance is the slight morphological variation in the short arm of the heterochromatic X in a good portion of normal and tumor epithelioid forms. In the majority of fibroblast-like derivatives, this heterochromatic segment or X_2 is slenderer and undergoes late DNA synthesis. In epithelioids, this portion appears intermediate in dimensions or X_{1-2}. If this morphological feature is substantiated by a corresponding intermediate type of tritiated thymidine uptake pattern, epithelioid derivatives would then have an X_1X_{1-2} status, whereas fibroblast-like cells feature the more differentiated or X_1X_2 status.

These observations suggest that some chromosomal segments may reflect patterns of nuclear and cellular differentiation, possess intermediate levels of heterochromatization (gene expression), and govern rates of CPE caused by viruses.

6.14. The Chromosomes of A- and B-spermatogonia of the Mouse. EVA HANSEN-MELANDER (Lund, Sweden).

In the tubules of the mouse two types of spermatogonial cells are found, the A- and B-spermatogonia, which differ considerably in their chromosomal behaviour. The chromosomes of the A-spermatogonia resemble from prophase to telophase the chromosomes of any other dividing somatic cell. The A-spermatogonia give rise to new-A-spermatogonia or to B-spermatogonia.

From the onset of prophase the chromosomes of the B-spermatogonia behave differently from those of the A-spermatogonia. The differences are visible already during prophase and become striking during full metaphase. When the division of the B-spermatogonia is over the cells enter meiosis.

The heteropycnosis of the sex chromosomes, particularly of the Y, is conspicuous during prophase in both kinds of spermatogonia.

6.15. Origin of Sex Chromatin and Related Phenomena. YNGVE MELANDER (Lund, Sweden).

The sex chromatin of the female rabbit originates during early embryogenesis. The long arm of one X chromosome becomes positively heteropycnotic during interphase as a result of a

heavy attenuation during anaphase. The stretching is due to a delayed separation of the daughter chromosomes in a terminally located segment. The formation of sex chromatin in mammals is not an isolated occurrence as similar events cause cell differentiation at embryogenesis of other animals. Thus, in planarians (*Paludicola*, *Tricladida*) chromosome segments of the blastomeres become allocyclic, forming chromocenters during interphase, after an attenuation during anaphase. Similarly, more or less long-lasting chromocenters originate in the blastodermic cells of a higher fly (*Calliphora erythrocephala*). Another result of chromatid attenuation in planarians and *Calliphora* is diminution of small chromosome fragments, also obviously differentiating the somatic cells.

All these events are initiated by a delayed separation of daughter chromosomes during anaphase of embryonic cells as is also the well known elimination of chromosomes in lower dipterans (Nematocera).

These observations will be published in full in *Hereditas* during 1963.

6.16. The Pattern of DNA Synthesis in the Chromosomes of the Marsupial Potorous tridactylis[1]. KIRSTEN H. WALEN (Berkeley, U.S.A.).

The marsupial, *Potorous tridactylis*, has 12 chromosomes in the female, and 13 in the male (XY_1Y_2). The chromosomes are large and all pairs are morphologically distinguishable. Kidney and testis cells can be cultured indefinitely and the cell population remains largely diploid. Tritiated thymidine labeling trials have shown the mitotic cycle to be about 32 hr from metaphase to metaphase. Early sampling of labeled female material showed that silver grains were present over all the chromosomes except the short arm of one X chromosome. The same procedure with male kidney cells resulted in the same type of asynchronous uptake of triated thymidine into the single X chromosome. In general both chromatids were labeled in the first metaphase after exposure to tritiated thymidine, whereas only one chromatid was labeled in second- and third-cycle metaphase figures. Frequently, however, it was observed that the labeling switched from one chromatid to the other. No open chromatid breaks or gross chromosomal aberrations were observed in any of the division cycles.

Sponsored by the Office of Naval Research under the terms of a contract with the Regents of the University of California.

6.17. The Ciliate Macronucleus. B. R. SESHACHAR (Dehli, India).

The electron microscope has revealed, in the macronucleus of two Spirotrichous ciliates, *Blepharisma* and *Spirostomum*, a system of greatly elongated filaments disposed inside it in a random manner. The thickness of the filaments is variable and each individual filament shows varying thickness along its length. Sections of the filament resemble very strikingly those of the chromosomes of higher organisms. They are interpreted as chromosomes of the macronucleus. Each filament is made up of many microfibrils about 150 Å thick, also disposed in a random manner but with a slight orientation in the long axis of the filament. Earlier findings of the author show that on centrifugation and cyanide action, the macronucleus of these two ciliates yield long Feulgen positive filaments, corroborating the observations with the electron microscope.

These greatly elongated chromosomal filaments would have to be interpreted in the light of the special attributes of the ciliate macronucleus: its origin and differentiation from a diploid micronuclear product, its large size (believed to be due either to polyploidy or to its compound nature), its capacity for growth and regeneration, its high DNA content, its amitotic division and its genic and metabolic functions. It would also have to be integrated with our knowledge of DNA structure and mitotic chromosome generally.

6.18. Behaviour of Nucleoli in Isolated Nuclei. L. F. LACOUR (Hertford, Great Britain).

Nuclei have been isolated from HeLa cells without appreciable loss of nucleic acids (Fisher and Harris, 1962). In isotonic solutions the isolated nuclei swell and the nucleoli disappear but reappear when the nuclei recover normal size in buffered saline. No nucleolar material can be detected in swollen nuclei in the electron microscope (Crawley and Harris, 1963). This behaviour is being investigated by labelling of the nucleolar RNA with ^{3}H-cytidine before isolation of the nuclei.

6.19. The Variable Characters of Chromosomes. ATIF SENGÜN (Istanbul, Turkey).

The chromosomes carry the hereditary units, the genes, and are responsible for the continuity of life and of the characters of an organism. Therefore, it has been accepted for many years that the chromosomes should have a static organization represented by a thread with a definite number of chromomeres, varying in size and which occupy a special place on the chromonema. But a comparison of the homologous chromosomes shows a variation of chromosome morphology, e.g. in corresponding phases of mitotic and meiotic divisions or in different tissues of the same animal, etc. The morphological or structural differences of the homologous chromosomes described in relevant literature are: (a) the degree of spiralization, (b) the length and (c) the thickness (the diameter) of the chromosome, (d) the number and the localization of differentiated, specific, regions such as the heterochromatic parts, the puffs and the macrochromomeres, etc., (e) the amount of the non-hereditary substances including the metabolic DNA and RNA. All these variable characters together form the investigated and visible chromosome.

A survey of the literature reveals: (1) the above-mentioned characters are controlled by internal and external factors—including the microenviromental ones, (2) an alteration of a character of the chromosome is correlated with certain, even with definite physiological variation of chromosome, (3) Some substances are formed and given off into the surrounding medium by the chromosome, (4) certain structures of the chromosomes are responsible for the formation of these non-genetical, temporary substances. Therefore, it may be suggested that the visible structure or the morphology of the chromosomes represent the phenotype of a chromosome which is merely a product of non-visible internal and external factors. According to the above-mentioned conclusion the question "What is the genotype of a chromosome?" is still obscure.

6.20. Heterocyclicity of the System of Cell Nucleus. A. PROKOFIEVA BELGOVSKAYA (Moscow, U.S.S.R.).

The study of the behaviour of the parent chromosomes of the nuclei in *Drosophila melanogaster* salivary gland cells has shown that in a number of cases lack of conjugation of homologous regions is caused by a difference of the cyclic state of the chromosomes or their corresponding regions. Heterocyclicity of chromosome regions becomes increased in the cases of rearrangements. Distal regions IABI brought near to heterochromatic region 20A in the X-chromosome of homozygous female sc^8 differ by their states and do not conjugate in 27 per cent of the nuclei.

The study of the cell nucleus on early stages of development of *Salmo salar*, *Corregonus baeri* and *Cyclops serrulatus* showed that parent chromosomes till to the stage of middle gastrula remain isolated and up to the late blastula differ from each other in many respects, such as: character of spiralization of prophase chromosomes, time of their mitotic reorganizations and sometimes the intensity of the nucleal reaction. During prophase the parent chromosomes form two separate skeins of threads oriented towards opposite cell poles. Parent metaphase chromosomes in many blastomeres are oriented in different plates. In late blastulae parent chromosomes distinctly differ in their ability to form the material of nucleolus.

While investigating chromosomes of irradiated embryos of *Salmo salar* and *Misgurnus fossilis* (X-ray, 800r, stage of 8 blastomeres) differential damage of parent chromosome complexes could be stated. This could be connected with a difference of their cyclic states at the time of irradiation.

Heterocyclicity of parent chromosome sets is clearly expressed in a primary culture of human embryonic fibroblasts. It leads to the formation in a number of cells at various stages of mitosis of separate chromosome complexes. With the aid of thymidin - H^3 and autoradiography it could be shown that homologous parent chromosomes differ in relation to the reduplication cycle of their DNA as well. This peculiarity is highly characteristic for the homologues of the X-chromosome pair, and slightly expressed for some autosomes in the primary culture of embryonic fibroblasts and leucocyte cultures of periferal blood. So far as observations show the high grade of heterocyclicity of parent chromosomes present during early stages drops down in the succession of cell generations. It is possible that the levelling of parent chromosome-cycles presents one of the factors of chromosome conjugation and transformation of mitosis into meiosis.

The data obtained confirm the assumption of former cytologists concerning prolonged independency of parent chromosome sets (Häcker, 1895; Amma, 1911). These data are considered in the light of the hypothesis offered by the author in 1948, according to which heterocyclicity of the system of the cell nucleus is evolutionary fixed by the peculiarities of the

development of gamete nuclei and plays a fundamental role as a factor constituting the high metabolic properties of the cell.

6.21. Natural and Experimental Modification of Chromocenters in Interphase Nuclei of Hymenolepis diminuta. L. T. DOUGLAS (Baltimore, U.S.A.).

The distribution and size of DNA-positive centers (i.e. chromocenters) within interphase nuclei of *Hymenolepis diminuta* appears to be a function of the tissue in which the nuclei are located. Chromocenters of nuclei in early blastomeres and embryonic tissues are numerous and small while those in fully differentiated tissues tend to be larger and less numerous. Intranuclear contraction centers accentuated in freshly isolated nuclei by 10^{-2}M arginine correspond morphologically to foci which are positive for DNA and basic nucleoprotein in fixed material.

It is tempting to conjecture that variation in distribution and size of intranuclear chromocenters in different tissues has physiological significance; and, for example, it might be that the imposition of a confining microscopic geometry on specific regions of a submicroscopic component (e.g. DNA double helices) would alter the phenotypic expression of a genome in such a way as to provide, via coding, for different cytoplasmic and nuclear enzymes in different tissues.

6.22. Functional Changes in the Polytene Chromosomes of Drosophila melanogaster. B. M. SLIZYNSKI (Edinburgh, Great Britain).

A recessive autosomal mutant gene in *Drosophila melanogaster* located in the second chromosome at locus 12.0, called "fat" symbol ft, produces besides its described effects (short abdomen, thorax and wings with some abnormalities in the morphology of the posterior crossvein) also numerous vacuolae in the cells of salivary gland. The vacuolae are formed at the end of the second instar. The formation appears to proceed in a wave starting from the distal end of the gland. Later the vacuolae disappear and still later new vacuolae are being formed. The formation of vacuolae is accompanied by varying degree of puff formation in the band or bands corresponding to the cytological location of the gene, namely in the subdivision 24D/E of the cytological map of salivary gland chromosome. The neck cells of the gland do not form any vacuolae and their chromosomes do not show any evidence of puff formation in the region.

6.23. Pulse Labeling Studies of Nucleoprotein Synthesis on Drosophila Polytene Chromosomes. W. PLAUT (Madison, U.S.A.).

Drosophila salivary glands excised from advanced larvae have been incubated in modified Ringer's medium with tritiated thymidine, lysine, uridine, and cytidine. Results to date indicate that autoradiographically detectable chromosomal labeling can be obtained in $2\frac{1}{2}$ min of incubation. The labeling patterns resulting from brief incubations in DNA, RNA, and protein precursors are strikingly different. DNA labeling, in particular, is frequently discontinuous along the chromosome. The possible significance of these synthesis patterns relative to chromosomal structure and function will be discussed.

6.24. Variability of RNA Synthesis in Polytene Tissues. CLAUS PELLING (Tübingen, Germany).

Investigations of RNA synthesis in salivary gland chromosomes of *Chironomus* larvae have demonstrated that the rate of uridine H^3 incorporation into nuclear structures is highly variable.

Larvae of the same batch, taken at approximately the same time under the same experimental conditions, may sometimes differ by more than 3 orders of magnitude in the amount of incorporated uridine determined by grain counts of a given nuclear structure (Balbiani Ring II).

Different batches, grown up in different culture vessels, may also differ in the overall level of uridine uptake.

Salivary glands are not the only polytene tissue showing variation in RNA synthesis. Autoradiographs of the anterior part of the intestine, the malpighian tubules, and the rectum also reveal large alterations in uridine incorporation from animal to animal. Activities in different tissues vary independently. Striking differences have also been observed between cells within the same tissue. A mosaic-like pattern of nuclear uridine incorporation is typical for some areas of the intestine, whereas activity gradients seem to occur along the malpighian tubules.

In contrast to the polytene tissues, embryonic diploid nuclei seem to incorporate at a more or less constant rate.

All observations point to the conclusion that

variation of RNA synthesis is a characteristic feature of polytene cells of *Chironomus*. Attempts to control the rhythm of synthesis in salivary glands by modifying light and temperature conditions have so far failed.

6.25. Localization of Deoxyribonucleic Acid exclusively in the Bands of Drosophila Salivary Chromosomes. DALE M. STEFFENSEN (Urbana, U.S.A.).

Drosophila salivary chromosomes were labeled with H^3-thymidine to study the distribution of radioactive DNA by quantitative autoradiography. Stretched salivary chromosomes were examined for the distribution of silver grains (radioactivity) produced from tritium over bands and interbands. The evidence leaves little doubt that DNA is primarily if not exclusively in the bands. On the other hand the interbands would appear to contain no more radioactivity than background and therefore little or no DNA.

A variety of physical considerations were examined and it was calculated that DNA must be only in the bands and probably absent in interbands. The theoretical evaluation showed that radioactivity would have been detectable if DNA were in interbands. The presence of DNA in interbands proved to be highly unlikely. All in all it would appear that Bridges was right all along in his hypothesis that genes (DNA) are located only in the bands.

As might be expected there was intense radioactivity from heterochromatic regions indicating a high concentration of DNA. Regions with few bands and "puffs" exhibited little radioactivity. In some puffs, DNA may be absent altogether. The temporal inactivity of "heterochromatin" is discussed in light of recent evidence about localized RNA synthesis and chromosome differentation.

This study was supported by the National Science Foundation (NSF G 17600).

6.26. Structure and Function of the Y-heterochromatin in Drosophila. G. F. MEYER and O. HESS (Tübingen, Germany).

In spermatocyte nuclei of *Drosophila* structural modifications of the Y-chromosome occur which have been shown to be homologous to the loops of lampbrush chromosomes. Several pairs of such structures can be distinguished on a morphological basis (e.g. in *D. hydei*, "threads", "clubs", "pseudonucleolus" and "tubuli"). By analogy with lampbrush loops these structures have been considered as regions of the Y-heterochromatin whose activity is increased enormously. This can now be directly demonstrated by autoradiography and by experiments using actinomycin C. Evidence for the genetic functions of loop-forming loci comes from a study of the correlation between the involved regions of the Y-chromosome and the length of spermatozoa. Spermatozoal length varies between and within species according to the total mass, the degree of differentiation, and the number of the individual types of Y-chromosomal loops. It seems that almost any alteration in the quantity of Y-heterochromatin results in changes of the sperm length. In a X-ray induced Y-autosome translocation in *D. hydei* the two translocated fragments of the Y-chromosome were found to carry two loop-forming loci each. In the translocation stock males occur which are deficient for one or the other Y fragment and which, correspondingly, lack two of the four loop pairs. These Y-deficient males produce immotile sperm which are shorter than normal sperm. Males with partial or complete duplications of loops are also found and show the expected increase in sperm length. The chromosomal modifications described here are unique in that they are formed only by the (heterochromatic) Y-chromosome.

6.27. Amounts of DNA and RNA in the Chromosomal and Nucleolar Regions of Isolated Plant Nuclei. JOHN MCLEISH (Bayfordbury, Great Britain).

Amounts of both DNA and RNA were determined in nuclei isolated from the root apices of different species of higher plants. A new method of isolation was used [1] by which approximately 50 per cent of the nuclei known to be present in the roots could be recovered. There was no significant loss of nucleic acids from these nuclei during the isolation procedure. The plants chosen included both closely and distantly related species; a polyploid series within one genus; and the diploid and tetraploid forms of a single species. The results demonstrated the absence of any correlation between the amounts of DNA and total nuclear RNA; the high DNA: RNA ratios found in most species suggested that, at any given time, a lot of the DNA is not associated with RNA.

A new technique for the isolation of nucleoli from these nuclei has now made it possible to estimate the amounts of DNA and RNA in both the chromosomal and nucleolar regions. Although it can be stated that there is no correlation

between the amounts of DNA and RNA in the chromosomal regions, the precise quantitative relationship between DNA and RNA in the nucleoli themselves is still under investigation.

1. McLeish, J. (1963) *Proc. Roy. Soc. B* (in the press).

6.28. Chiasma Distribution at Normal and Elevated Temperatures in a Locust. S. A. Henderson (Cambridge, Great Britain).

Using the technique of directly measuring chiasma position along each chromosome, an analysis of the normal chiasma distribution has been made for all members of the complement of the desert locust, *Schistocerca gregaria*, where a wide range of chromosome size is present. On subjecting this species to high temperature treatment the pattern of chiasma distribution is greatly modified. Reduction in chiasma frequency is accompanied by a change in the position occupied by those chiasmata which do form. This shift, to the chromosome ends, may culminate in univalence. Chromosomes of different sizes differ in their responses to heat-treatment.

6.29. Some New Principles Governing Chromosome Pairing—The Spatial Relations of Chromosomes and Nuclear Membrane. K. Pusa (Helsinki, Finland).

It has been shown by others that in many instances in the soma the chromosomes attach very firmly to the nuclear membrane (NM) by their (1) heteropycnotic regions or (2) proximal or (3) distal parts. This has been demonstrated by micromanipulation or ultracentrifugation which thereby produces characteristic configurations. There are many observations on mere attachment by direct inspection of parts (2) and (3) and the situation of (1) is well known. The questions are: Can those findings be generalized? How is it just before and at chromosome pairing?

A study was undertaken on a number of flowering plants, mainly Cruciferae (conspicuous heteropycnosis at centromeric regions), Tradescantia, Liliaceae and Plantago as well as scattered samples from various families. Centrifugation, both normal and ultraspeeds (up to 150,000 *g*) were employed. In all cases, in the archespore and early PMC's before pairing the situation is the same as in the surrounding parenchyma: very typical configurations ensue whereby the tips and often also the proximal regions attach, the more movable parts being strongly displaced in the centrifugal direction. The more indifferent parts can be displaced at very low speeds, e.g. 1000 *g*, especially in interphase and early prophase, less well in later prophase when condensing chromosomes become rigid but in prometaphase they are deposited *in toto*. Breaks of chromosomes with tips still anchored are found and also indentations in the NM at points where tips, especially many of them, attach. In most plants studied there is no bouquet orientation. Just prior to pairing the chromosomes detach themselves and then pair immediately. In early pachytene the "standard" situation is resumed, i.e. reattachment. The bouquet of Tradescantia and Liliaceae is complicated: the chromosomes behave as if they were totally loose. Yet, there seems to be a very weak bond—they seem to glide towards the bouquet arrangement as has been observed by others before. Pachytene shows attachment of the tips. Condensed chromosomes in diakinesis are peripheral *in toto*, as is well known. The attachment is firm in pachytene but in most cases will become strikingly labilized at diplotene though they seldom detach themselves. Nonhomologous associations, if present, to some extent interfere with the pachytene reattachment.

6.30. Chromosome Pairing at Meiosis. C. R. Burnham (St. Paul, U.S.A.).

Previous studies (M. Tabata in maize and K. J. Kasha in barley) indicate that pairing is initiated at or near the ends of chromosomes and not at the centromeres. This information has come from hybrids between stocks of interchanges in which the breaks in both chromosomes were in opposite arms in the two parental interchanges. In the previous studies in maize the total length of the "between-breaks" segments was equal to or less than the total length of the interchanged segments. In the combinations now planned the total length of the "between-breaks" segments will be greater than that of the interchanged segments.

Observations (O. L. Miller) on asynaptic (*as as*) plants in maize show that the members of partially synapsed pairs are associated at the centromeres. The two seemingly divergent observations can be reconciled if it assumed that in *as as* maize plants pairing at the ends of the chromosomes is prevented, but that this may or may not extend to the remainder of the two homologues.

6.31. Differential Rates of Meiotic Development in Desynaptic Hordeum vulgare L. E. B. WAGENAAR (Ottawa, Canada).

Cytological analysis of the first meiotic division of desynaptic barley (var. Huskey, $2n = 14$; homozygous for gene *ds*) produced evidence that the developmental rate was not equal for all metaphase cells. This differential rate was apparently related to two cellular characteristics which simultaneously affected metaphase development. These characteristics were the degree of irregularity and the chiasma frequencies per bivalent. Cells with numbers of bivalents approaching that of the normal condition (7 bivalents), as well as cells with highest average chiasma frequencies per bivalent, tended to proceed most rapidly through metaphase 1. Possible underlying factors inducing the observed developmental pattern are discussed.

6.32. Cytogenetical Studies of Oryza sativa L. and its Related Species. 5. Differential Condensation and Chromosome Pairing in the Hybrid O. sativa × O. australiensis. H. W. LI, C. C. CHEN, H. K. WU and KATHERINE C. L. LU (Taipei, China).

The chromosomes of *O. australiensis* were made up partly of heterochromatin whereas those of *O. sativa* were almost completely euchromatic.

In the F_1 hybrid of *O. sativa O. australiensis*, there was differential condensation in these two morphologically different types of chromosomes. The ones with partly heterochromatin and partly euchromatin condensed early, starting off presumably from pachynema, on till diakinesis. The ones with only euchromatin seemingly started their condensation later but condensed more complete at first metaphase. Thus before diakinesis, the *australiensis* chromosomes were darker in staining, while at MI-AI they were 2-4 times the size of the *sativa* chromosomes.

At either diakinesis or MI-AI, about two bivalents could be found per PMC. These bivalents could be separated into two types, at MI-AI by size, or by difference in having taken up the stain at diakinesis:

1. Autosyndetic.
2. Allosyndetic (multivalents were also found but very rarely).

All these bivalents were proved to be authentically true bivalents. The evidences were:

1. There were loosely paired chromosomal segments observed repeatedly in many PMC's at pachynema.
2. At diplonema, allosyndetically paired bivalents were found to have one chiasma mostly, or two or more chiasmata in some PMC's.
3. At diakinesis, these allosyndetically paired bivalents were found to be ring-shaped as well as end-to-end ones.
4. Closed allosyndetic bivalents with two chiasmata were frequently observed at MI-AI.

Pairing of the homologous segments in these allosyndetic pairs was assumed to be carried out at the euchromatic regions of the two chromosomes concerned. Presumably, these euchromatic regions of the chromosomes from two different species might have the same rate of condensation at various stages of meiosis.

6.33. Meiosis of Luzula purpurea. H. NORDENSKIÖLD (Uppsala, Sweden).

Luzula purpurea belongs to the materials, the chromosomes of which have diffuse or polycentric centromeres. Somatically it has six equal-sized chromosomes. During early prophase the chromosomes pair and chiasmata are formed. The chiasmata are easily studied during diakinesis, and one or two per bivalent is the most common number. They may be interstitial or more or less terminalized at first metaphase. During that stage each chromosome of the bivalents is arranged in the equatorial plane, i.e. is showing auto-orientation.

The stage at which the individual chromatids can best be studied is the transition between first anaphase and telophase. At this stage the two halves of each side of the equatorial plane always form mirror-images when compared with each other. This phenomenon illustrates the cytologically equational mode of the first meiotic division. Relict chiasmata may occur between the chromatids of the half-bivalents. This phenomenon demonstrates the fact that the two chiasma-forming chromatids are able to move towards the same pole. As chiasmata are generally assumed to be formed between non-sister chromatids the separation of chromatids in the points of the original exchange in such cases ought to have occurred between the sister-chromatids. The separating half-bivalents are composed of two chromatids which may be kept together by the relict chiasmata or their cast formations, and/or by the ends of the chromatids belonging to the same original chromosome, the terminal parts of which seem to separate at a comparatively late stage in meiosis. At interkinesis the homologous chromatids of the half-bivalents pair again and these pairs form two very

contracted metaphasic plates before the separation during second division.

To prove the validity of the described type of meiosis in a material with a heteromorphic bivalent, the meiosis of plants with one broken chromosome have been studied. One heteromorphic association is then formed in each meiotic cell, originating from the pairing between the two fragments and their homologous unbroken partner. At first metaphase this association is always an open chain with the large chromosome in the middle of the association and the two small ones on each side. This association separates equationally during first anaphase giving one long and two short chromatids on each side of the equator. At interkinesis the two fragments pair again with their long partner, the result of which can be studied at the end of the separation during second telophase. In the four cells of the tetrad the two small chromosomes regularly substitute the originally broken chromosome, giving rise to four cells, two with three large chromosomes and two with two large and two small ones.

6.34. Meiosis in the Sex Organs of the Biflagellatae. E. SANSOME (Zaria, North Nigeria).

Critical cytological evidence showing that meiosis occurs in the oogonia and antheridia is given for *Pythium debaryanum*, *Phytophthora cactorum* and an *Achlya* sp. In the case of *Pythium debaryanum* ($n = 18$ *ca.*) an association of four chromosomes was observed in metaphases in the sex organs. In the case of *Phytophthora cactorum* ($n = 9$ *ca.*) and *Achlya* ($n = 8$ *ca.*) multivalents were observed in polyploid nuclei in the oogonia and antheridia after treatment with natural camphor. Additional evidence in the case of *Phytophthora cactorum* was the observance of a bridge and fragment at anaphase, evidently resulting from crossing over in an inversion.

The fact that two successive divisions occur and that the size of the nuclei at the end of the two divisions is approximately half that of the vegetative nuclei is in full accordance with the other cytological evidence in indicating that these two divisions constitute meiosis.

The finding that meiosis occurs immediately before and not after fertilization has important genetical implications. A survey of the scanty genetical data in this group shows that they can be more readily explained on the basis of the organisms being diploid rather than haploid. Further genetical investigations should be facilitated by the knowledge that the organisms are diploid as shown by the cytological evidence, and not haploid as previously assumed.

See SANSOME, E., *Nature*, **191**, 827 (1961); SANSOME, E., and HARRIS, B. J., *Nature*, **196**, 29-292 (1962); SANSOME, E., *Trans. Brit. Myc. Soc.* in the press.

6.35. Which are the Specific Biocatalizers for the Multiplication of Chromonemata and, consequently, of the Genes or of the DNA Molecules in Higher Plants? F. RESENDE (Lisbon, Portugal).

Growth in a pluricellular plant is the result of the growth of its constituent cells. This growth is till now considered as of 2 types: (a) cell multiplication and (b) membrane distension.

In some species of the succulent genera *Bryophyllum* and *Kalanchoë*, growth is extremely sensitive to photoperiodism, being much greater under LD (long day) conditions than in SD (short day). This LD effect results from a much enhanced growth of the stem internodes, of petiole length and of leaf area. The amount of growth biocatalizers is consequently greater in LD than in SD, IAA (3-indolyl-acetic-acid) concentration, for instance, may be 20 times greater (Linskens and Resende).

In plants with arrested growth (i.e. in rosette) through SD action, *nuclei are*, however, *larger than in plants under LD* and show endopolyploidy, which may reach 32×, whereas under LD nuclei never show more than 8× (Von Witsch and Flügel).

It all seems to function as if biocatalysis governing cell multiplication and cell membrane distension does not affect the growth and the multiplication of the chromonemata and, hence, the synthesis of genem aterial, DNA, and protein. This synthesis in LD is at most equal (probably even inferior) to that in SD, and therefore not related to the general plant growth biocatalizers known up to the present. Hence, it is logical to postulate the existence of *specific biocatalizers for the growth and multiplication of the chromonemata, and which are independent from those governing multiplication of the nucleus and cell and the distension of its membrane*. This hypothesis further explains the cases of ectomitosis (Resende), i.e. nucleus multiplications without multiplication of chromonemata.

6.36. Functional Structures in Cytoplasm and Chromosomes of D. hydei Salivary Gland Cells. H. D. Berendes (Leiden, The Netherlands).

Each salivary gland of *D. hydei* contains 100-150 cells. Larval development at 25°C under optimal food conditions is completed in 121 hours, both first and second instar lasting 25 hours and the third instar 71 hours. The development of the gland was observed using phase contrast microscopy of whole glands mounted in 0.7 per cent saline at various stages of all instars, and sectioned as well as squashed glands at stages of the third instar and prepupa. Nuclear diameters increase from 4.5 μ at hatching, to 55 μ. About 20 hours after beginning of the third instar, a differentiation can be seen between proximal and distal part of the glands. First sign of this differentiation was a faster enlargement of the distal nuclei, in connection with a higher polyteny of the chromosomes. The cytoplasm of these distal cells then produces a large number of granules with diameters of 1-2 μ. These granules consist of PAS positive material and are not stained by toluidine blue. At the end of the third instar these granules disappear again. At this moment the nuclei of the distal cells have a diameter of 45-55 μ whereas the proximal nuclei have a diameter of 28-35 μ. During the third instar the cells of the proximal part never contain PAS positive granules, but much smaller particles instead. These particles are positively stained by toluidine blue. They are also present in the distal cells during the third instar.

The puffing pattern within the five chromosome arms has been studied in relation to the development of the cells in proximal and distal part of the gland during the third instar. Differences in puffing pattern between both parts have been found.

6.37. A Technique for Obtaining Chromosome Preparations of Rat Embryo. M. T. Biola (Fontenay-aux-Roses, France).

During the examination of chromosome aberrations of the rat embryo after irradiation of the mother, we have determined a technique for obtaining chromosome preparations without tissue culture.

With this technique we can examine the chromosomes of the cells which were in mitosis when the animal was killed and observe the real anomalies of the embryonic cells.

Embryos are taken, minced into small fragments which are digested by the combined action of both trypsin and hyaluronidase.

After 45 min at 37°C, serum is added to inhibit the enzymes.

The undigested residues are allowed to settle and the cells of the supernatant are carefully removed. The cells are washed with serum and incubated a few hours at 37°C in a nutritive medium, supplemented with colchicine.

The mitoses are stopped at metaphase. The cells are submitted to hypotonic expansion, they are fixed and spread on slides and air dried.

With this technique we have been able to obtain metaphase cells with well-spread chromosomes allowing good analysis of the caryotype of the rat embryos.

6.38. Mode of Action of Metal Blocking Agents in Nuclear Dissolution. Fernando Silió and Dolores Angulo (Madrid, Spain).

Versenate and other chelating substances induce a strong chromatic dissolution and a remarkable increase of cell volume. From the beginning we thought that this action might be due to an indirect process of activation of proteolytic enzymes. In order to confirm this we used cyanide that does not have any chelating action, but is an inhibitor for (heavy metals containing) terminal oxidases.

The cytological effects obtained with cyanide were equivalent in some aspects to those induced by versenate, which confirms our hypothesis work based on the following facts.

1. Papain, and other papainlike enzymes, suffer strong activation by cyanide, versenate and other peroxidase inhibitors. Thyolic compounds and many other reducing agents, increase this activation while oxidants inhibit it.

2. The EDTA presents a much greater affinity for Fe^{+++} or Cu^{++} than for Zn^{++}, which proves its selectivity at low concentrations. The affinity for Ca^{++} and Mg is still lower. Therefore it is to be supposed that it will react with Fe before it will do so with Ca and Mg.

3. As the lysis is already verified at very low concentrations of EDTA or CNK, a very selective action on trace elements may be assumed, rather than on macroelements like Ca or Mg.

The enormous increase of cell volume observed means a great imbibition which may be attributed to a great increase of the osmotic pressure. All this would confirm an intense despolimerization of macromolecules.

With coenzyme A we have obtained similar effects.

6.39. Nucleic Acid Profiles of Germ Cells of the Mouse. JOHN H. D. BRYAN (Ames, U.S.A.).

Primordial germ cells may be distinguished both from their definitive counterparts in the adult, and from adjacent somatic cells by their large nuclei and chromosomes. The present work forms part of an attempt to determine the probable chemical basis underlying these size differences. The results of these experiments should also contribute to our understanding of chromosome structure. The distribution of DNA and RNA in sections of acetic-alcohol fixed fetal and adult gonads and liver tissue has been studied following simultaneous visualization by means of azure B staining(1).

In certain cases staining was carried out following treatment with enzymes (trypsin and/or RNAase), or chemical extraction of RNA (cold 10 per cent perchloric acid). The results obtained, though qualitative in nature, suggest that there may be differences between the nucleic acid-protein complexes of primordial germ cells and their definitive counterparts. Somatic cells, on the other hand, appear to react to the staining experiments in a manner essentially identical with that of the definitive germ cells.

Supported by grant CA 05591-02 from the National Cancer Institute, U.S.P.H.S.

1. FLAX and HIMES, *Physiol. Zool.* **25,** 297-311, 1952.

6.40. (D.) Endomitosis during Megasporogenesis in Saccharum. G. BREMER (Wageningen, The Netherlands).

In many interspecific hybrids of this genus it was found by Bremer in 1920, and afterwards also by other investigators, that the somatic chromosome number of such hybrids equals the sum of twice the haploid number of the female parent plus once the haploid number of the male parent. It appeared to Bremer that this phenomenon is based on endo-duplication of chromosomes during megasporogenesis after a normal first meiotic division reducing the chromosome number to the half.

The endo-duplication will be shown in microscopical slides with drawings to match.

6.41. Sites of Formation of the Extra Nucleoli during Early Oocyte Growth in the Freshwater Teleost Salvelinus fontinalis Mitchill. L. A. CHOUINARD (Quebec City, Canada).

The behaviour of nuclear structures during early oocyte growth in *Salvelinus fontinalis* will be described with special attention focused on the sites at which the extra nucleoli first appear. In the species investigated, the process of extra nucleoli formation begins at about the mid-pachytene stage of meiotic prophase and proceeds in an uninterrupted fashion until the late strepsitene stage is reached; during that period of time, close to a hundred extra nucleoli form within the nuclear cavity. From the mid-pachytene up to about the mid-strepsitene stage, our observations suggest that, while some of the extra nucleoli arise by successive growth and detachment from a single large heterochromatic body adjacent to the oocyte's original nucleolus, the others develop in contact with minute chromocenters located in the peripheral portion of the nuclear cavity. The relevant observational evidence would seem to favour the view that the heterochromatic body adjacent to the oocyte's original nucleolus contains one of the four alleged pairs of nucleolar organizing regions of the usual nucleolar chromosomes. During the mid-strepsitene stage, on the other hand, our observations reveal that, while the heterochromatic body and the chromocenters disappear from view, additional extra nucleoli develop at various points along the length of a number of chromosomes. These observations are taken to indicate that non-heterochromatic chromosomal regions distinct from the nucleolar organizing regions of the usual nucleolar chromosomes are, during that period of meiotic prophase, instrumental in the formation of extra nucleoli. The significance of the above findings will be discussed in relation to the observations of other workers on the sites of formation of nucleoli in general.

6.42. An Investigation of DNA Replication in Bovine Sex Chromosomes. STANLEY M. GARTLER and BARBARA E. BURT (Seattle, U.S.A.).

Both the X and Y chromosomes in cattle can be unambiguously distinguished from the autosomes. The X is a large sub-metacentric chromosome, the Y a small metacentric and all the autosomes are acrocentric. DNA replication studies using tritiated thymidine in cell cultures have shown the following: in the male the Y is the last chromosome in the complement to replicate while the short arm of the X replicates shortly before it. The long arm of the X replicates at a distinctly earlier period. In the female one X replicates as in the male while the other X

begins replication a little earlier but both arms replicate in a relatively short period. Thus, as in man the X chromosomes in the female replicate asynchronously and the chromatin body is apparently derived from the X in which the long arm replicates last.

6.43. (D.) Effects of Digitonin on Dividing Meristematic Cells and Cell Wall Formation. L. V. OLAH (Carbondale, U.S.A.).

Demonstration of the mitotic cycle of "digitonin" mitosis is represented by a series of microphotographs. The ultrastructure of "digitonin" mitosis is shown by a series of electron microphotographs. Alteration of the geometry of mitosis and the origin and function of the phragmoplast is shown by a series of schematic drawings. Phases of "digitonin" mitosis are demonstrated by microscopical slides.

6.44. Reciprocal Translocation obtained after Colchicine Treatment in Phlox drummondii. T. S. DHILLON (Hong Kong).

Seedlings of *Phlox Drummondii* were raised in clay pots. After two to three leaf pairs had developed, the growing points of seedlings were smeared with 0.5 per cent emulsion of colchicine in lanolin. Twenty out of fifty treated seedlings survived and produced main stem from the colchicine treated growing point, as well as lateral branches from nodes below the level of colchicine application. First meiotic division was studied in the PMCs of buds taken from the main stem as well as the lateral branches. Eighteen treated plants and fifty untreated plants were found to be meiotically normal and showed seven bivalents at M.I. Main stem of one of the treated plants was found to be autotetraploid whilst the laterals originating from first two nodes of this plant were diploid. In the remaining treated plant, main stem showed an interchange complex involving four chromosomes and five bivalents. The first and second node lateral branches of this plant were meiotically normal, showing seven bivalents at M.I. Whilst the lateral branches of this plant were completely fertile, the main stem and lateral branches originating from fourth node and above, showed 40 to 50 per cent aborted pollen grains and had reduced seed set.

6.45. (F.) Mitosis with Undivided Chromosomes in Irradiated Cells. GUNNAR ÖSTERGREN and JADWIGA MOLÈ-BAJER (Lund, Sweden).

In irradiated endosperm of *Haemanthus* various disturbances of mitosis occur. An interesting deviation consists in nuclei entering into mitosis with undivided chromosomes each consisting of a single chromatid only. These are often associated by means of a presumably unspecific pairing mechanism that suddenly stops working with the start of anaphase.

6.46. (D.) Cytological Studies of Nothoscordon, Allium and Molium. S. E. EID (Shatby, Egypt).

I. *Centromere Structure:* Following hydroxyquinoline treatment of chromosomes of *Nothoscordon inodorum* (Ait) Asch et Gr., the median centromere could show up to 6 or more pairs of chromomeres. The terminal centromere, being a free end, may have a greater chance for extension, showing up to 8 or more pairs of chromomeres. At anaphase I the centromere may show two structures directed towards the near pole. These two structures can be seen transversely connected. (To appear in *Bull. of Faculty of Science*, Alexandria University, 1962.)

II. *Effect of the Environment on Meiosis, in Nothoscordon inodorum (Ait.) Asch et Gr. and Some Allium Species:*

A. High temperature treatment of flower buds on *Nothoscordon inodorum*, leads, in certain cases, to increase of the chromosome number from 19 to 32, in the pollen mother cells. This is attributed to duplication of the 13 metacentrics but not the 6 telocentrics, of the complement.

B. Cold leads to asyndesis and high temperature may lead to asyndesis or equational division with transitional conditions. (To appear in *Journal of Botany of the United Arab Republic*, 1962.)

III. *Cytological studies in section Molium of the genus Allium.*

6.47. (D.) Chromosome Reconstruction for Studies on Mutagenesis and Other Cytogenetical Problems. I. I. OSTER, R. SCHWARZ and R. BINNARD (Philadelphia, U.S.A.).

Several new stocks and techniques have been devised in order to facilitate studies on mutagenesis and other cytogenetical investigations by

combining mutations currently maintained at the National Science Foundation-supported Drosophila Stock Center in Philadelphia with specific chromosomal disarrangements induced by radiation in experiments precisely designed for such purposes. These include: "multi-purpose" strains for detecting chromosome loss, sex-linked recessive lethal mutations, translocations, and mutations at 8 specific loci in the offspring of the same treated flies; improved methods for detecting mutations arising in the female germ line; strains for determining the genetic components of somatic damage induced by low doses of radiation; stocks for detecting mutations arising at 20 specific loci in the female germ line and at 37 specific loci arising in the male germ line; a method for determining how often homologous loci mutate simultaneously following various types of treatment; several ring-shaped X chromosomes containing portions of the Y chromosome; several ring-shaped Y chromosomes with and without visible markers; new marker-inversion stocks for "isogenizing" the whole (or parts of the) genome; and combinations with visible effects in the immature stages to facilitate recognition of different genotypes during very early life for developmental studies.

Diagrams of these stocks and techniques, the manner in which they can be utilized, and the applicability of the principles in methodology involved to other organisms will be shown.

This work has been supported by grant G14148 from the U.S. National Science Foundation.

6.48. (D.) Cytological Observations on Drosophila and Human Material. I. I. Oster, G. Balaban and R. Binnard (Philadelphia, U.S.A.).

A modified method for observing somatic chromosomes recently described in *Drosophila Information Service* [1] has been utilized for the following projects:

1. Additional evidence was obtained that ring-shaped chromosomes in *Drosophila* are highly susceptible to loss following irradiation as shown by their breakage and frequent involvement in anaphase bridges. This is in agreement with our previous results derived from breeding tests and confirms our preliminary cytological observations. These effects can be demonstrated even after the cells have been exposed to relatively low doses of rays.

2. Examination of the somatic cells of *Drosophila* larvae which had been fed chemical mutagens (e.g. urethane, nitrogen mustard) or compounds currently under investigation as "chemosterilants" (e.g. "Mapo": tris(1-(2-methyl aziridinyl) phosphine oxide) by the U.S. Department of Agriculture have revealed the presence of fairly large numbers of broken chromosomes. In line with the theoretical considerations concerning the mode of action of such alkylating agents as the latter, these results tend to indicate that chemicals which are capable of causing sterility in insects act primarily via the induction of genetical damage.

3. Analyses of structurally altered X and Y chromosomes, particularly newly-synthesized ring-shaped chromosomes, in *Drosophila*.

4. Karyotypic analyses of the housefly (*Musca domestica*) and the honey bee (*Apis mellifera*).

5. Detection of the Barr body in human buccal smears (by the use of some portions of the technique only). This method yields preparations which are relatively less distorted than slides made by the techniques currently in use (e.g. thionine, cresyl violet).

Data, slides, and photomicrographs illustrating these areas of research will be shown.

This work has been supported by grant G 24261 from the U.S. National Science Foundation and grant AT(30-1)-2618 from the U.S. Atomic Energy Commission.

1. Oster, I. I. and G. Balaban No. 37, 142-144 (1963).

SECTION 7

CYTOGENETICS

7.1. Tomato Chromosomes and Their Relationship to the Linkage Maps. B. SNOAD (Hertford, Great Britain).

The tomato is a rather exceptional plant since it has been for many years a subject for intensive genetical research and also it has chromosomes which are morphologically distinguishable at pachytene. It is only recently, however, that attempts have been made to correlate the linkage maps with the chromosomes themselves. Each chromosome is composed of distinct heterochromatic and euchromatic regions and it is therefore possible to compare the genetic activity of these two regions.

Such a comparison is being made by the use of X-ray induced reciprocal translocations. The broken chromosomes are identified at pachytene and test-crosses are made with appropriate genetic marker stocks. In this way linkage between the visible points of chromosome breakage and the selected genes can be estimated.

The evidence so far indicates that crossing-over does not occur in the heterochromatin and that the active genes studied are located in the euchromatin. The great majority of chromosome breakage occurs in the heterochromatin so that linkage between the genes and such points of breakage is really a measure of the amount of euchromatin separating them rather than the total amount of euchromatin and heterochromatin.

7.2. Localizing Genes by means of Induced Chromosomal Deficiencies in Tomatoes. GURDEV S. KHUSH and CHARLES M. RICK (Davis, U.S.A.).

The induced deficiency method, previously applied (*Genetics*, **46**, 1389-1393) to genes on chromosome 11, has been extended successfully to localize other seedling marker genes. Stocks homozygous for unlocated recessive genes are pollinated with X-rayed pollen of a normal, non-mutant parent, and all mutant progenies are searched cytologically at pachytene for chromosomal alterations. More than 95 per cent of such individuals are deficient for that part of the chromosome in which the gene in question resides. In this fashion *hl* of chromosome 11, *dl* of 8, and *ful* and *clau* of 4 were assigned to the achromatic regions of the short arms (S) of their respective chromosomes; likewise a_1 to the achromatic of 11L and l_1 to 8L. No unequivocal evidence has been obtained for genes in the chromatic regions. More than half the deficient plants are monosomic, but simple monosomics have been found only for chromosome 11, the remainder being translocated monosomics, in which one element of a reciprocal translocation has been lost. Of ten such deficiencies, the lost element nearly always comprises the shorter arms of the respective chromosomes, longer deficiencies doubtless being less viable. Exceptional was a case in which centromeric breaks led to translocation and loss of 8L and 9S. Since this plant had l_1 phenotype and appeared in the progeny of *dl-bu-*l_1 X-irradiated + + + pollen, l_1 clearly lies in 8L and both *bu* and *dl* in 8S. No deficiencies were transmitted to any of 1429 progenies of female parents deficient for arms of 11 and to 131 offspring of haplo-11 ♀.

Research partly supported by Grant GM 06209 of the U.S. Public Health Service.

7.3. Cytogenetic Correlations in Tetraploid Tomatoes. PETER B. MOENS (Toronto, Canada).

Five markers (dv, d, m, aw and wv) on chromosome no. 2 of the tomato, the satellited chromosome, were found to segregate 1: 30 in the progeny of duplex tetraploids. The individual ratios did not differ significantly from each other ($\chi^2=0.40$, d.f. 4) and an average ratio of 1: 30.257 was established for the markers (3.20 per cent recessives ± 0.05 per cent, based on 110,000 observations). The fact that the ratios do not vary with the distance from marker to centromere, as is normally the case in tetraploids, results from the specialized structure and behaviour of chromosome no. 2.

Recessive zygotes in excess of 1: 35 are the result of genetic non-disjunction in quadrivalents. The frequency of chromosome no. 2 quadri-

valents was found to be 0.30. Random pairing of homologues was ascertained through the use of plants having two homologues with long satellites and two homologues with short satellites. Quadrivalent formation was found to be of a specialized type: only the long arm of chromosome no. 2 participates. For example, in a ring quadrivalent each long arm must have at least two chiasmata and one partner exchange. Due to the space requirements the proximal chiasma always lies near the centromere. Consequently, all genetic markers on chromosome no. 2 will segregate independently of the centromere.

The predicted frequency of recessive zygotes is 1/29.8 for markers separated by one chiasma from the centromere when the frequency of quadrivalents is 0.3.

7.4. A Cytogenetic Study of the Tumour-bearing Hybrid Nicotiana glauca X N. langsdorffii. M. R. AHUJA (Philadelphia, U.S.A.).

It is well known that the cross *N. glauca* X *N. langsdorffii* gives 100 per cent tumors in the F_1 progeny. The triploid and the tetraploid combinations also yield an entirely tumorous progeny. Kehr and Smith (*Brookhaven Symp. Biol.* **6**, 55-78, 1954) and Smith and Stevenson (*Z. Vererb.* **92,** 100-118, 1961) have shown that tumor formation depends on the interaction of many genes in the hybrid. Furthermore they found that with one or several *glauca* chromosomes on diploid *langsdorffii*, tumors do not form. On a different hybrid between 4*n* *N. debneyi tabacum*×*N. longiflora* (Ahuja, *Genetics*, **47**, 865-880, 1962) tumor production was found to be a function of an alien *longiflora* chromosome or a fragment of it on the *debneyi-tabacum* background. Since Kehr and Smith did not examine the cytology in the *glauca-langsdorffii* hybrid combination it was considered worthwhile to check the cytology in the triploid generation (having two *langsdorffii* and one *glauca* genomes). A study of meiosis revealed that in 15 plants not all plants in the triploid generation carried a full complement of 30 chromosomes ($9_{II} + 12_I$). Three plants were found with 29 chromosomes ($9_{II} + 11_I$). Somatic elimination of a chromosome is ruled out as buds on different branches of the same plant had the same chromosome number. All the three deficient plants are tumorous indicating that tumor production in this hybrid may not be function of the complete genomes of the parental species. This opens the way to a more complete analysis of the role of chromosomes in tumor forming process.

This work was supported by Grant DRG 622 from the Damon Runyon Memorial Fund for Cancer Research.

7.5. A Pseudo-supernumerary Chromosomein Collinsia heterophylla. E. D. GARBER (Chicago, U.S.A.).

Progeny of plants of *C. heterophylla* ($n=7$) but not of six other species in the genus treated with colchicine included individuals either with an extra chromosome (trisomy) or with a heterozygous reciprocal translocation. Thirteen independently obtained trisomics had the same extra chromosome which behaved cytogenetically as did supernumerary chromosomes in two other species. Primary trisomes from an autotriploid plant of *C. heterophylla*, however, were orthodox in their cytogenetic behavior. Although a trisome for one of the seven chromosomes in this species simulates a supernumerary chromosome, this chromosome cannot be lost (monosomy) from the complement. The pseudo-supernumerary chromosome presumably responds to colchicine by undergoing non-disjunction during mitosis and may be responsible for the unexpected chromosome breakage induced in this species by colchicine.

Research supported by the National Science Foundation.

7.6. Genetics of B-chromosomes. A. RUTISHAUSER (Zürich, Switserland).

Most accessory or B-chromosomes of higher plants show some kind of boosting-mechanisms: the B-chromosomes are preferentially included in the generative or sperm nucleus (directed non-disjunction in pollen grain-mitosis) or in the functioning megaspore (preferential segregation). In *Crepis capillaris*, where plants with one B-chromosome rarely occur in nature, a new type of boosting-mechanism has been found: the number of B's is doubled in the inflorescences.

The B-chromosomes of *Crepis capillaris* are by no means genetically inactive. Although partly heterochromatic they exert a variety of effects on the carrier plants. The flowering time is retarded, the shape of inflorescences changed, the pollen fertility lowered and, depending on the number of B's, seed setting reduced to $\frac{1}{2}$ (1B) or 1/4 (2B's). Even more striking is their effect upon bivalents of the A-chromosomes:

the number of chromatid-bridges accompanied by acentric fragments of first divisions of PMC's, and accordingly the number of tetrads with micronuclei and persisting bridges, rises considerably if B-chromosomes are present. In 2B-plants B-chromosomes themselves form bridges.

From these findings it may be inferred that B-chromosomes of *C. capillaris* enhance variability and therefore may well have an evolutionary effect.

7.7. Frequency and Geographical Distribution of Rye (Secale cereale) with Accessory Chromosomes in Korea. Woong-Jik Lee (Seoul, Korea).

Frequency of accessory chromosomes in population of rye from Korea was known to be exceedingly high. Extensive present work for frequency and geographical distribution of rye with accessory chromosomes in Korea has been carried out and the result has revealed that the frequency is ranged from 39 to 53 per cent. This figure, smaller than the result in previous report, is still higher than any other region in the world. Correlation between frequency of accessory chromosomes and edaphic factor (type and pH of soil) will be discussed.

7.8. The Cytogenetic Effects of a Paracentric Inversion in Neurospora crassa. Patricia St. Lawrence and Jesse R. Singleton* (Berkeley and Lafayette, U.S.A.).

The mutant S1325 carries a paracentric inversion which is inseparable from a niacin requirement and involves about 70 per cent of the known genetic length of the right arm of linkage group I. Recombination between the *nic-2* and *lys-3* loci is much reduced in viable progeny from crosses of S1325 to normal stocks. In immature asci from such crosses a conspicuous bridge and fragment is seen at anaphase I. Persistence of the bridge is occasionally observed leading to fusion of the daughter nuclei or to aberrant chromosome segregations in subsequent divisions. Migration of the nuclei is irregular and non-identical sister spores may be formed. It has not been possible to determine whether a breakage-fusion-bridge cycle is operative. At maturity most of the asci are completely abortive; the major class of asci with ripe spores has one ripe pair in each half of the ascus. Analysis of isolates from asci of crosses of S1325 to a stock carrying centromere markers in linkage groups II, III, and VI has given genetic evidence of nuclear passage; coincident second division segregation for all markers is observed in one type of ascus. No such coincident segregations were found in other types of asci from this cross nor in asci from control crosses.

*Deceased.

7.9. Polyploidy and the Interchange-heterozygote. Keith Jones (Kew, Great Britain).

The nature of chromosome pairing and its genetic consequences are well understood in diploid interchange-heterozygotes. Permanent hybrids of the *Oenothera* type have maintained heterozygosity by control of chromosome segregation and the elimination of homozygotes. Polyploidy is also an efficient means of maintaining heterozygosity and its role in the evolution of *Anthoxanthum odoratum* ($2n=20$) is discussed.

7.10. Genically Controlled Variability of Chromosome Number in Pennisetum Hybrids. P. Gildenhuys and K. Brix (Pietermaritzburg, South Africa).

Crosses between diploid *Pennisetum typhoides* ($2n=14$) and tetraploid *P. purpureum* ($2n=28$) yield sterile triploids ($2n=21$) with regular mitotic behaviour. The colchicine induced hexaploids (amphidiploids) are fertile but show instability in somatic chromosome number, even in cells of the same root, as do the C2 plants ($2n=36$ to 49 with $2n=42$ occurring most frequently). These hexaploids produce pollen grains which contain from 12 to 21 chromosomes. When back-crossed to *P. typhoides* the resultant progenies, as expected, have $2n=21$ to $2n=28$ chromosomes, but, in addition, the crosses also yield offspring with chromosome numbers in excess of $2n=28$. These arise from unreduced egg cells in *P. typhoides*.

Whilst C1 and C2 plants all show similar ranges of intraplant variability in somatic chrosome number, this is not so in the back-crosses, where the range of variation is highly significantly different from plant to plant. For example, in one plant numbers range from 18 to 29 (28 occurring most frequently), in another from 32 to 38 (35 most frequent), whereas in another plant the range is only 34 to 36

(35 most frequent) whilst in yet another the number is constant at $2n=35$. It is suggested that intraplant aneuploidy, known to exist in other intergeneric and interspecific hybrids, is in this case under genetic control, probably with similar gene action to that which we have demonstrated in the amphidiploid of the cross between *P. typhoides* and the mitotically unstable *P. dubium*.

To be published in the *South African Journal of Agricultural Science*.

7.11. Colchicine-induced Somatic Chromosome Reduction in Sorghum. J. G. Ross, C. H. Chen and G. M. Simantel (Brookings, U.S.A.).

As a result of colchicine treatment of diploid sorghum seedlings of certain genotypes, true-breeding and segregating diploid mutants have been observed. A laboratory method by which light, temperature and humidity are controlled was developed to ensure the appearance of the phenomenon in "colchicine reactive" genotypes. To explain the appearance of true breeding mutants a mutational effect was postulated in conjunction with somatic chromosome reduction followed by restoration to the original chromosome number. This hypothesis was tested by treating tetraploid seedlings of "colchicine reactive" genotypes. Out of 80 treated seedlings there were 9 diploids (one a chimera of diploid and tetraploid tissue) among the 17 survivors, while no diploids occurred among the 118 control tetraploid plants. Histological study indicated that diploid cells occurred at the base of the apical dome 2 to 3 days after colchicine application and that these may proliferate to take over the growing point at the 5th to 6th day. As an additional test, 441 seedlings containing structural chromosome markers (reciprocal translocations) in the heterozygous condition were treated. Of 258 survivors 6 were true-breeding mutants homozygous for the structural markers and 4 appeared to be chimeras of mutant and original tissue and still heterozygous. No homozygosity occurred in the 108 surviving untreated controls. These tests indicate that after colchicine treatment of sorghum seedlings of a reactive genotype under certain environmental conditions somatic chromosome reduction occurs. Cells so formed may take over the growing point to form a plant containing half the original chromosomes or homozygous if restored to the original number.

7.12. Cytogenetic Studies of Perennialism in Derivatives of Interspecific Hybrids of Zea*. Donald L. Shaver (Upton, U.S.A.).

Perennialism is recovered in 4x derivatives of hybrids between 4x maize and 4x perennial teosinte by sib-mating among the most perennial segregates of each generation. Similarly, only 3 generations of selection at the 75 per cent maize level have resulted in a progressive recovery of the perennial expression. A high degree of maize-likeness therefore appears compatible with the perennial expression at the 4x level.

Diploid derivatives of maize and perennial teosinte are produced by crossing the 30 chromosome triploid hybrid of 4x perennial teosinte $\times$2x maize back to 2x maize, and then intercrossing among the resulting array of euploid and aneuploid plants. The average chromosome number in the first post-triploid generation is 24.6, and in the second post-triploid generation is 20.7. The third post-triploid generation is almost entirely euploid. The study of pollen abortion and seedling lethality in euploids of the post-triploid generations indicates the presence of genetic lesions which are functionally viable in 4x teosinte but which act as diploid-lethals. In spite of the high degree of preferential segregation of markers in the triploid indicating that post-triploid material should carry a large proportion of perennial teosinte genes, derived diploids are very maize-like. The apparent tendency to lose teosinte genes is probably connected with the elimination of diploid-lethal factors. However, even among euploids and aneuploids of the first post-triploid generation which have not yet suffered elimination of teosinte genes, it has not been possible to demonstrate true perennialism. One exceptional 21 chromosome plant has been found which appears truly perennial. It has been cloned up through the fourth generation of culms. Data on its cytology and genetic progeny ratios will be presented.

---

* Research carried out at Brookhaven National Laboratory under the auspices of the U.S. Atomic Energy Commission.

7.13. The Use of Hypoploids in Identifying Naturally Occurring Duplications in Maize. J. R. Laughnan (Urbana, U.S.A.).

We have commenced using various hypoploids in an arm-by-arm search for naturally occurring duplications in the maize genome. Assuming that

such duplications occur (there is some independent genetic evidence for this) it is argued that during meiosis in the hypoploid individual, the chromosome arm that is in haplo condition should synapse occasionally with segments of other chromosomes representing duplications of chromatin in the haplo arm. Crossing over in such "illegitimately" paired regions should yield gametes carrying reciprocal translocations; the identification and analysis of interchanges originating in this way might be expected to reveal the nature and extent of naturally occurring duplications.

In our scheme, hypoploid individuals among the progeny of pollen parents carrying an A–B translocation, are used as pollen (or egg) parents in crosses with vigorous single-crosses. The progeny of such crosses are grown on a large scale and at harvest are searched for the occurrence of scatter-grain ears, an indication of heterozygosity for a reciprocal translocation. These are routinely analyzed to confirm the presence of an aberration and to identify it cytologically.

Preliminary results based on an analysis of progeny of TB-9a and TB-9b hypoploids indicate the occurrence of reciprocal translocations which appear to involve preferentially the haplo arm of the hypoploid as one member of the interchange. On the other hand, the haplo arm is not always involved in the identified interchange, but even in these cases it appears that certain arms may be involved more frequently than others. It may be that the haplo arm in the hypoploid plant leads to secondary "illegitimate" associations, or that chromosomes are more prone to break in hypoploids and that in this respect some regions are more unstable than others. Preliminary results indicate that certain hypoploids yield higher frequencies of interchanged progeny than do others.

7.14. Inversion Polymorphisms in Teosinte. Y. C. TING (Chestnut Hill, U.S.A.).

During last few years, five varieties of Guatemalan teosinte and eight varieties of Mexican teosinte have been cytologically investigated. Chromosome inversions were identified in most of these varieties. *In9* in the short arm of chromosome 9 was present in the Guatemalan varieties, Florida, Jutiapa, El Valle and Lake retene; but not in the variety Huixta of northern Guatemala. Measurements at pachytene showed the length of this inversion to be about 60 per cent of the length of the short arm. Bridges and fragments were found at both anaphase I and anaphase II of the microsporocyte divisions. Among Mexican teosinte, the varieties, Chalco, Xochimilco, Durango, Nobogame and perennial teosinte were observed to have the same *In9*. In addition, *In8* was also present in these Mexican varieties; but not in perennial teosinte. The average length of this *In8* at pachytene occupied about 50 per cent of the total length of the short arm of chromosome 8. It was further found that the variety Xochimilco had, in addition to *In8* and *In9*, an *In3* in the long arm of chromosome 3. This *In3* being equivalent to about half of the length of the long arm, manifested interchromosomal effect on the frequency of crossing over within the inverted segments of both *In8* and *In9*. The convincing evidence of this effect was that at pachytene the frequency of loop formations of both *In8* and *In9* was higher than this in in plants having only *In8* and *In9* but not *In3*. (Detailed report of this study will be published in a monograph under preparation.)

7.15. An Evolutionary Significance of a Pericentric Inversion in a Barley Trisomic for Chromosome 6. TAKUMI TSUCHIYA (Yokohama Japan).

The seven primary trisomic types have been produced in *Hordeum spontaneum* var. *transcaspicum*. A new trisomic type has been found in the progeny of Purple, the trisomic for chromosome 6. The extra chromosome of the new trisomic type had two constrictions located at subterminal positions of opposite sides. The study of mitotic chromosomes showed that the one of the two constrictions was the kinetochore and the other the secondary constriction of the chromosome 6. Thus the changed chromosome was assumed to be derived from the chromosome 6. The length of the changed chromosome was about 18 per cent shorter than that of the normal chromosome 6. The trivalent chromosomes associated with the nucleolus at diakinesis. Thereby it has been confirmed that the changed extra chromosome might be derived from chromosome 6. Meiotic behaviour was almost normal resembling that of the primary Purple trisomics. From the somatic chromosome morphology and the meiotic behaviour it has been assumed that the structural change in the new Purple trisomic would be a pericentric inversion accompanied by a deletion. The new Purple trisomicd showed vigorous growth with intermediate characteristics between normal diploid and primary Purple trisomics and almost normal fertility. Tetrasomic and some other chromosomal types have been obtained in the sibling and/

or progeny of the new trisomic plants. Some problems in the linkage studies using trisomics are considered in connection with the occurrence of such pericentric inversions accompanied by a deletion as has been reported here in this paper.

7.16. Genetic Recombination and Chromosome Disjunction in a Balanced Tertiary Trisomic of Barley. R. T. RAMAGE (Tucson, U.S.A.).

The interchanged chromosome 2^{7}_{a} is broken close to the centromere in the short arm of chromosome 2. The Ms_2ms_2 locus is tightly linked with the breakpoint of 2^{7}_{t} and is carried on chromosome 2^{7}_{a} (arm not known). The Vv locus is carried on the long arm of chromosome 2 and, in normal stocks, exhibits about 26 per cent recombination with the Ms_2ms_2 locus. A balanced tertiary trisomic was set up so that the two normal chromosomes carried the v and ms_2 alleles and the interchanged chromosome 2^{7}_{a} carried the V and Ms_2 alleles. In the balanced tertiary trisomic the frequency of recombination between the genes V and ms_2 was measured by determining the frequency of male gametes carrying the V allele. Chromosome disjunction types, which are dependent upon the type of pachytene pairing, were obtained by determining the frequencies of microspore abortion and of primary trisomics in the progeny of the balanced tertiary trisomic. Microspore abortion and the production of primary trisomics are results of the same pachytene pairing event and should be found with the same frequency. If genetic recombination is dependent upon an exchange of chromosome parts at pachytene, the frequency of microspore abortion, or of the production of primary trisomics, should equal one-half the frequency of male gamete carrying the V allele. In these studies, much more genetic recombination was observed than can be accounted for by an exchange of chromosome parts at pachytene.

7.17. Chromosome Segregation in a Tertiary Trisome of Secale cereale in its Three Karyological Backgrounds. J. SYBENGA (Wageningen, The Netherlands).

Tertiary trisomes originate from reciprocal translocation heterozygotes. The extra chromosome can occur in three "backgrounds": the translocation homozygote, the translocation heterozygote and the normal type. All three forms may be found in the progeny of a selfed translocation heterozygote, that is trisomic for a translocation chromosome.

Meiotic configurations depend on the "background", and chromosome segregation depends on these configurations. In the translocation homozygote background the trisome is in fact a primary trisome. In the normal background as largest configuration a chain of five can be formed. In the heterozygous background this is again a chain of five that, with zigzag orientation, segregates into the translocation complement with the extra chromosome against the normal complement without it, or the reverse. With equally complete pairing a bivalent and a chain of three may be formed, segregating into the translocation complement without the extra chromosome against the normal complement with it. The third possibility is a ring of four with a univalent, that leads to loss of the extra chromosome, or inclusion in either group with equal probability. Less complete pairing or non-zigzag orientation leads to increased loss of the extra chromosome, increased randomness of its distribution and increased sterility.

In the homozygous backgrounds no segregation in regard to the translocation occurs. The type of configuration affects the frequeny of the extra chromosome in the progeny and the sterility. Meiosis and chromosome segregation were studied in one tertiary trisome in all three backgrounds in *Secale cereale*. Orientation of the multivalents was predominantly zigzag. Preference for type of segregation in the heterozygote could not be demonstrated. Apparently the translocation did not tend to favour pairing between completely homologous chromosomes only. This agrees with the results of Ahloowalia on the autotetraploid translocation heterozygote. Data on meiosis and chromosome segregations will be presented.

7.18. Transmission of Rye Chromosomes in Monosomic Addition Lines of Rye Chromosomes in Wheat. SHAMS-UL-ISLAM KHAN (Lahore, Pakistan).

In the monosomic addition lines of rye (*Secale cereale* L.; "Swat Rye", $2n = 14$) chromosomes in wheat (*Triticum aestivum* L.; C271, $2n = 42$), transmission of individual rye chromosomes has been studied, both through male and female gametes. Ratios (42/43) through male and female gametes are: I (4.78 : 1/5.0 : 1), II (2.8 : 1/3.0 : 1), III (2.0 : 1/2.25 : 1), IV (12.0 : 1/10.0 : 1), V (3.0 : 1/2.9 : 1), VI (8.0 : 1/6.0 : 1), VII

(6.0 : 1/8.0 : 1) and total (5.5 : 1/5.3 : 1). These ratios lead to the following conclusions:

(a) Transmission is almost the same through male and female gametes when all seven chromosomes are considered together.
(b) In spite of differences in transmission these seven chromosomes fall into five groups: (1), I; (2), II and V; (3) III; (4) IV; and (5), VI and VII.
(c) Groups (1), (3) and (4) consist of single chromosomes, and show almost the same transmission through male and female gametes.
(d) In groups (2) and (5), each consists of two chromosomes, and the transmission ratio of one through the male gamete is the same as that of the other through the female gamete.

From the above transmission data of rye chromosomes it is tempting to infer that the rye progenitor may have had 5 and not 7 pairs of chromosomes and that the evolution of genera and species in the *Triticeae* has been from an ancestral type with 5 pairs of chromosomes: undoubtedly through alterations in the physical constitution of certain chromosomes only.

7.19. The Nature of a Spontaneous Transfer of Hairy Neck from Rye to Wheat. C. J. DRISCOLL and E. R. SEARS (Columbia, U.S.A.).

A series of backcrosses transferred the rye character "hairy neck" (pubescent peduncle) to Chinese Spring wheat from the Cornell wheat selection 82al-2-4-7, a derivative of a wheat–rye hybrid. This dominant character exhibited monogenic inheritance. By means of monosomic analysis it was located on chromosome 4A (IV).

When heterozygous plants, with one entire and one telocentric chromosome 4A, were selfed, non-hairy offspring were obtained that possessed an entire 4A. Since the hairy-neck gene was carried by the entire chromosome in the parent plant, this result demonstrated that the gene is located on the arm homologous to the telocentric. In the plants heterozygous for hairy neck and the telocentric, pairing (to form a heteromorphic bivalent) was observed in only about 30 per cent of pollen mother cells. When unpaired, the two chromosomes often exhibited co-orientation, suggesting that synapsis had occurred but that crossing over and chiasma formation had not ensued. As regular pairing occurs between normal 4A and this telocentric, it may be assumed that the hairy-neck gene is located in a rye segment of substantial length in which crossing over with the telocentric does not occur.

Heterozygotes with the heteromorphic bivalent were backcrossed as males to Chinese for determination of the distance of the hairy-neck gene (or rye segment) from the centromere. As desynapsis leads to fewer functioning non-cross over gametes, a correction factor, calculated as 0.56, will presumably need to be applied to the obtained value.

7.20. Cytogenetic Studies Bearing on the Nature of the Centromere. L. M. STEINITZ-SEARS (Columbia, U.S.A.).

Telocentric chromosomes resulting from misdivision were studied in hexaploid wheat to determine whether their somatic instability might be due to deficient centromere regions. Among 3134 offspring of monosomic 3B, which carried the hemizygous-ineffective gene *v* (virescent) as a marker of the short arm, 28 telocentrics and 7 isochromosomes for the short arm of 3B were found. Eighty additional short-arm telocentrics were presumably present in the virescent seedling but were lost before meiosis. A few of these plants, virescent as seedlings though green and monosomic at the time of meiosis, had 41 normal plus 1 telocentric chromosomes in their root tips.

In order to prevent meiotic loss, a telocentric for the long arm of chromosome 3B, which carries a gene for synapsis, was combined with the different telocentrics for the short arm. Thus lines were established possessing 20 normal and 2 telocentric pairs, in which the very stable pair for the long arm of 3B was identical but the pairs for the short arm differed in origin. Observations of leaf sectors suggest differences in the degree of stability of the short-arm telocentrics.

In the meiosis of individuals with only one of each of the telocentrics, varying amounts of co-orientation were found at metaphase I. The most stable short-arm telocentric was co-oriented with the long-arm telocentric in approximately half of the analysable cells. Co-orientation is interpreted to be a consequence of an overlap of centromeric regions of the two different stable telocentrics. Centromeric regions are believed to be complex and specific for each chromosome.

7.21. Gene Evolution in Polyploid Wheat. E. R. SEARS (Columbia, U.S.A.).

Where genes are duplicated, as in polyploids, mutations to a different level of activity–even

to the zero level—may not be detectable, because of the masking action of the duplicates at other loci. Harland [1] suggested that a duplicated gene could escape this masking effect by mutating to an allele with a divergent function. An example of this kind of mutant may be Neatby's virescent (*v*) on chromosome 3B (III) of common wheat, a hexaploid.

Increase in dosage of *v* from 2 to 3 results in greater abnormality (less chlorophyll), whereas increased dosage of the normal allele *V* or either of the homologous (related) chromosomes 3A (XII) and 3D (XVI) shifts the phenotype toward normal. This suggests that *v* is antimorphic to *V* and that *V* (or *V1*) has duplicates, *V2* and *V3*, on chromosomes 3A and 3D.

That *V3*, and therefore presumably *V1* and *V2*, are involved in chlorophyll production was shown by inducing a deficiency for *v1* and combining this with nullisomes 3A and 3D, respectively, thus reducing the dosage of *V* from the normal 6 to only 2. Nullisomic-3D plants (*V2V2*) had normal chlorophyll, whereas nullisomic-3A plants (*V3V3*) were of reduced chlorophyll content. Thus *V2* is more potent than *V3* in promoting chlorophyll development. On the other hand, *V3* is more effective than *V2* in reducing the expression of *v*. Although *v* may be a mutant with a divergent function, another explanation is possible; namely, that it is less efficient than *V* in producing chlorophyll but more efficient in competing for substrate.

1. *Biol. Rev.* **11,** 1936.

7.22. Induction of Autosomal Crossing Over by α-heterochromatin and Demonstration of Chromosome Interference in Phryne cincta. B. E. Wolf (Berlin, Germany).

Investigating the internal causes of crossing over in the euchromatic chromosomes of *Phryne cincta* the compact- or α-heterochromatin was recognized to be a decisive factor. Obviously the so-called "α-quantum difference", i.e. the difference in the amount of α-heterochromatin (or DNA) between the homologues of the X-chromosome, is causally related to the crossing-over-process ("α-differential-effect"; Wolf, *Chromosoma*, **13,** 646, 1963). It was supposed, that by way of a potential function it effects crossing over not only within the "α-differential" tetrad but also in nonhomologous and unrelated pairs of chromosomes in the same meiotic cell.

This hypothesis could be ascertained in cytological crossover studies by means of either two independent inversions coupled in autosome 2 and in autosome 3. Both pairs of inversions permitted to establish the influence of the "α-quantum-difference" in the X on crossing-over-frequency in the autosomes. Brother and sister males and females heterozygous for one pair of the inversions or for both together, furthermore characterized by particular X-chromosomes or X-chromosome-combinations (a/Y, b/Y, c/Y in the male and a/b, b/b, c/b in the female: a and c with much α-heterochromatin, b with less), have been backcrossed to homozygous animals of the standard stock (b/b resp. b/Y, free from inversions).

In the progeny of the males only the parental inversion-combinations in the autosomes 2 and 3 were found ($n = 356$ and 270 resp.). In the test cross progeny of the females the expected types of recombination appeared: with only one inversion in autosome 2 resp. 3 or simultaneously in both autosomes. There appeared a very high difference in linkage dependent on the present X-chromosome-combination in the mother. Females homozygous for the X-chromosomes (type b/b) exhibited only little recombination in chromosome 2 (3.0 per cent, $n = 430$) and no in chromosome 3 ($n = 318$) though the tested segments approximately involve a third of the total length in both chromosomes. Sister-individuals due to the presence of "α-differential" X-chromosomes (type a/b or c/b) gave a high rate of recombination in chromosome 2 (31.0 per cent, $n = 434$) as well as in chromosome 3 (11.4 per cent, $n = 784$).

Interchromosomal effects have been detected by observation of crossing over in the autosomes 2 and/or 3 and in the X-chromosome simultaneously. The frequency of contemporary crossing over in both autosomes as well as in the X and in one of the autosomes, based on independent occurrence, is significantly reduced. The results represent a cytological parallelism to genetic data on *Drosophila* of Schultz (1933) and Schultz and Redfield (1951).

7.23. Inherited Partial Sterility in Habrobracon. R. C. von Borstel (Oak Ridge, U.S.A.).

Inherited partial sterility has been shown to be the consequence of meiotic segregation of normal, translocated, and duplication-deficiency chromosomes in individuals heterozygous for a translocation. In the wasp *Habrobracon*, these can be detected by hatchability methods and confirmed by outcrossing and progeny-testing surviving haploid males from females giving low hatchability. One-half of the surviving males should

carry the translocation. After X-irradiation, twenty-seven cases of inherited partial sterility were found in *Habrobracon*. Of these, twelve of the females had hatchabilities between 30 and 40 per cent. Six were below 30 per cent, four were above 60 per cent, and five were between 40 and 55 per cent. By classic theory, it can be assumed that the 40-55 per cent class involved adjacent I segregation and the 30-40 per cent class involved mostly adjacent I and adjacent II segregation. The other classes require additional interpretations such as multiple chromosome involvements for those with hatchabilities lower than 30 per cent, and translocations involving chromosome ends for those with hatchabilities above 60 per cent. The time of death of the duplication-deficiency products is extremely regular among all of the induced cases, occurring at midembryonic stages of development. On the other hand, embryonic recessive lethal mutations are expressed at all stages of embryonic development from the blastula stage to hatching. Very few cases of inherited partial sterility are found in progeny from females irradiated in prophase I or metaphase I; inherited partial sterility is commonly induced after irradiation of males with mature sperm.

7.24. Crossing-over in the Sex Bivalent of Male Mammals. J. WAHRMAN and U. RITTE (Jerusalem, Israel).

A knowledge of the pairing relationships between the X and Y chromosomes of mammals and man is essential for an understanding of the heredity of X-borne genes, and especially of partial sex-linkage.

In the meiosis of *Apodemus mystacinus*, a murine species of Israel, pre- and post reductions occur normally side by side. Chiasmata can be directly demonstrated at first meiotic prophase. The occurrence of two disjunctional types may be observed at first anaphase and is confirmed by the inspection of secondary spermatocytes. Some of these contain X-dyads or Y-dyads resulting from pre-reduction, while others clearly exhibit the heteromorphic X-Y dyad awaiting post-reduction.

In *Apodemus sylvaticus* the sex bivalent is less favourable for analysis but a large proportion of secondary spermatocytes displays the X-Y dyad, which suggests the previous existence of a chiasma. Thus the examination of secondary spermatocytes may furnish critical evidence for the existence of chiasmata in species where these cannot be directly observed.

In certain mammalian species a small fraction of primary spermatocytes exhibit a chiasma between X and Y, although none can be seen in the majority of cells. Finally, in most mammalian species, including man, the X and Y appear in end-to-end configuration, which is generally attributed to non-chiasmic association. This point requires re-examination.

The occurrence of chiasmata in the male sex-bivalent, and therefore probably of genetic recombination, is firmly established in at least certain mammalian species.

7.25. Interchromosomal Effects on Crossing-over in Drosophila melanogaster. DAVID T. SUZUKI (Edmonton, Canada).

Crossing-over was measured in chromosome 3 of females heterozygous and homozygous for the X-chromosome inversions y^{3p}, y^{4}, sc^{4}, sc^{8}, sc^{9}, *dl-49*, w^{m4}, and rst^{3}. Crossover frequencies were increased in all inversion heterozygotes and in all but the sc^{9} and *dl-49* homozygotes. The lack of effect in sc^{9} and *dl-49* homozygotes indicates that the interchromosomal effects of inversion heterozygotes are not due to the intrinsic properties of the rearrangement *per se*. Since the inversions which had effects when homozygous have the X-tip apposed to heterochromatin (y^{4}) or heterochromatin located distally, it was suggested that the position of heterochromatin adjacent to the tip region was responsible for the interchromosomal effects of the inversions. Fragment-1 ($Y^{S}X^{\cdot}$), a normal X chromosome with the short arm of the Y chromosome attached to its tip, was tested and crossing-over was shown to be significantly increased in chromosome 3 of Fragment-1 homozygotes and heterozygotes. Since crossing-over was not changed in females homozygous and heterozygous for a normal X with Y-short attached to its centromere ($X^{\cdot}Y^{S}$), the distal position of the Y heterochromatin appears to be important for the interchromosomal effects of a Y-arm attachment.

7.26. The Mode of Interchromosomal Effect of Inversion Heterozygosity on Crossing-over Frequency in Drosophila melanogaster. TARVO OKSALA (Turku, Finland).

The mechanism by which inversion heterozygosity affects crossing over in heterologous chromosomes is still unknown. In order to elucidate this problem the author has carried out a few experiments in which the (structurally nor-

mal) X chromosome is the affected chromosome, the large autosomes representing different types of inversion heterozygosity. The X was divided into nine regions, each of which was tested separately in four parallel experiments: the standard autosomal homozygote as a control, the In(2L+2R)Cy heterozygote, the In(3L+3R)P heterozygote, and the combination of these two inversions. It turned out that in all regions the Curly+Payne combination is very much more effective in increasing the crossover value than these two inversions used separately. When the effect curves of Curly-, Payne-, and combined experiments are computed, all three prove to be nicely parallel, showing conspicuous peaks at both ends of the X and in the very middle of this one-armed chromosome (more or less around vermilion). This result suggests that all these types of inversion heterozygosity affect the X in basically the same way: they simply enable the X to produce more crossover points than normally. As there are now – instead of one or two crossovers – quite often three in the same tetrad, it is only natural that, owing to interference, they should be situated as far from each other as possible at regular intervals, i.e. at both ends and in the middle. This state of affairs is reflected in the three-peaked curves obtained.

Research supported by PHS Grant RG-6780.

7.27. The Centromere Effect on the Distribution of Exchanges in Drosophila Females. PETER E. THOMPSON (Ames, U.S.A.).

Tests of crossing-over with homozygous translocations have shown that a distal region attached near a centromere undergoes a reduction in exchanges. Conversely, a region normally lying near a centromere shows an increase in crossing-over when translocated to a distal position. This phenomenon is widely known as "the centromere effect on crossing-over".

In studies with these same translocations made heterozygous, the finding has generally been that crossing-over is reduced adjacent to the break, possibly as a result of synaptic ambiguities. The present investigation has shown, however, that marked regions very near the centromere produce *more* crossing-over if one of the homologues has a translocation break between the centromere and the markers. This has been strikingly true for chromosome 4, which normally does not undergo exchange in diploids, but which has produced appreciable frequencies of crossing-over in the presence of a translocation having a break near the centromere of 4.

This increased crossing-over in translocation heterozygotes applies only to those centric intervals which show a great disparity in cytological and genetic (crossover) length. Since the translocation has in effect removed only one of the adjacent centromeres, it is suggested that the normal distribution of exchanges is strongly influenced by some condition arising out of actual centromere pairing. The credibility of a repulsion of homologous centromeres prior to exchange is being examined.

These findings suggest a new interpretation of the "interchromosomal effect", based on switch pairing of homologous and non-homologous elements when rearrangements are present

7.28. Distal Crossing-over in Free-X and in Reversed Acrocentric Attached-X Triploid Drosophila melanogaster. J. D. MOHLER and JOHN C. NEELEY (Corvallis, U.S.A.).

Free-X triploids having normal chromosome structure give an estimated 6.0 per cent crossing-over for all X-chromosomes in the *y-w* region. The increase over the diploid frequency of 1 per cent accounts for nearly all of the distal increase reported for the longer *y-rb* region (Bridges and Anderson, 1925). In free-X triploids having crossing-over suppressed in one chromosome by *Ins* (1) sc^8, *dl-49*, the frequency of crossing-over in the *y-w* region between the normal chromosomes is 9.3 per cent, which is equivalent to a mean recombination frequency of 6.1 per cent for all X-chromosomes. Apparently two of the three chromosomes pair distally and yield cross-overs in roughly 9 per cent of the disjunction products. In uninverted triploids the non-pairing, non-crossover chromosome may be any of the three randomly; but in the inversion heterozygote the inverted X is usually the excluded chromosome.

The possibility of new investigation of this distal increase is opened by studies involving a reversed acrocentric attached-X triploid strain. The distal normal member of the attached-X probably pairs with the normal, free homologue, leaving the inverted (*Ins* (*1*) sc^8, *dl-49*), proximal chromosome unpaired. In this case the recombination frequency in the *y-w* region is 16.5 per cent. This secondary increase may be causally related to the initial increase over the diploid; but since our attached-X is deficient for some large part of the X-heterochromatin we are not yet able to distinguish between the various mechanical and physiological hypotheses that

have been offered previously (Schultz and Redfield, 1951).

The work of the senior author was done while receiving the support of a National Science Foundation Grant.

7.29. Crossing-over in Irradiated Females and Males of Megaselia (Phoridae). F. MAINX and E. DOSCHEK (Vienna, Austria).

Megaselia scalaris has become an interesting object for genetic studies in many respects. A new type of sex determination was discovered in this fly and reported on recently. Crossing-over occurs also in males. The crossing-over values are considerably reduced in males. The differing rates of this reduction depend on the localization of a given zone in the chromosome. Crossing-over values increase after irradiation of the pupae to a certain extent. This increase again differs for particular chromosomal zones in female and in male. Established on these results a new theory of crossing-over could be considered.

7.30. Experimental Analysis of Interchromosome Distribution and Genetic Significance of Chiasmata. H. K. JAIN and S. L. BASAK (New Delhi, India).

Observations on chiasma formation in structurally homozygous and heterozygous plants of *Delphinium ajacis* and other species, in which two or more groups of chromosomes could be recognized on the basis of size differences, have indicated the factors which control interchromosome distribution of chiasmata. An intraplant analysis first showed that chiasma frequency in an organism like *Delphinium* can be divided into an autonomous fraction which the different chromosome pairs claim independently of one another and a correlated fraction for which they show interrelationships. It has been found that the relative magnitudes of these two fractions depend primarily on the karyotype, particularly its symmetry. A highly asymmetrical karyotype such as in *Delphinium*, favours a relatively large interrelated fraction, which generates negatively correlated interchromosome distribution. A symmetrical karyotype on the other hand, like the one in *Chrysanthemum*, favours a disproportionately large autonomous fraction which is associated with absence of interchromosomal effects, or with positively correlated chiasma formation in different pairs. It has been found possible to induce an interchromosome redistribution of chiasmata in *Delphinium* following elimination of one or two of the chromosome pairs from forming chiasmata—a condition brought about in a number of plants, raised from X-irradiated seeds. The interchromosomal effects for chiasma formation in the normal and the X_1 plants find a close parallel in the "Schultz-Redfield" effect for genetic recombination and the present analysis, thus, provides further evidence for a correspondence between chiasmata and genetic recombination. A one to one relationship between chiasma formation and chromatid exchange has also been demonstrated in two interchange heterozygotes of *Delphinium*, in which it was found possible to record observations on chiasma formation in the interstitial segments of the interchange multiple and on anaphase separation of the chromatid parts involved.

7.31. Autosomal Behavior in Claret-nondisjunctional Drosophila Females. GALE DAVIS (Oak Ridge, U.S.A.).

High frequencies of exceptional behavior of chromosomes X and 4 were found by Lewis and Gencarella (1952) in progenies of female *Drosophila melanogaster* homozygous for ca^{nd}. Studies reported here were undertaken to determine the extent of chromosome-4 nondisjunction and to clarify patterns of chromosome behavior under the influence of ca^{nd}.

Mating systems were devised to test nondisjunction and loss of chromosomes X and 4 simultaneously. Females having y/y; ca^{nd}/ca^{nd}; ci^{D}/spa^{Cat} were mated to males having a compound chromosome 4. The resultant progeny reflected 63.4 per cent nullo-4, 3.2 per cent diplo-4, and 33.4 per cent mono-4 egg types, and 18.3 per cent nullo-X, 2.7 per cent diplo-X, and 45.5 per cent mono-X egg types among recovered offspring. The X-chromosome classes contained an apparently nonrandom distribution of chromosome-4 types, leading to the conclusion that X and 4 were involved coincidentally in nondisjunction as well as loss. Nondisjunction and loss of chromosome 2 was studied by mating ca^{nd} females to males having a compound chromosome 2. Estimates of frequency of chromosome-2 nondisjunction were not possible because behavior of compound autosomes in the male was poorly understood. The ratio of recovered nullo-2: diplo-2 egg types (2:1) con-

trasted to those observed independently for nullo-X: diplo-X (6:1) and nullo-4: diplo-4 (20:1), yet these same ratios of X and 4 egg types concomitant to chromosome-2 nondisjunction were essentially unchanged.

This investigation was supported by U.S. Public Health Service Predoctoral Fellowship No. GPM-13,926-C1 from the National Institutes of Health.

7.32. Nondisjunction in Drosophila hydei. T. G. GREGG (Oxford, U.S.A.).

A previous report on nondisjunction in *Drosophila hydei*[1] indicated that the rate of primary nondisjunction in *hydei* females was somewhat higher than the rate in *Drosophila melanogaster* females. On the basis of recent work[2] it has become apparent that primary nondisjunction in *melanogaster* females results from the failure of two x-chromosomes from a no exchange tetrad to engage in distributive pairing during meiosis. It also seems that distributive pairing depends largely, although not exclusively, on the centric heterochromatin. On the basis of these latter observations one might expect the rate of primary nondisjunction in *hydei* females to be much lower than the rate in *melanogaster* females, provided the mechanisms involved in meiosis are similar in the two species. This expectation is based on the unusually large amount of heterochromatin in the *hydei* X which should facilitate distributive pairing, and on the fact that the genetic length of the *hydei* X is twice as great as the *melanogaster* X which should result in fewer no exchange tetrads. Data have been collected that indicate that the rate of primary nondisjunction in *hydei* females actually is markedly and significantly lower than the rate in *melanogaster* females. The difference in rate between this report and Spencer's report probably lies in Spencer's use of the markers bobbed and Notch, which he himself suggested might increase the rate of nondisjunction. Data in this report also indicate that in *hydei* females the rate of nondisjunction does not increase with age.

1. SPENCER, *J. Ohio Acad. Sci.* 1930.
2. See especially GRELL, *Proc. Natl. Acad. Sci. U.S.* 1962.

7.33. Evidence from Some Unusual Mutants Concerning the Nature of t-alleles in the Mouse. M. F. LYON (Harwell, Great Britain).

"Mutation" of *t*-alleles in the mouse results usually from crossing-over in an abnormal chromosome region. Study of the "mutants" should therefore reveal whether the different properties of *t*-alleles can be ascribed to linearly arranged factors or depend on the whole length of abnormal chromosome. The properties of the allele t^6 include: modification of expression of dominant alleles at the *T*-locus, homozygous lethality, male sterility, abnormal male segregation ratio, and crossover suppression. Mutants derived from t^6 show that the lethal factor is not at the *T*-locus but is close to the nearby locus of *tf*, while the *T*-modifying factor is very close to the *T*-locus. The allele t^{h7}, derived from t^6, decreases the expression of *T* rather than enhancing it as t^6 does, and can mutate back, by crossing-over, to the *T*-enhancing form. This suggests that it carries a duplication of the *T*-modifying factor, and hence that this factor has a gene activity which is reduced, but greater than half normal. Another mutant, t^{h15}, also derived from t^{h7} by crossing-over, has a new lethal factor, complementary to the t^6 lethal factor.

These facts suggest the hypothesis that the fundamental chromosome abnormality in *t*-alleles is reduced gene activity combined with loss of specific pairing. This would lead to crossover suppression, unequal crossing-over, and hence to duplications and deficiencies. A functional change in nucleic acid (e.g. to heterochromatin) would provide a better explanation than a structural one (e.g. inversion).

7.34. Cytological Investigation on the T-locus in Mus musculus L. IRENE GEYER-DUSZYŃSKA (New York, U.S.A.).

The T-locus in mice is known as a most complex and odd one. Cytological analysis has revealed, that this complexity is due to the fact that lethal t-alleles are not point mutations but deficiencies located in various regions of the chromosome. The size and the position of the deficiency is for a given t-allele constant. The analysis of compounds and translocations T190 and T138 gave additional evidence that the deficiencies found are located in the chromosome of linkage IX group, and connected with t-alleles. Cytogenetically this clromosome can be described as follows: centromere—T—tf—Ki—Fu—H2—small deficiencies (t^{w18} and t^{w6})—

large middle deficiencies (t^{-} and t^{w1} groups) of terminal deficiency (t^{12}). The spatial order — deficiencies on the chromosome fits well with the pattern of action of t-alleles which, when homozygous are embryonic lethals, and indicates that genes involved in different embryonic processes are grouped in different regions of the chromosome of.

Lethal t-alleles suppress recombination in the region of 8-10 crossover units between T and tf; it is suggested that this effect is due to asynapsis accompanying a deficiency and spread on other chromosomal regions. The possibility that in the origin of a new t-allele which is connected with the presence of an old t-allele and with exceptional recombination within the region where recombination usually is suppressed by this allele, effective pairing occurring in the time of chromosome reduplication can be involved, will be discussed.

SECTION 8

CYTOTAXONOMY AND EXPERIMENTAL TAXONOMY

8.1. Cytology, Morphology, and Evolution in the Proteaceae. L. A. S. JOHNSON and BARBARA G. BRIGGS (Sydney, Australia).

The Proteaceae have a predominantly Southern Hemisphere distribution, in Australia, Africa and South America, extending into both eastern and western tropics. A fairly complete survey, chiefly at the generic level, suggests that the "Proto-Proteaceae" had 7 pairs of large chromosomes, as found today in *Placospermum*, *Persoonia*, and *Garnieria*. Reduction to 5 pairs of moderate size is represented in *Bellendena*, with subsequent tetraploidy to 10 pairs in *Symphionema*. In the remainder of the family, ancestral doubling of the original 7 is indicated; all of these genera have very much smaller chromosomes. $n = 14$ occurs in both subfamilies Proteoideae and Grevilleoideae, but repeated and independent reductions to $n = 13$, 12, 11 and 10 appear to have taken place. So far as known, numbers within genera are constant, except for one case of tetraploidy (*Persoonia*) and one of aneuploid increase (*Orites*, $n = 14$, 15). The changes in chromosome number and size appear to have occurred at early stages in the evolution of the family.

Comparative study of all morphological features, together with cytology, has made it possible to reconstruct the probable ancestral conditions for the family and for its various subgroups – a condition most nearly represented today in the relict *Placospermum* (N.E. Queensland). A scheme of relationships and evolutionary trends has been worked out, taking into consideration adaptive changes in relation to habitat, pollinators, and distribution. A tropical origin is probable, with the temperate members in Australia and South Africa independently derived from tropical sources, although the phytogeographic history is complex.

See *Austr. Journ. Bot.* **11**, No. 1, in press.

8.2. The Role of the Plastome of Oenothera in Evolution. W. STUBBE (Cologne, Germany).

Cleland (1957, 1958) has elaborated the outlines of the phylogenetic relationship between the complex heterozygotic races of the subgenus *Euoenothera* and their homozygotic ancestors. The diversity of species was derived from three fundamental groups of complexes carrying, respectively, the genes for (a) the hookeri-strigosa-phenotype, (b) the grandiflora-biennis-phenotype and (c) the argillicola-β parvi-flora-phenotype.

Independent investigations of Stubbe (1959) demonstrated that the same major groups of genome complexes stand out when attention is confined to the co-operation between genomes and plastomes. Five plastid types can be identified, their distribution being of systematic importance. On the basis of recent results the following facts can be established about the role of the plastome in evolution:

1. All known kinds of damage to the normal plastid development lead to a negative selection. Sometimes these damages arise by spontaneous mutation, but more frequently we find them after interspecific hybridization. Since the pollen contributes plastids to the zygote, though less than the egg cell, we get a somatic segregation-pattern of different plastid phenotypes, these being green or chlorophyll-deficient, depending on a good or bad compatibility between genome and plastome. The viability of such chimaeras depends upon the size of their green foliage portion.

If not only one but both kinds of parental plastids are incompatible with the hybrid genotype, the plastid differences result in a barrier for hybridization, i.e. a factor establishing isolation. Such plastome-dependent incompatibility has been demonstrated in crosses between the homozygous species of the hookeri-, elata- and grandiflora-group on one hand and the argillicola-group on the other.

2. In hybrids in which the genetically different plastids of both parents develop normally, there will be an invisible somatic segregation-pattern. Concerning evolution, another factor brings its influence to bear, namely plastid competition (Schötz, 1954). This is due to differences in the multiplication rates of the plastid types taking effect in the "mixed" cells. If outcrossing is guaranteed, repeated mixing of the different

plastids occurs in the zygotes, and the subsequent plastid competition will lead to a gradual elimination of the plastid type which multiplies more slowly. It is a proven fact that this process did happen during phylogeny, since wild species which have been shown to be compatible with more than one plastid type, now possess the faster multiplying plastid type.

3. Sometimes the incompatability between certain genomes and plastomes is expressed even in the microspores by inhibited germination of living pollen grains or by early cessation of pollentube growth. As this character shows up mainly after hybridization, Renner (1919) regarded it as the mechanism responsible for the elimination of one complex in the pollen. This mechanism would tend to make the complex-heterozygotic species heterogamous. Species of the parviflora-groups would seem to offer examples for this mechanism. Furthermore, based on experimental results about plastid competition and about the compatibility correlations between the five wild type plastids and the genotypes of the subgenus *Euoenothera*, the phylogeny of the five plastomes of this subgenus are being inferred step by step in an unambiguous manner.

8.3. Chromosome Differentiation in Genomes of Gossypium. M. S. Brown (College Station, U.S.A.).

Chromosomes of species and F_1 hybrids of *Gossypium* are compared at pachytene, diplotene, diakinesis and metaphase. At pachytene, all chromosomes pair closely, even in sterile hybrids between species with chromosomes of unequal size and low bivalent number at metaphase. At diplotene, genome differences become apparent; and at diakinesis and metaphase, size differences in chromosomes are shown to be correlated with amount and distribution of chromatic material. In monosomics of *Gossypium hirsutum*, an allotetraploid, chromosomes of A and D genomes can be distinguished in like manner. These differences in chromosome size and composition can be correlated with variation in the cytosine/5-methylcytosine ratios as determined in A, B and D genomes by Ergle and Katterman.

8.4. Signification of Cytological Data for the Taxonomic Conception of the Leguminosae. J. A. Frahm-Leliveld (Wageningen, The Netherlands).

Chromosome studies in several leguminous genera made by various investigators suggest heterogeneity within the genera as to base number, chromosome dimensions and shape.

Investigations in *Indigofera* from West and East Africa, and in a few Himalayan and East Asiatic species, reveal differences in base number, shape and size between the various species, and a few cases of polyploidy.

Recent data for the genus *Vigna* show two base numbers, 10 and 11, this in accordance with earlier reports, and considerable differences in chromosome dimensions within the *Vigna unguiculata* complex. Grafting experiments between some cultivars of the cultivated cowpeas resulted in a success of 80-90 per cent when using types with equal chromosome dimensions, and a success of below 50 per cent in the case of deviating dimensions.

There exist several other genera, e.g. *Trifolium*, *Astragalus*, *Lespedeza*, in which either ranges of base numbers or entirely different base numbers are present. Sometimes such a difference agrees with different gene centres. In some case loss or increase of chromosomes has been suggested.

Another line of thought next to those mentioned above may be the acceptance of polyphyletic evolution: the same lines of development acting on different original substrates under similar circumstances, but at various points of time may result in convergent morphological types brought under an equal denomination by the taxonomists.

8.5. Desynapsis in Lotus Hybrids. William F. Grant (Quebec, Canada).

In meiotic analyses of several embryo cultured interspecific hybrids between six diploid ($2n = 12$) species of *Lotus* (Leguminosae), closely related to *L. corniculatus*, univalents, resulting from precocious separation of the bivalents, were observed at diakinesis and metaphase I. In some cells up to 50 per cent of the chromosomes were observed as univalents. Univalents, however, were present in only 40 per cent of the cells examined. Desynapsis is considered to be primarily of the "weak" type as defined by Prakken. In some cases, at metaphase I, the univalents were observed to be loosely connected. At anaphase I, lagging chromosomes were observed, most of which consisted of univalents. Occasionally lagging chromosomes were observed to remain behind in the cytoplasm at telophase I and to fail to be incorporated within the restitution nuclei. In some cases the telophase I chromosome complements consisted of more, or less, than the

normal chromosome number as a result of unequal distribution of the chromosomes at anaphase I. The loose bivalents observed in a number of cells at metaphase I signify some lack of specificity in pairing of the homologous chromosomes which may represent the visible remnants of the effects of earlier translocations through which species differentiation has occurred. The high percentage of pollen sterility and the low seed set in the hybrids may partly be caused by extensive intrachromosomal rearrangements, since it is difficult to reconcile the very low fertility with the relatively low frequencies of visible cytological anomalies only. The precocious division of the bivalents is considered, therefore, to be the result of segmental and genetic differences between the parental species. The hybrids exhibiting this desynaptic behavior were found in crosses between *L. japonicus* × *L. schoelleri*, *L. krylovii* × *L. filicaulis*, *L. japonicus* × *L. alpinus*, and *L. schoelleri* × *L. krylovii*.

8.6. The Phylogenetic Riddle of Astragalus (Leguminosae). G. F. LEDINGHAM (Regina, Canada).

Chromosome counts of 252 species of *Astragalus* have been made. The species counted fall into two distinct groups; the 163 Old World species are based on an 8-chromosome series with 23 per cent polyploidy while the 89 New World species counted form an "aneuploid" series with $n = 11$ (39 species), $n = 12$ (34), $n = 13$ (12), or $n = 14$ (3) and $n = 22$ (1), the last species being the only polyploid. Ten Old World species (6 with $2n = 16$ and 4 with $2n = 32$) have spread recently, probably during the Pleistocene, from N.E. Asia into N.W. America, but these are still easily recognized by their morphological similarity to other Old World species. There is no indication of an aneuploid series among Old World species of *Astragalus* and no deviation from the 8-chromosome number series except for *A. boeticus* ($2n = 30$), *A. pentaglottis* ($2n = 28$) and *A. somalensis* ($2n = 20$) and these occur in the Mediterranean area or in Africa. Thirty species of *Oxytropis* have been counted and these all have $n = 8$ or some multiple of 8, 36 per cent polyploidy. *Oxytropis* seems to be an integral part of Old World *Astragalus*. The origin and significance of the consistent differences between New and Old World phylogenetic lines of *Astragalus* is discussed.

8.7. Cytogenetics of Vicia cracca and V. tenuifolia. ARNE ROUSI (Piikkiö, Finland).

Vicia cracca L. consists of three chromosome races ($2n = 12$, $2n = 14$ and $2n = 28$). The two latter ones are morphologically close to each other and have basically similar karyotypes. The karyotype of the 12-chromosome race suggests an origin from the 14-chromosome race as a result of one or probably two interchanges and the subsequent loss of one chromosome. Systematically it seems to tally with the taxon *V. cracca* subsp. *Geraldi* (All.) Gaudin. Spontaneously arisen albinotic and inviable seedlings with 13 chromosomes were interpreted as hybrids between the 12- and 14-chromosome races on the basis of their karyotypes and hemagglutinating properties. This suggests that these races are effectively isolated by means of hybrid inviability. Hybridization between the self-incompatible 14- and 28-chromosome races was attempted by placing solitary potted individuals of the former among populations of the latter. The seed set of the diploids was very low, but one viable triploid and one tetraploid arose, the latter being apparently a result of an unreduced egg cell. Although there is a selection against triploids the isolation between the 14- and 28-chromosome races is therefore not complete. *V. tenuifolia* Roth occurs as a diploid ($2n = 12$) and a tetraploid ($2n = 24$) race, the diploid being rare and somewhat distinct. The karyotypes of both are basically similar to the 12 chromosome race of *V. cracca*. Certain morphological resemblance between this race and tetraploid *V. tenuifolia* suggests a close evolutionary relationship.

8.8. Cytological Studies in Melilotus Polyploids. J. JARANOWSKI (Poznań, Poland).

Inducement of artificial polyploids in the genus *Melilotus* was mainly intended to utilize them in the attempts to overcome the barriers of reproductive isolation in species hybridization. Owing to their peculiar properties these polyploids were closely examined and cytologically analysed to obtain some information on the relationship of species.

The basic number of chromosomes for the genus *Melilotus* is $n = 8$. Fourteen species, 7 varieties 3 species hybrids (*M. alba* × *M. officinalis*), and 33 different forms of backcrosses of the species hybrids were treated with colchicine (0·2-0·7 percentage conc.) to induce polyploidy.

The number of polyploids obtained in the different species varies greatly. Biennials (belonging to the section *Eumelilotus*) and annuals (belonging to the section *Micromelilotus*) responded quite differently to colchicine treatment

The latter forms gave a much larger proportion of polyploids. In the biennials it was more difficult to induce polyploidy among others because—in cases when the action of colchicine on the hypocotyl was not strong enough—their second-year spring growths were diploids. In addition, there often occurred mixoploid forms. Some of the species are exceptionally resistant to the mutagenic action of colchicine (e.g. *M. suaveolens*, *M. volgica*, *M. taurica*). A 0.7 per cent solution of colchicine failed to bring about changes in these species. Progressive rises of the colchicine concentrations up to a strength, resulting in a total destruction of plants, did not induce polyploidy.

In regarding pollen viability as a criterion of fertility, the induced polyploids greatly varied in this respect. Some autotetraploids like *M. officinales* and *M. alba* yielded nearly 100 per cent of viable pollen, while others like *M. messanensis* only 10–15 per cent; *M. hirsuta*, *M. segetalis* and *M. italica* about 50 per cent. Frequency in the occurrence of quadrivalents, univalents and polyvalents in meioses accounts partly for this situation.

Analyses of pollen viability *in vitro* show that polyploidization changes the physiologic reaction of pollen grains to the medium and to the optimal temperature of germination. Differences between species are great.

Inducement of polyploidy failed to overcome self-incompatibility in *M. officinalis*.

8.9. (D). Evolution in the Genus Mentha from a Genetic Viewpoint. M. J. Murray (Kalamazoo, U.S.A.).

The fertile species in the spicate-flowered section of the genus are *Mentha rotundifolia* ($2n = 24$), *Mentha longifolia* ($2n = 24$), *Mentha spicata* ($2n = 48$) and *Mentha aquatica* ($2n = 96$). The sterile species are *M. spicata* ($2n = 36$), *M. niliaca* ($2n = 36$), and *M. piperita* ($2n = 72$). All colchicine-induced *4n's* are fertile. F_1 and F_2 hybrids between the basic species are intermediate in appearance and do not resemble *M. spicata*; but hybrids between *M. spicata* and *4n M. rotundifolia* or *4n M. longifolia* are perfectly fertile. *M. spicata* strains are highly variable in leaf size, leaf shape, crispness of leaves and plant habit. Contrary to popular belief, *M. spicata* individuals do not invariably have a spearmint odor with the ketones carvone and dihydrocarvone. Some strains have the ketones menthone and pulegone as also found in the species *M. aquatica* and *M. piperita* (genotypes ccAA of ccAa), while others have piperitone and piperitenone as found in the basic species (genotype ccaa). Selfed heterozygous strains (CcAa) give a ratio of 12:3:1 for the 3 ketone types. *M. aquatica* with 40 per cent menthofuran and the menthone–pulegone ketones can be hybridized to *M. spicata* (menthone–pulegone or piperitone-piperitenone genotypes) to resynthesize *M. piperita* ($2n = 72$). These peppermint-odored hybrids are of substandard commercial quality in having too low menthol, too high ester, and tremendous differences in minor constitutents (terpene hydrocarbons of which there are at least 20). They differ greatly in plant habit, leaf size, leaf shape, leaf crispness depending upon the *M. spicata* parent used in the cross. Spearmint-odored hybrids occur in all crosses where the *M. spicata* parent is spearmint-odored. Diploid chromosome numbers 60, 72, 84 and 96 may occur in other peppermint-odored interspecific combinations.

8.10. Cytological Studies in Section Molium of the Genus Allium. S. E. Eid (Shatby, Egypt).

The following results have been obtained:

I. *A. blomfieldianum* Asch. & Schweinf. has $2n = 14$ chromosomes with median (m) or submedian (sm) centromeres.

II. *A. neapolitanum* Cir. shows two forms, an allotriploid with $2n = 21$ m and sm chromosomes, and an allotetraploid with $2n = 28$ m and sm chromosomes. The triploid shows nonpairing of one set of seven chromosomes and is sterile.

III. *A. erdelli* Zucc. (including its v. *roseum* Boiss.) has $2n = 16$ m and sm chromosomes with four telocentrics.

IV. *A. roseum* v. *tourneuxiii* Boiss. shows two cytological forms, a diploid with $2n = 16$ m and sm chromosomes with two telocentrics; and an autotriploid with $2n = 24$ m and sm chromosomes with three telocentrics. *A. roseum* v. *bulbilliferum* Vis. is found to be an allopentaploid with $2n = 40$ m and sm chromosomes with five telocentrics. It is sterile and viviparous.

The basic number in the present species is shown to be seven, with centric breakage of metacentrics into telocentrics in *A. erdelli* and *A. roseum*.

8.11. Species Relationship in the Genus Avena. J. H. W. Holden (Durham, Great Britain).

The diploid species *Avena longiglumis* shows

extensive chromosomal differentiation from all other diploid species. The nature of this differentiation and its significance in the study of species relationships in diploid, tetraploid and hexaploid *Avenae* will be discussed.

8.12. Some Experimental Data on the Range of the Species Hordeum spontaneum C. Koch emend. Bacht. F. Kh. Bakhteyev (Leningrad, U.S.S.R.).

Until lately it was thought that *Hordeum spontaneum* includes only two-rowed forms. However, the discovery in 1958 in the Turkmenian Soviet Republic territory of a six-rowed wild-growing barley, conditionally called *Hordeum lagunculiforme* Bacht., made it necessary to reconsider this question, especially as somewhat later similar six-rowed wild barleys were found in the Azerbaijan and Tadjik Republics, as well as in some other localities of the U.S.S.R.

The author's own large original materials enabled the conclusion to be drawn that *H. lagunculiforme* is actually not an independent species, as one could suppose up to the present time, but represents another extreme link in the system of the species *Hordeum spontaneum* C. Koch emend. Bacht. The latter includes four strains: (1) var. *spontaneum* (= *H. spontaneum* var. *ithaburense* [Boiss.] Nábělek); (2) var. *ischnatherum* (Cosson) Thell.; (3) var. *proskowetzii* Nábělek; (4) var. *lagunculiforme* Bacht. Thus, we may consider it proved that *Hordeum spontaneum* C. Koch emend. Bacht. includes not only two-rowed individuals, as was thought since the first description of this species by C. Koch in 1848, but that it involves also six-rowed forms.

The above data should be regarded not only as a new illustration to the splended theoretical conception of N. I. Vavilov, "The Linnean species as a system", but as a real basis for further experimental investigations into the origin, phylogeny, and evolution of cultivated barley.

In principle one may probably agree with the conception, that the initial six-rowed and two-rowed cultivated barley forms originated from the genetically nearly-related *Hordeum spontaneum* C. Koch emend. Bacht.

8.13. Evolutionary Relationships between Two South American Species of Hordeum. Juan H. Hunziker and Leonor Maumús (Buenos Aires, Argentina).

Hordeum halophilum and *H. muticum* are two diploid sympatric species distributed along the mountains from Peru to Argentina. The morphology, chromosomal meiotic behaviour and karyotypes of these two species and of their natural sterile hybrid have been studied.

Meiosis in the parental strains is regular or nearly so. *H. halophilum* had the following mean chromosome associations: 6·99 II, 0·02 I, and *H. muticum* 6·81 II, 0·38 I, while the hybrid had 0·02 IV, 0·01 III, 6·63 II, 0·62 I. The meiotic behaviour of the chromosomes in the hybrid as well as the karyotypes indicate that the genomes of both species are largely homologous. They differ, however, in at least one reciprocal translocation and one paracentric inversion.

The main isolating mechanisms between both species seem to be the slightly different edaphic requirements and the absolute sterility of the hybrids that makes gene interchange impossible.

At anaphase II 8.5 per cent of the cells had 1–3 "dineocentric" bridges. These originated from activation of usually terminal or more rarely interstitial neocentromeres, each stretching the laggard and causing the formation of a bridge, with the centric region attenuated and stretched. This phenomenon has already been observed in other grass hybrids (Hunziker and Covas, *Rev. Investig. Agric.* **9** (3), 155-175, 1955; Walters, M. S., *Univ. of Calif. Public. in Bot.* **28** (6), 335-447, 1957, etc.).

8.14. Genome Construction Within the Triticinae. L. E. Evans (Winnipeg, Canada).

The two synthetic amphiploids *Triticum durum* var. Carleton—*Aegilops squarrosa* (AABBDD) and *Triticum durum* var. Stewart—*Agropyron elongatum* (AABBEE) when crossed produced a partially fertile F_1 having an average meiotic metaphase configuration of $14^{I}+14^{II}$. By means of cytological selection in the F_2 to F_7 generations 32 moderately stable, fertile, hexaploid derivates were obtained from this cross. These individuals should possess the 14 pairs of *T. durum* chromosomes (AABB) plus a mixture of *Aegilops* (DD) and *Agropyron* (EE) chromosomes in the third genome. Several of these lines were backcrossed to the original hexaploid parents and the backcross F_1 plants were cytologically studied in order to determine the number of *Aegilops* and *Agropyron* chromosomes in the third genome. The combinations that theoretically could be produced range from 7D+0E to 7E+0D chromosomes and the results indicate that the entire range may be produced. The identity of the spe-

cific D and E chromosomes in each derivative has not been determined.

If this phenomenon has occurred in nature it may explain the difficulty encountered in determining the exact parentage of such naturally occurring allopolyploids as *Triticum aestivum*.

8.15. DNA and Wheat Ancestry. H. Rees and W. I. C. Davies (Aberystwyth, Great Britain).

It is generally agreed that the AA chromosomes found in the AABB tetraploid and AABBDD hexaploid wheats are derived from *Triticum monococcum* or a closely related diploid species, and that the DD chromosomes are derived from *Aegilops squarrosa*. There is some doubt concerning the source of the BB chromosomes. The chief candidates are *Ae. speltoides*, *Ae. bicornis* and *Agropyron triticeum*. From an analysis of the DNA content of nuclei in the polyploid and diploid species our evidence shows that *Ae. speltoides* is the most likely contributor of the BB chromosomes. Its nuclear DNA content added to that of *T. monococcum* (AA) agrees with the values found in AABB (*T. durum*), and in combination with AA and DD values agrees exactly with the DNA value for AABBDD (*T. aestivum*). The DNA values for *Ae. bicornis* and *A. triticeum* are, compared with that for *Ae. speltoides*, too high and too low respectively.

8.16. Cytotaxonomic Studies in the Genus Agropyron Gaertn. Jurgen Schulz-Schaeffer, Peter Jurasits, Helmut Lorenz and Penelope W. Allderdice (Montana, U.S.A.).

Within the framework of a biosystematic investigation in the genus *Agropyron* studies of cytological characteristics are being carried out for taxonomic purposes. Karyotypes of 33 *Agropyron* species were determined from root tip cells and were compared. In general, centromeres are median or submedian. *Agropyron triticeum* ($2n = 14$) and *A. orientale* ($2n = 28$) are exceptions to this rule. Nuclear organizer chromosomes were compared. According to an evolutionary concept, one nucleolar organizer chromosome should be present for each basic genome. This was almost averaged in previous studies of the genus *Bromus*. Only about half of the expected number were observed in *Agropyron*. There seems to be a considerable difficulty involved in detecting certain nucleolar organizer chromosomes in mitotic metaphase. Investigations in meiosis are performed to supplement. Eighty-eight nucleolar organizer chromosomes were observed in 160 genomes. They can be classified as 11 different types. One type (F-1, F-2) occurs in 19 of the 33 species. Type F seems to represent one basic genome which probably is common to a great number of *Agropyron* species. The nucleoli as a possible measure of nucleolar organizer chromosome number were determined by the Rattenbury-method for 5 species. The average number was, in general, corresponding with the number of basic genomes. An average of 4 nucleoli was found for tetraploid *A. desertorum*. This may confirm unpublished evidence from coworkers that *A. desertorum* has a certain number of nucleolar organizer chromosomes.

8.17. Karyotypic Studies of South American Snakes. W. Beçak, M. L. Beçak and H. R. S. Nazareth (São Paulo, Brazil).

The sex elements can be readily identified by cytological means in the chromosome complements of most mammalian and avian species. In the lower vertebrates, however, the Z and W, or X and Y, may still be so undifferentiated as to be morphologically indistinguishable.

Karyotypic studies have been made of two species of South American snakes, *Boa constrictor amarali* and *Bothrops jararaca*. Both have 36 chromosomes: 16 macrochromosomes and 20 microchromosomes.

In *Boa constrictor amarali* the centromere is almost median in the first, third and fourth pairs, submedian in the second pair and subterminal in the fifth, sixth, seventh and eighth pairs. Neither quantitative evaluation nor direct observation of the karyotypes revealed the presence of a heteromorphic sex pair in either the male or female of this species.

In the male of *Bothrops jararaca*, the first, third and fourth pairs have submedian centromeres and the sixth and eighth pairs have subterminal centromeres. In the female, however, the fourth pair is distinctly heteromorphic. One element is much smaller than the other and has a subterminal centromere as well. Thus in this species the fourth largest pair of chromosomes appears to be the sex pair; the female is the heterogametic sex, and the sex-determining mechanism is the ZZ-ZW type. The fact that the W chromosome appearing only in the heterogametic female is seven-tenths the size of the Z, permits cytological identification of the sex chromosomes in *Bothrops jararaca*.

8.18. Chromosome Studies of Domestic Mammals. J. F. LOPEZ-SAEZ and G. GIMENEZ-MARTIN (Madrid, Spain).

In this work the chromosome complement(s) of the horse (*Equus caballus*), ass (*Equus asinus*), mule (*Equus caballus* × *Equus asinus*), bull (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*) and domestic pig (*Sus scrofa domesticus*) are studied. The karyotype of each species is as follows:

The horse (*Equus caballus*) $2n = 64$. The X chromosome has submedian centromere and is in size the second of the complement; the Y chromosome is telocentric or apparently telocentric, being in the complement two pairs smaller than it.

The ass (*E. asinus*) $2n = 62$. The X chromosome has subterminal centromere and is in length the fourth pair of the complement; the Y chromosomes, with submedian centromere, is the smallest chromosome.

The mule (*E. caballus* × *E. asinus*) $2n = 63$. The male has the X chromosome of the horse and the Y chromosome of the ass; the female has the X chromosome of the horse and the X chromosome of the ass.

The bull (*Bos taurus*) $2n = 60$. The sex chromosomes are the only ones with metacentric or submetacentric centromere. The X chromosome is the longest of the idiogram and the Y chromosome is among the five shortest chromosomes, but it has median centromere.

The sheep (*Ovis aries*) $2n = 54$. The X chromosome is the longest of the telocentric or apparently telocentric chromosomes and the Y, also telocentric, is the smallest of the idiogram.

The goat (*Capra hircus*) $2n = 60$. All chromosomes are telocentric or apparently telocentric. The X chromosome is the second in the haploid idiogram and the Y is the smallest of the complement

The pig (*Sus scrofa domesticus*) $2n = 38$. The X chromosome is metacentric and the Y chromosome is the smallest of the karyotype. This is characterized by a median centromere.

8.19. The Karyotype of Normal Pigs and of One Intersex. B. HENRICSON and L. BÄCKSTRÖM (Stockholm, Sweden).

The karyotype of a swine intersex has been analyzed in blood leucocytes grown in tissue culture. Also external and internal genitalia have been investigated.

As the karyotype of normal swine has been described in only a few earlier works, this is also presented here with suggestions for grouping and numerical classification.

The intersex investigated had a karyotype which contained 36 apparently normal autosomes with sex chromosomes of the XX type.

The external genitalia consisted of a vagina with a relatively big clitoris. There were two testicles of about normal size. One was situated in the scrotum, the other was abdominal. No germ cells were present, only Sertoli cells in well-defined tubuli and Leydig cells in the interstitium. No ovarial tissue could be detected. Priitive ductuli deferentes from the testicles went over in a rather well-developed uterus.

Pictures are presented.

8.20. Classification of the Chromosomes of the Mouse Karyotype. MARCO CRIPPA (Rome, Italy).

Actively proliferating cultures of cells obtained by trypsinization from embryonic kidneys of C3H and albino Swiss mice were submitted to the techniques usually employed for chromosome studies: colchicine and hypotonic treatment, air-drying and orcein staining. By arranging the 40 chromosomes of the normal mouse diploid number according to the criterion of decreasing length, it was possible to subdivide them into five distinct groups, each composed by chromosomes of similar length. The first group includes 4 chromosomes, the second 7 chromosomes in the male and 8 in the female, the third 20 chromosomes, the fourth 6 chromosomes and the fifth 3 chromosomes in the male and 2 in the female. According to this classification it seems reasonable to assume that the X chromosome belongs to the second group and the Y chromosome to the fifth group. The X chromosome was never observed to have any peculiarity like heterochromatic traits or secondary constrictions which could differentiate it from the autosomes of the same group. Nor was it possible to identify the Y chromosome within the small chromosomes of the last group. The length of each chromosome was measured and the data obtained were statistically evaluated by using the analysis of variance. This analysis showed that the differences in chromosome length among the five groups are highly significant, although a variability among different mitoses is present to a certain extent. Further details concerning the data obtained by the statistical analysis will be discussed.

8.21. On the Chromosomes of the Geometrid Moths Cidaria. E. SUOMALAINEN (Helsinki, Finland).

In the species-rich Geometrid moth genus

Cidaria, the chromosome number of 40 Finnish species has been determined, i.e. of about half the number of species found in Finland. The chromosome number has been determined from oogenesis, but in some species spermatogenesis has also been studied. The commonest (haploid) number among the *Cidaria* species so far investigated is 31, which is likewise the next commonest number among the Lepidoptera as a whole. Thirteen species had this number. Most (32, or 80 per cent) of the species had a chromosome number between 28 and 32, like most of the other groups of Lepidoptera. No species had more than 32 chromosomes, whereas eight had less than 28. The smallest chromosome numbers found were 25 (two species), 19, 17 and 13 (three species and probably also a fourth one). The great differences in chromosome number between closely related species is of interest. Such discrepancies are shown by the subgenera *Thera* (*variata* and *obeliscata* 13, *firmata* 19, and *juniperata* 30), *Lampropteryx* (*minna* 17 and *suffumata* 32), and *Hydrelia* (*testaceata* 13 and *flammeolaria* 30). The chromosomes are clearly bigger in the species with a low chromosome number than in those with a high one. Photometric measurements revealed that the DNA-content of closely related species is almost the same, in spite of great differences in chromosome number. This indicates that one chromosome of a species with a low number corresponds to two or more chromosomes of another one with a high number. Consequently, we are not concerned with polyploidy, although the higher number is close to a multiple of the lower one. It is probable that this kind of chromosomal evolution—found among the Lepidoptera—is rendered possible by the fact that they obviously have a diffuse kinetochore. Contrary to earlier assumptions, chiasmata are not formed in the bivalents during oogenesis in the Lepidoptera. This is especially evident in preparations stained with Feulgen, when the elimination chromatin contained by the bivalents in the female remains unstained.

8.22. Cytotaxonomy of Syrphid Flies. Janny M. van Brink and J. W. Boyes (Utrecht, The Netherlands).

The chromosomes of 56 species of Syrphidae from the Netherlands have been examined. Species of most genera in the subfamily Syrphinae have four pairs of chromosomes, including a small heteromorphic pair and three larger metacentric to subtelocentric pairs (*Platychirus*, *Melanostoma*, *Sphaerophoria*, *Doros* and *Scaeva*). A *Xanthogramma* species has five pairs of chromosomes; six species of *Syrphus* have four pairs and three others have five pairs, but in one species counts of four pairs and five pairs were obtained. Two *Chrysotoxum* species in the subfamily Chrysotoxinae had five pairs. In the subfamily Cheilosiinae, species in the genera *Neoascia*, *Pipizella* and *Cnemodon* have four pairs; in *Rhingia*, *Myolepta* and *Cheilosia* five pairs; and in *Chrysogaster* and *Liogaster* six pairs. One *Volucella* species of the subfamily Volucellinae has six pairs but another has five pairs plus a few microchromosomes. In the subfamily Eristalinae, seven species of *Eristalis* have karyotypes consisting of five metacentric-subtelocentric pairs, apparently autosomal, plus one or two additional pairs, or plus a peculiar complex of small elements which will require detailed analysis for accurate interpretation. Six pairs were found regularly in species of *Eristalinus*, *Merodon*, *Eurinomyia*, *Helophilus* and *Parhelophilus* but only five pairs in *Myiatropa florea*. In the Xylotinae single representative species of *Tropidia*, *Syritta*, and *Zelima* have five pairs. Many variations in chromosome morphology were also found and will be mentioned briefly. This is a preliminary report on an extensive analysis of karyotypic variation in this family.

8.23. The Anal Plates of Drosophila Larvae in Different Species. Suavi Yalvaç (Erzurum, Turkey).

Exposing 3rd instar larvae of *Drosophila* to 70 per cent alcohol causes a pigmentation which begins on the posterior end of the larval body and spreads forward. This occurrence helps to make two symmetrical plates appear around the anus of the larva. Otherwise it is too difficult to notice these plates because of their transparency.

After comparing about 30 species of Drosophilidae it has been established that different types of anal plates exist in the larvae of these flies. Some of them, like *D. victoria*, *D. buskii*, *D. mulleri*, *Chymomyza procnemius* and others, have anal plates so typical for their own species that one can easily identify their larvae by means of these plates.

It seems that anal plates of *Drosophila* larvae might be considered as a taxonomic factor.

8.24. Levels of Speciation and Reproductive Affinities in the Willistoni Cryptic Group of Drosophila. Helga Winge (Porto Alegre, Brazil).

The intercrossing of strains from different

localities, belonging to *D. Willistoni* and sibling species, disclosed several levels in the speciation process. A total of 90 different intercrosses, with five repetitions, each with 10 pairs of flies, gave 450 replications, which were periodically transferred to new vials until the death of the females (about 60 days).

Amongst the most significant results is the production of F_1, fertile *inter se* from *paulistorum* males x *willistoni* females strains from Southern Brazil, edge of *D. paulistorum* distribution. The reciprocal cross yielded few sterile individuals.

The offspring of *paulistorum* males x *equinoxialis* females showed a large excess of fertile females and rare, sterile and always mutant males (eyes, wings or bristles). The reciprocal crosses yielded few descendants, the sex-ratio being normal, but all males sterile and mutants (eyes, wings and bristles).

The degrees of affinities in regard to the cytoplasm allowing the development of interspecific hybrids, at least until larvae, can be stated very briefly as follows: *insularis* (all other tested) > *willistoni* (*tropicalis*, *paulistorum*, *equinoxialis* and rarely with *insularis*) > *paulistorum* (*equinoxialis*, *tropicalis*, some strains of *willistoni* and rarely with *insularis*) > *equinoxialis* (*paulistorum*, *tropicalis* and rarely with *willistoni*) > *tropicalis tropicalis* from Palma (other *tropicalis* and *paulistorum*) > *tropicalis cubana* and *tropicalis* from Tefé and Trinidad (*inter se* only).

D. tropicalis cubana (Townsend, 1954) can be ranked to *D. cubana* with sub-species distributed over the Antilles, Trinidad and the Amazon basin (Tefé to Belém). Males of *D. cubana* from Cuba, Trinidad or Tefé crossed with *D. tropicalis tropicalis* from the type locality, Palma, yielded few descendants, the males being sterile and females fertile with paternal strains only. The reciprocal crosses produced *no* descendants.

The *D. cubana* "complex" of sub-species from Cuba, Tefé and Trinidad produced, *inter se* abundant F_1 with fertile females and sterile males in both ways.

8.25. Cytological Studies in the Cardini Superspecies of Drosophila. WILLIAM B. HEED and JEAN S. RUSSELL (Tucson, U.S.A.).

In order to determine the phylogenetic relationships among the 15 species in the cardini superspecies of *Drosophila*, a salivary gland chromosome map has been constructed from a homozygous population of *D. acutilabella* from Cuba. Nine species have been analysed thus far and their inversions have been compared with the standard sequence from Cuba. Eight of the nine species form a natural subgroup in that their population structures are dependent upon the position and size of the islands in the Caribbean Sea. A total of 26 inversions were found among 34 separate laboratory populations and strains. Fifteen inversions are homozygous and 11 inversions are heterozygous. *D. acutilabella* is homozygous for the standard gene sequence in Florida (4 localities), Jamaica (6 localities) and Cuba (1 locality). It is highly polymorphic, however, in Haiti (3 localities) with 8 heterozygous inversions. Five separate populations of *D. dunni* from 3 localities in Puerto Rico are homozygous, and they are all fixed for 3 inversion differences from standard in the X chromosome. Analysis of the polymorphism in the Haitian *acutilabella* is in progress.

8.26. Cytotaxonomy in the Drosophila melanica Species Group. HARRISON D. STALKER (St. Louis, U.S.A.).

On the basis of morphology and reproductive isolation the six New World species of this predominantly Nearctic group may be arranged in a sub-group of four sibling species: *D. melanica*, *D. paramelanica*, *D. euronotus* and *D. melanura*; and two more distinct species: *D. micromelanica* and *D. nigromelanica*. *D. micromelanica*, considered to be the most nearly ancestral, has a rod-shaped, single-element X in most strains; the other five species have V-shaped, two-element X chromosomes. Salivary chromosome differences in banding sequence were studied by hybridization and by the comparison of photographs and composite photomaps. In all six species chromosome II is the most variable, showing 57 inversion differences within species. Chromosomes XR, XL, III and IV show 10, 6, 1 and 2 intraspecific inversion differences respectively. Chromosomes showing the largest number of heterozygous inversions within species also show the most homozygous inversion differences between species. Chromosome comparisons indicate the following phylogenetic sequence for the group: *D. micromelanica*—*D. euronotus* and *D. melanura*—*D. paramelania*—*D. melanica*. (*D. nigromelanica* is apparently derived from *D. micromelanica* independently of the *D. euronotus*, *D. melanura* pair.) Within the group of four sibling species, the ratio of heterozygous inversions within species to homozygous inversion differences between species is approximately 3.5: 1, suggesting a rapid isolation of the four sibling species, followed by an extensive period

of chromosomal diversification within each of them.

8.27. Attempt on the Biological Significance of the Chromosome Numbers. MIHAI SERBAN and CANTEMIR RISCUTIA (Bucharest, Rumania).

In a paper read at the XVI International Congress of Zoology, Washington, 20-27 August 1963, we analyzed the distribution of the chromosomes, from the grandparents (P_2) in the genome of a certain grandchild (F) using the "indicators of stability" (I.S.), calculated with the formula (1) for even, and (2) for odd numbers of chromosomes.

$$2/2N \sum_{m=0}^{N/2-1} m/n.\ C\frac{m}{N} + 1/2N.\ C\frac{N/2}{N}\ ;(1) \text{ in which } m + n = N$$

$$2/2N \sum_{m=0}^{[N/2]} m/n.\ C\frac{m}{N}\ ;\ (2) \text{ in which } (N/2) \text{ means whole part of } N/2$$

A table of the I.S. values is given calculated for the numbers N=2-25. We analyzed the distribution of frequency of the haploid chromosome numbers (N) for 2374 species of flowering plants (Dobzhansky, 1937) using the I.S. and the values of I.S. generalized for the generations P_4 and P_6. We found a significantly greater frequency of numbers N divisible by 8 than by 4, divisible by 4 than by 2, and generally a greater frequency of even numbers compared to odd ones.

The species in which the interactions between organism and environment are more complex have generally a greater number N, of chromosomes, which assures a greater stability. The growth of the stability can be explained by the I.S. values, which augment with the increase of N.

8.28. Chromosomes of Swiss Ants. E. HAUSCHTECK (Zürich, Switzerland).

Chromosome sets of a number of ant species from the subfamilies Myrmicinae, Formicinae and Dolichoderinae have been investigated. Diploid numbers between 8 and 40 to 50 have been found. The diploid numbers of the Formicinae are usually higher than those of the Myrmicinae. There is only one genus of the Dolichoderinae studied which has 22 chromosomes. Species with low chromosome numbers have longer chromosomes than species with high chromosome numbers. For instance, *Lasius niger* has 30 small chromosomes in metaphase, each one to two microns. In *Stenamma brevicorne* there are only 8 chromosomes but these are five microns or longer. *Leptothorax* is intermediate. It has 18 chromosomes, each two to six microns. The karyotype of *Leptothorax* exhibits two striking constrictions on one pair of chromosomes. In the genus *Lasius* four species have a diploid number of 30, and one a diploid number of 28 chromosomes.

8.29. Hybrid Population Analysis by means of Differential Staining of Chromosomes. HUMIHIKO ONO, and BUNZO SAKAI (Tokyo, Japan).

Crepidiastrum keiskeanum (Compositae) is distributed along the Pacific coast of southern Japan. In Izu Peninsula, the northern limit of its distribution, many hybrids of this species and some allied ones are found. To analyse the karyotypes of the hybrids, the differential staining method using cold treatment was applied. This treatment reveals special beaded patterns in the chromosomes of *Lactuca squarrosa* and faintly staining segments in the chromosomes of *Paraixeris denticulata*. In the hybrids each chromosome or its segments are clearly identified. Analysis of minor karyotypes of these hybrids revealed the following extraordinary characteristics:

1. The karyotypes of the hybrids are not always the sum of the genomes of their presumed parents. The maternal chromosomes remain intact in the hybrid karyotypes. Marked diminutions in number and length are often observed in the paternal chromosomes. Many hybrids of *Crepidiastrum keiskeanum* (n=5) and *Lactuca squarrosa* (n=9) had 10 chromosomes. But minor karyotypes revealed by the differential staining showed individual differences.

2. Some of these hybrids were observed to be mixtures of the cells with different karyotypes. Fragmentation and elimination of paternal chromosomes cause the alteration of karyotypes. Such alterations occur among the somatic cells of a single individual. With the lapse of time, cells with the most balanced karyotype overgrow less balanced ones. This phenomenon is ascertained by observing the karyotype of a single plant at different ages and by measuring the pollen fertility of each stem of an individual. The pollen fertility is remarkably divergent according to the position of the stem even in a single plant, although it recovers considerably in successive years.

8.30. Cytogenetics of South American Amphibians. FRANCISCO A. SAEZ and BRUM DE NADIR ZORRILLA (Montevideo, Uruguay).

South American species of amphibia offer a wide scope for investigation due to their abundance and their distribution in different regions of the continent. Up to present we have investigated more than 80 species belonging to eight families.

BUFONIDAE; In eleven species of the genus *Bufo* the diploid number is $2n=22$. *CERATOPHRYDAE;* in the genus *Odontophrynus* numerical diversity was found, six species with $2n=22$ and seven species with 24, 30, 42, 44, 50 and 60 somatic chromosomes. The genus *Ceratophrys* is unique because in the same species *C. ornata* were found four different numbers, 26, 92, 96 and 108 chromosomes. The genus *Lepidobatrachus* presents $2n=30$.

LEPTODACTYLIDAE; eight species of the genus *Leptodactylus* have $2n=22$; three species of *Pleurodema*, $2n=22$; two species of *Physalaemus*, $2n=22$; two species of *Elogia*, $2n=26$; two species of *Crossodactylus*, $2n=22$; three species of *Eleutherodactylus*, $2n=22$; three species of *Telmatobius*, $2n=22$; *Calytocephalella*, $2n=26$; *Throropa*, $2n=26$; *Eusophus*, $2n=22$; *Cycloramphus*, $2n=26$; and *Pseudopaludicola*, $2n=22$.

PSEUDIDAE; one species of the genus *Pseudis* has $2n=22$.

HYLIDAE; Fifteen species with $2n=24$ and one with 30.

MICROHYLIDAE; one species of *Elachistocleis* with 22 chromosomes.

BRACHYCEPHALIDAE; two species with $2n=22$ and one with 26.

RANIDAE; R. pipiens and *R. palmipes* from Costa Rica and Perú respectively with 26 chromosomes. None of the studied species shows cytologically differenciated sex chromosomes. Important problems of evolution, geographical distribution and taxonomy will be dealt with by cytogenetical analysis in a future publication that will appear elsewhere.

SECTION 9

POPULATION GENETICS

9.1. The Diversity and Complexity of Ecotypic Differentiation within Plant Species in Response to Soil Factors. R. W. SNAYDON (Reading, Great Britain).

The Park Grass experimental plots at Rothamsted provide a unique opportunity for studying ecotypic differentiation in response to controlled differences in soil factors over short distances and relatively short periods of time. The plots, which measure approximately 20 m × 35 m, have continuously received different fertilizer treatments since 1856 and different liming treatments since 1903. The various treatments have led to wide differences in the physiognomy and botanical composition of the vegetation, yet the grass species *Anthoxanthum odoratum* is present on most plots.

Populations samples of *A. odoratum* from six plots were grown in spaced plant trials and greenhouse experiments. The results indicate that the populations differ significantly in a number of morphological and physiological characters. The response of populations to contrasting soils was closely correlated with native soil pH. Vegetative height, inflorescence height, inflorescence posture and rust susceptibility of the populations were closely correlated with vegetation height on the Park Grass plots. Data of flowering, number of panicles and mildew susceptibility were partially correlated with vegetation height but also with fertilizer treatment. Yield, panicle size and leaf size were to some extent correlated with vegetation height, but the relationship was evidently complex. These results, together with results of the physiological analysis of ecotypic differentiation in response to soil factors in *Trifolium repens*, demonstrate the complexity of ecotypic response to initially simple soil differences, both through the interactions of soil factors and through the secondary and tertiary effects of the changing physiognomy and botanical composition of the vegetation.

9.2. The Analysis of Evolutionary Processes involved in the Divergence of Plant Populations. A. D. BRADSHAW (Bangor, Great Britain).

It is perhaps usual to imagine that evolutionary divergence of distinct populations within a species can only occur overc onsiderable differences. However, in a number of plant species this can be shown not to be true. In two species, *Agrostis tenuis and Anthoxanthum odoratum*, populations occurring on toxic soils associated with old mine workings show a tolerance to the toxicities which is not present in normal populations. This characteristic shows a remarkable localized distribution. Populations only a few feet away from the toxic areas fail to show the characteristic. In the same manner, in *Agrostis stolonifera*, populations occurring in exposed maritime habitats are found on cultivation to possess a dwarf growth form not found in populations occurring in protected habitats a very short distance away.

In such situations it can be demonstrated that there is considerable gene flow between contrasting populations tending to obliterate the differences between the populations. However, since the differences between the populations are retained, strong selection must be occurring to remove the effects of gene flow. Evidence for this is available. It appears that the manner and timing of the selection may be very different in the adjoining populations, but in general it occurs in the seedling stage.

Gene flow between populations appears to occur even between populations much farther apart. From this it may be argued that the continuous interplay of strong selection pressures and gene flow are a normal part of the evolutionary processes determining the occurrence of distinctive-populations within species.

9.3. Variability of Growth of Ryegrass (Lolium spp.) Progenies under Artificial Environments. S. O. FEJER (Ottawa, Canada).

Responses of inbred and outcrossed New Zealand ryegrass progenies to some factors of artificial environments in the phytotron of the California Institute of Technology, Pasadena, are reported. Growth was relatively little affected by night temperatures, except in some

L. perenne inbreds, and in a hybrid with *L. perenne* cytoplasm, which were strongly depressed by high night temperatures. Responses to day temperature depended on the genotype, supporting earlier results on such interactions in ryegrass. Long days (16hr) produced more growth in all material than did short days (8 hr). Unnatural conditions, such as very short days (4 hr), cold days and warm nights, or changes in temperature at noon, caused depressions. Stress conditions caused by water and nutrient shortage, excessive defoliation, and very low light intensity also depressed growth, and interacted with the genotypes in various measurements. Heterosis and inbreeding depression were enhanced by such conditions. Under any one condition, variation within clones was relatively low, depending on plant type but not on heterozygosity, and variation between progenies was generally higher than within progeny variation. The latter conformed to expectations of the homeostasis theory as the mean coefficients of variation over all environments increased linearly with decreasing heterozygosity, but it also depended on genotype and environment. Conversely, growth of individual clones under different environments was in some cases most uniform in the low producing inbred lines.

9.4. Ecotypic Variation in Response to Photo - and Thermoperiod of Seedlings of Douglas - fir (Pseudotsuga menziesii (Mirb.) Franco).
H. Irgens-Moller (Corvallis, U.S.A.).

The response of young Douglas fir seedlings to long and short photoperiods varies with the geographic origin of the seed. Seedlings from maritime areas show less sensitivity to changes in photoperiod than do seedlings from continental areas. Similarly, the response to different thermoperiods also varies with seed origin. At a constant temperature of 20°C and 8-hr photoperiods a large percentage of seedlings from Arizona go through 2-3 periods of dormancy as opposed to only one in seedlings from Vancouver Island, B. C. Low night temperature (3°C) causes not only depression of growth for all sources tested but an earlier induction of dormancy in the seedlings from continental areas than in those from coastal areas.

After dormancy is induced and the seedlings are exposed to 16-hr photoperiods and greenhouse temperatures, growth is resumed considerably earlier in the seedlings from continental areas than in those from coastal areas. However, the seedlings previously exposed to a constant 20 C remain dormant for a considerably longer period than those exposed to a low night temperature. It is suggested that ecotypic differentiation in long-lived species such as forest trees may profitably be studied by short-term studies of differences in seedling responses to photo- and thermoperiod.

Supported by grants from National Science Foundation, Washington, D.C.

To be published in *Forest Science*, 1964-65.

9.5. Genetics of Drosophila subobscura Populations in Greece. C. B. Krimbas (Athens, Greece).

Data concerning the type of inversions and the indexes of free recombination (IFR) of several populations of *D. subobscura* in Greece are reported. An ecological description of the sites in given. Some of these data are the following:

In Mount Parnes a population living in a fir forest (altitude 1100m, rainfall 1000mm) had an IRF of 85·6 ±1·2 and of 85·2 ±1·2 in surveys of two successive recent years.

In Mount Ainos, Cephallonia, a fir forest the population (alt. 1200m, rainfall 1200mm) had an IFR of 84·3 ±1·2.

Three populations living in citrus orchards, in Athens (alt: 100m, rainfall 400 mm), in Alikianou, Creta (alt. 100m, rainfall 1040mm) and in Poros (alt. 50m, rainfall 400 mm) had IFR's of 83·8 ±1·5, 85·0 ±1·2 and 77·0 ±1·6 respectively in 1962. The previous year a small sample of Poros population had an IFR of 80.1 ±3.8.

Attempts to correlate IFR with ecological characteristics of these and other European and Middle East populations are reported.

Results are also given of experiments, now in progress, concerning the measurement of body weight, wing length, developmental time, dessication resistance, longevity and fertility of the individuals of these populations, their inter-populations F_1's and F_2's.

These data are discussed in relation to

(a) the Dobzhansky-Carson hypothesis of the pattern distribution of the inversion polymorphism within the area of geographical expansion of the species,

(b) the existence of an integrated gene pool in each population as far as the measured fitness components are concerned and

(c) the natural selection of the complex group

of related characters body size, developmental time, dessication resistance and fertility.

This research is supported by a grant of the Royal Hellenic Institute of Research.

9.6. Polymorphism in the Leopard Frog (Rana pipiens). DAVID J. MERRELL (Minneapolis, U.S.A.).

The dominant *burnsi* mutation is shown to be present in natural populations of the leopard frog in an area of about 100,000 square miles centering on the Anoka Sand Plain of Minnesota. This distribution represents only about 2 per cent of the total range of *Rana pipiens*. The highest frequencies of the mutant type reach only 5-10 per cent. Evidence will be presented suggesting that the present distribution of this mutation is related both to the glacial history of the area and to the fitness of the mutant in its present environment.

9.7. Intra-specific Interaction in Rice. SUBODH KUMAR ROY (Calcutta, India).

When two varieties of rice are grown together, each may influence the yield of the other. The nature of interaction may differ with the kind of mixture, the genotype mixed, the proportion of the components and the ecological conditions. The effect is as often favourable as unfavourable. However, cooperation occurs, and certain pairs outyield the means of the respective components when grown in monoculture and occasionally the better components.

The factors involved in such genotype interactions seem to be very complex and are not fully understood. However, experimental evidences suggest that biologically active chemical substance or substances produced by the roots may be responsible for stimulation or inhibition at least in certain pairs. The two varieties which cooperate may make different demands on the soil or the water and may suffer from complementary metabolic deficiencies. Diverse nature of roots, and of lodging and disease resistance of the components, may have considerable advantage in a mixture over the pure stand.

The usual breeder's -practice of growing several varieties of rice experimentally side by side without any bond of separation between them may lead to quite false conclusions as to the yields of the varieties in question in pure stand because under such a situation interactions may be expected. This finding demands the formulation of a new set of principles for the design of experiments on rice.

It is not yet possible to assess the exact economic value of this finding but the cumulative results seem indicate a hopeful future possibility.

9.8. Distribution, Numbers and Natural Selection in a Butterfly Population. M. S. B. SEIGER and R. H. T. MATTONI (Lafayette, U.S.A.).

The Lycaenid butterfly, *Philotes sonorensis*, tends to form isolated or semi-isolated colonies throughout its distribution range. We selected a relatively isolated population in the San Gabriel Canyon, California, to study population size, ecology, movement and phenotypic variability in the six component colonies of this population. The colonies are no more than 80 m in diameter and are restricted to those areas where the larval food plant, *Dudleya lanceolata* (Crassulaceae), grows. The two closest and two farthest colonies are 136 and 473 m apart respectively. For three years the population was sampled regularly throughout the flight period by a capture-mark-release-recapture system. The captives were classified according to sex, spot pattern, area and dates of previous captures if the specimen had been recaptured.

Very few individuals moved from their original capture sites. Most of the interchange of genetic material among the colonies was due to the movement of the males. Thus gene interchange was facilitated while distribution was localized. Small ecological barriers such as rocks and small streams acted as strong deterrents to the movement of *Philotes*. Evidence indicating isolation by minor ecological barriers was derived from differences in sex ratio and spot pattern frequencies among the six colonies. The effects of birth and death rates and rates of incoming and departing individuals on fluctuations in daily colony sizes were determined for each of the colonies. The effects of natural selection were determined by comparing the population parameters for the three years.

9.9. Ecological Genetics of Tribolium and Houseflies. ROBERT R. SOKAL (Lawrence, U.S.A.).

The ecological components of natural selection in *Tribolium castaneum* populations are examined under two different population systems. (1) The (more natural) overlapping of generations and

concurrent competition of all life history stages. (2) Competition among developing larvae and pupae only, with the adults separated from the flour at regular intervals. This model simulates natural selection in simpler univoltine species. The fate of two alleles (*sooty* and *black*) under both systems of selection seems deterministic and analytical experiments are reported which try to isolate the separate components of selection. Among the findings of interest are: Proof of gene frequency dependent changes of adaptive value in the *sooty* and *black* locus, proof of gene frequency and zygotic frequency dependent facilitation of growth processes by the *sooty* allelomorph, genetic differences in response to environmental conditioning and differences in medium conditioning produced by different genotypes (in the case of the *black* locus). Density dependent differences in adaptive values are apparently responsible for changes in the direction of selection in population system (2). Related experiments have been carried out with houseflies and are contrasted with the *Tribolium* findings.

9.10. Mimetic Multilocus Polymorphism in South American Butterflies (Heliconius spp.) (Lepidoptera, Nymphalidae). JOHN R. G. TURNER (Oxford, Great Britain).

Heliconius melpomene and *H. erato* have about thirty morphs each—though they are monomorphic over large areas—and are mutually mimetic. Experiments (Brower, Brower and Collins) showing that birds find the insects distasteful and confuse the two species, and the existence of beak-marked specimens, showing that birds attack the butterflies in the wild, show that this similarity involves synaposematism; that theory predicts that aposematic species become monomorphic, that some "mimetic" pairs are allopatric, and that some morphs are non-mimetic, shows that other factors affect the polymorphism. The only one whose action has been shown is visual sexual selection. Features advantageous in courtship are usually dominant, and butterflies lacking red (the chief releaser) are rare; all-black insects are very scarce. There are several autosomal loci, most of them linked; two constitute a supergene. Population studies show strong epistasis and linkage disequilibrium at this superlocus; never are all four superalleles found together, and one of them is very restricted geographically in *melpomene* and extremely rare in *erato*. In *erato* a third locus is included in the supergene. One cannot tell what maintains the polymorphism, although visual predation alone can produce strong epistasis, and there is some correlation between gene distribution and climate; note the similarity to the Rhesus blood-groups, also supergenic and also maintaining their polymorphism despite an unstable equilibrium.

Some results were obtained jointly with JOCELYN CRANE and P. M. SHEPPARD. Papers on *Heliconius* from the New York Zoological Society's Tropical Research Station appear in *Zoologica* (N.Y.).

9.11. The Evolutionary Potential, as Measured by Seed Germination, of Chromosome Races of Mimulus (Scrophulariaceae). ROBERT K. VICKERY JR. (Salt Lake City, U.S.A.).

The purpose of this investigation was to explore one facet of the norm of reaction of *Mimulus*, specifically, the range of temperatures under which seed germination could occur and to see whether there were different responses in races of different chromosome numbers. Seeds of over 20 races were tested in various combinations of temperatures ranging from minus 7 to 50°C. They were placed in one temperature for 16 hr and in another for 8 hr to simulate a daily periodicity of temperature changes.

In the *Mimulus glabratus* complex, the diploid ($n = 15$) and tetraploid ($n = 30$) races germinated in combinations of cooler temperatures, from 4° to 21°, and the hexaploids ($n = 45$) from 4° to 25°. Aneuploids of the diploid and tetraploid races exhibited ranges that extended 5° to 10° higher than their respective euploid forms. The aneuploids of the hexaploid level regularly tolerated combinations involving 35° and one race even grew well in a combination involving −7°. The *M. glabratus* complex aneuploids germinated and grew under a wider range of temperatures than their euploid relatives and the ones with the highest numbers showed the widest range of tolerance.

The results for the closely related *M. guttatus* complex suggest a note of caution to too easy generalizations. In this group the diploids exhibited a far wider range of temperature tolerance than did the tetraploids or aneuploids. However, in both groups the range of temperature tolerance of the races was often wide and not necessarily correlated with the temperature ranges of their native environments which suggests that *Mimulus* has a wide norm of reaction and much unexploited evolutionary potential at each chromosome number level.

9.12. An Instance of Interaction of Genotype and Environment at the Population-level. F. E. BINET (Werribee, Australia).

The interaction of genotype and environment is usually considered on the level of the individual; it denotes the phenomenon of different environmental effects on the phenotypic expression of different genotypes.

In the terminology of Analysis of Variance this may be expressed as the "non-additivity" of the "average phenotypic value", and the "environmental contribution".

Considering a population *as a whole*, another influence of its environment on its phenotypic composition needs consideration. Even without any difference in overall allele-composition the zygotic genotype-composition of two populations may be different. In presence of any dominance and/or epistasis different "average phenotypic values" follow from that difference.

Environmentally conditioned mating-systems assemble identical gene-material into different zygotic genotype-collections. The conditioning factor is ecological (in wild populations) or human action (in domesticated populations).

This lecture attempts to account for findings (Clifford and Binet, 1954), contrary to anticipations, based on Anderson's (1949) models.

We measured certain characters of trees in three stands; their estimated dispersion-matrices define quadratic forms, whose cartesian images are hyperellipsoids, with centroids, representing (as position-vectors) the estimated mean-vectors. In this representation (cf. Pearson, 1901) Anderson's model leads to anticipating the major axis of the hybrid-stand's hyperellipsoid to lie on the line connecting the parent-stands' centroids. Contrariwise, we found the major axes of the three hyperellipsoids nearly parallel.

It is submitted that these findings do not contradict Anderson's theory on the formation of the "recombination-spindle" and on subsequent "introgression"; rather, it appears that in the "hybridized habitat" natural selection may favour the hybrid individuals so strongly over the pure offspring of both parents that the hybrid stand consists almost exclusively of hybrids. From those hybrid stands whose representative hyperellipsoids *are* such recombination-spindles, such pure offspring have not been eliminated by natural selection. We assume that in our case the effect of natural selection resulted in a hybrid stand of a very similar constitution to one at which a breeder, crossing individuals from *different* parent strains *only*, would aim.

ANDERSON, E. *Introgressive Hybridization*, New York, 1949: Wiley.

CLIFFORD, H. T., and F. E. BINET. A quantitative study of a presumed hybrid swarm between *Eucalyptus elaeophora* and *E. goniocalyx*. *Aust. J. Bot.* **2**, 325-36 (1954).

PEARSON, K. The lines of closest fit to a system of points. *Phil. Mag.* **2**, 559 (1901).

9.13. Selection toward an Optimum and Linkage Disequilibrium. S. WRIGHT (Madison, U.S.A.).

The author (1935, 1937) gave an approximate formula for change of gene frequency from selection under random mating, assuming random combination among multiple interacting factors $(\triangle q = q(1-q)\frac{\delta^w}{\delta^q} 2^w$ where the W's are selective values of total genotypes, assumed constant). This is only approximate because selection with interaction usually maintains deviations for randomness. Exact treatment (1944, 1952) of an extreme two locus case (W falling off symmetrically from an optimum: $W = 1$ for two plus factors, $(1-s)$ for one or three, $(1-4s)$ for none or four, recombination c), showed considerable linkage disequilibrium at (1/2, 1/2) if $s = c$ (0.1465 AB or ab 0.3535 Ab or aB) but little if $s/2c$ is small (gamete frequencies about $1/4\,(1 \pm \frac{s}{2c})$). This paper considers three linked loci, ABC, both recombination fractions c, complete interference, $W = 1$ for three plus factors and $(1-s)$, $(1-4s)$, $(1-9s)$ for increasing deviations. If $s = c$, and sc is small, the equilibrium frequencies are 0.0495 ABC (and abc), 0.1387 ABc, etc., 0.1731 AbC (and aBc) but 0.1097, 0.1278 and 0.1347 respectively if $s = 0.1c$. If $s = c$, the disequilibrium (^{q}Ab^{q}aB^{-q}AB^{q}ab), for loci A,B in presence of C,c is 59.7 per cent of that with no C,c segregation while disequilibrium of loci A,C in presence of B,b is only 26.5 per cent of that with BB or bb. The approximate formula for $\triangle q$ may be considered reasonably satisfactory, in spite of the extreme interactions of the optimum model, with respect to selection coefficients of lower order than the recombination fractions.

9.14. Monte Carlo Investigation of Interaction between Linkage and Selection under Dominance Model. N. R. BOHIDAR, D. G. PATEL and R. L. HURST (Logan, U.S.A.).

The mathematics of simulation of genetic

systems associated with the problem at hand have been developed and a fairly general computer program has been written for the high speed computer IBM 1620. The experiment consisted of three levels of selection intensity, eight levels of linkage condition, four replicates and three dominance models, no dominance, complete dominance and over-dominance. Each combination of the levels of the five factors was subjected to 15 generations of selection with an arbitrary initial population in linkage equilibrium. The chief interest of the study was the progressive changes in mean and variance of the phenotypes at each generation. The statistical analyses consisted of fitting a quadratic regression to the curves to evaluate the rate of genetic gain per generation. Analysis of variance procedure was adopted to gain information on the main effects and particularly the interaction of linkage and selection. Results showed that there was a strong indication of presence of interaction under no dominance model. No significant interaction was detected for the other models. The optimum combinations for the maximum selection gain per generation were determined under all models. A full account of the study will be published in *Biometrics*.

9.15. The Theoretical Study of Gene Flow between Sub-species, using a Digital Computer. J. L. Crosby (Durham, Great Britain).

The representation of plant or animal populations by abstract models within an electronic computer allows the theoretical investigation of evolutionary systems which are too complex for mathematical treatment.

One problem which has been studied in this way concerns gene flow between two related subspecies which come together again and interbreed, after a period of genetical divergence during geographical isolation. It has long been held that where such genetical divergence is great, there should be selection of factors tending to inhibit interbreeding (and thus accelerate speciation), although the theoretical demonstration of the course of such selection is not easy.

The present series of experiments has considered one aspect of this problem—the selection of pre-fertilization barriers to interbreeding in plants. The example chosen was that of flowering season in two sub-species which flower simultaneously and interbreed, with partial sterility of the hybrids. Slight differences in the time of flowering arise in the first place by random fluctuations, but where the hybrid sterility is high enough these differences become subject to selection; eventually, the flowering periods of the two sub-species hardly coincide, and at the same time each becomes shorter in duration. Interbreeding is thus very greatly reduced, and gene flow between the two sub-species is almost eliminated; there has thus been a significant step in the direction of speciation.

The problem of the selection of post-fertilization barriers to gene flow is a much more difficult one, but it is certainly amenable to the same kind of theoretical treatment.

9.16. Biometrical Parameters of Self-fertilizing Diploid Populations. Dewey L. Harris (Ames, U.S.A.).

The mechanism of inheritance in diploid populations is examined for the situation where all reproduction is by self-fertilization. This study suggests certain parameters for a general representation of the means, variances and covariances involved. Letting $\mu^{(k)}$ represent the population mean in generation k and letting $\mathrm{Cov}(k;r,s)$ represent the covariance of the rth generation progeny means of generation k individuals with the sth generation means of the same individuals, we have

$$\mu^{(k)} = \sum_{x=0}^{n} \frac{1}{2^{kx}} (\delta x)$$

and

$$\mathrm{Cov}(k;r,s) = \sum_{x_{12}=0}^{n} \sum_{w=0}^{n-x_{12}} \sum_{x_1=x_{12}}^{n-w} \sum_{x_2=x_{12}}^{n-w} \frac{2^{kx_{12}-0w}}{2^{rx_1+sx_2}} \left(1-\frac{1}{2^k}\right)^{w} \binom{x_{12}w}{x_1\, x_2} Q$$

where n is the total number of segregating loci which influence the quantitative trait under consideration and Q^{xuv} and $\mu_u^{(0)}$ are the basic parameters of this parametrization. The meaning of these parameters and their relation to the types of gene action occurring in the population is discussed. The relation of this parametrization to those previously suggested by Horner and by Kempthorne is indicated. The implications to these results to the design of experiments for the selection of genetically superior material in such populations is emphasized.

9.17. A Theoretical Study of Population Changes Under Stabilizing Selection. S. K. Jain and R. W. Allard (Davis, U.S.A.).

The "intermediate optimum" and the "homeostatic" models of stabilizing selection require

fitness of an individual to be related to its metric value and heterozygosis respectively and therefore have different consequences on the distribution of gene frequencies and the level of heterozygosity at equilibria. Data from a predominantly inbreeding population of barley, however, did not allow a clear choice between these alternative models. It would be particularly significant to find clear evidence for the operation of homeostatic model in this case in view of the probable role of heterozygote advantage in inbreeding populations postulated earlier.[1] The present study was taken up in order to characterize changes in various statistics (mean, inter-family and intra-family variances, fitness, hybridity, linkages) of a population under different forms of stabilizing selection. The case of complete selfing was analysed with the use of generation matrices and of mixed mating with the help of numerical work on a digital computer. In the "variable" parameter runs, the selective values and the proportion of selfing were made random variables in turn and the initial values of "fixed" parameters were varied among the parallel runs. These results allow a distinction between the consequences of different models and seem to have interesting bearing on the mode of selective changes in inbreeding populations.

1. S. K. JAIN and R. W. ALLARD, *Proc. Nat. Acad. Sci.* **46**, 1373-1378, 1960.

9.18. On the Theory of Genetic Loads. HOWARD LEVENE (New York, U.S.A.).

Morton, Crow and Muller and Crow have suggested a method of determining whether the genetic load is mainly due to recurrent deleterious mutations or to genes with heterozygous advantage. The method is based on the comparison of individuals from random matings with inbred individuals. A number of restrictive assumptions are made in the derivation of the method, and additional assumptions must be made to apply it to actual data. The effect of relaxing these assumptions, and the degree to which valid conclusions may be drawn from the types of data so far employed will be discussed. The broader implications of the concept of a "genetic load" will also be considered.

9.19. Optimal Genetic Systems in a Fluctuating Environment. RICHARD LEVINS (Rio Liedras, Puerto Rico).

A mathematical study of natural selection in a fluctuating environment was carried out by analytic methods and computer simulation. The average fitness of the population was determined for different genetic systems, and the optimal genetic system was found for different pattern of environmental heterogeneity. Genetic variance increases the average fitness provided the environmental variation is large compared to the tolerance of individual genotypes, or when the correlation between the environments of successive generations exceeds a threshold that depends on the model. If the first condition holds but not the latter, the optimal system has no additive variance but much epistatic and dominance variance. If only the second condition holds, most of the variance will be additive. In a fluctuating environment the average frequency of a heterotic lethal is below its optimal value so that mutation to the lethal may increase fitness. Mutation also reduces the variance of gene frequency and increases the correlation between the state of the population and its optimal state. The optimal rate of recombination was also shown to depend on the environmental variance and autocorrelation.

9.20. Linkage in Selection Programmes. A. ROBERTSON (Edinburgh, Great Britain).

Linkage is obviously a retarding factor in artificial selection programmes. The extent of this retardation is dependent on several factors between which there may well be important interactions. The population size in the selection programme may well have an effect at intermediate values but not with very large or very small populations. We may expect the effect to be greater (i) when linkages are primarily in repulsion in the initial population, (ii) when we are concerned to fix a large number of genes, and (iii) when the initial frequencies of these genes are small. Possible interactions between the factors will be discussed theoretically and with reference to results of computer programmes. Finally a selection experiment with *Drosophila*, in which crossing-over was suppressed in one series of lines, will be discussed.

9.21. Heterosis and Genetic Balance. N. V. TURBIN (Minsk, U.S.S.R.).

At the present time there can be no doubt that the two conflicting concepts of heterosis–the

hypothesis of dominance and the hypothesis of overdominance—are not mutually exclusive, but the causes of heterosis with which they deal may act, and as a rule do act, simultaneously. The heterosis effect cannot be explained by a single genetic cause, by one type of interaction of hereditary factors, but is the result of the total similar action of various genetic processes. Not one of the proposed hypotheses determined for any one type of interaction of hereditary factors can be accepted as a general theory of heterosis, although certain of them, and, in particular, the two hypotheses mentioned, are in good agreement with determined experimental data. They contain the elements of exact knowledge and can be considered as fragments of the general theory of heterosis.

It is represented as extremely probable that the concept capable of helping to unite these fragments into a single whole and of supplying the missing elements, taking into account the types of interaction of hereditary factors (allelic and non-allelic) and also the role of conditions of the surrounding medium in the development of characters in hybrids, is the theory of genetic balance.

The value of any character in each parent variety (line) is a result of a determined balance (equilibrium) worked out in the course of selection, in the variously directed action on this character of many hereditary factors and conditions of the surrounding medium, in which the development of the organism takes place. In the cross-breeding of parent varieties (lines) which differ in their heredity there takes place in hybrid offspring a change in the genetic balance with relation to a larger or smaller proportion of the characters which can cause a deviation in the value of one or another character in the direction of an increase or decrease by comparison with the parent forms (positive or negative heterosis).

Departing from what has been said above, an attempt is made to interpret the causes of heterosis and in particular the role in this process of various types of allelic or non-allelic interaction of hereditary factors on the basis of the theory of genetic balance.

9.22. Population Structure and Dynamics in a Complex Scilla scilloides (Liliaceae). TUTOMU HAGA and SHOZO NODA (Fukuoka, Japan).

In a perennial plant *Scilla scilloides* Druce, two basic genomes A ($x=8$) and B ($x=9$) are clearly distinguishable by karyotype and by meiotic chromosome pairing. Up to the present, the following 9 cytogenetic types have been identified in natural populations. Plants of the constitution AA ($2n=16$), BB ($2n=18$), and AA BB ($2n=34$) are propagated sexually by seeds as well as vegetatively by bulb multiplication. Plants of the constitutions BBB ($2n=27$), BBBB ($2n=36$), AAB($2n=25$), ABB ($2n=26$), ABBB ($2n=35$), and AABBB ($2n=43$) set some seeds, instable sexually, but propagate by cloning by bulb multiplication.

These various cytogenetic types are found usually in juxtaposition in a population. An index of homogeneity of the populations as to these cytogenetic types ranged from 0.82 to 0.22, from population to population, where 1.00 indicates a pure stand of a single cytogenetic type. The indexes were computed as follows. Let a be frequency of a type A, b that of another type B, c that of another type C, and so forth. Simple computations $(a^2+b^2+c^2+\ldots)/(a+b+c+\ldots)^2$ give the index values ranging from 1 to certain values approaching 0.

9.23. Progress From One Generation of Selection in Nicotiana tabacum. Dixie Bright 244 × Coker 139. D. F. MATZINGER, T. J. MANN, and C. CLARK COCKERHAM (Raleigh, U.S.A.).

Within the F_2 generation of a cross of 2 naturally self-fertilizing flue-cured tobacco varieties, Dixie Bright 244 · Coker 139, individual plants were self pollinated and crossed to random members of the population. Only the cross-bred material was evaluated and estimates of additive genetic and dominance variances were obtained assuming no epistasis. Parental plants high in per cent total alkaloids were identified on the basis of mean cross-bred performance. From remnant seed, selfs of these superior plants were intercrossed to form a new population. In this first selected cycle, both self and cross-bred families were evaluated. Estimates of additive genetic, dominance, and additive × additive epistatic variances were obtained.

In both the initial and selected populations, significant estimates of additive genetic variance were obtained for cured leaf yield, per cent total alkaloids, days to flower, number of leaves, and plant height. Additive genetic variance was not significant for leaf value in either population. The only significant estimate of dominance variance was for leaf value in the original population. None of the estimates of additive × additive variance were significant in the selected population. Selection for increased

per cent total alkaloids was effective; however, the yield of cured leaf decreased. The response to selection for total alkaloids and the accompanying decrease in leaf yield was in good agreement with the predictions.

9.24. The Evolution of Flower Colours in Populations of Tulipa schrenkii Rgl. according to Statistical Data. A. J. KUPZOW (Moscow, U.S.S.R.).

The area of *Tulipa schrenkii* Rgl. extends over the steppes of the European part of the U.S.S.R. and of Northern Kazakhstan. Individuals, in the wild populations of this tulip, have four main colours of their flowers: red (anthocyanine + anthoxanthine), yellow (anthoxanthine), rose (anthocyanine), and white (no pigment). There are also certain modifications of these: orange, light rose, violet, and cases of uneven distribution of pigments (striped). Red flowers are characteristic of most of the tulipa species, in sect. eiestemenes, and also of the bulk of individuals of *T. schrenkii* in the virgin steppes. The influence of man leads to a decrease of the amount of red flowers in the wild populations of *T. schrenkii* and, thus, to an increase of other colours, especially of yellow. The latter ultimately, becomes exclusive in the environs of large towns, such as Odessa. 31 populations of *T. schrenkii* from different parts of its area have been studied. The correlation between the amount of red flowers and that of yellow, the two main types in these populations, was found to be $-0.9 + 5 \pm 0.0150$. The percentage of red flowers can be taken as an independent variable and that of flowers with other colours as its function. Then, while the percentage of red flowers decreases from 100 per cent to 51 per cent, all its functions rise, and so the composition of the populations approaches the ratio: 9 red $\pm$ 3 yellow $\pm$ rose $\pm$ 1 white (with 10 - 15 per cent of modified colours among them, mainly orange and violet as variants of red). In the interval from 51 per cent to 0 per cent, of red flowers, the percentage curve of yellow flowers continues to go up whereas the proportions of rose flowers goes down, the curve of white ones ascends up to 30 per cent of the red ones and then also descends. The percentage of the modified colours tends to go down within this interval. The removal of anthocyanine (in yellow) is observed more often than that of anthoxanthine (in rose). Thus, in the former interval many populations have 5-10 per cent of yellow flowers and 0-9 per cent of rose ones. The aforesaid modifications are observed only in anthocyanine: orange and violet, as mutants of red being more frequent (up to 10.7 and 6.0 per cent) than light rose, as a mutant of rose (up to 1.5 per cent).

According to the available data, the following concept is suggested:

1. The initial colour of the flowers of *T. schrenkii* was red, and it subsequently produced distinctive mutants deprived of anthocyanine or anthoxanthine, and, also modifier genes (mutations affecting an anthoxanthine).

2. Individuals with red flowers have, in virgin steppes, gained success in the struggle for existence. However, under man's influence, various mutants, especially those with yellow flowers, have become the predominant forms.

3. High percentage of individuals with red flowers ensures a higher rate of mutation, but, if the amount of red flowers diminishes, this rate decreases in the population.

4. Any increase of individuals with yellow flowers in a population is accompanied by a decrease of the rate of mutation, and so all individuals with differently coloured flowers gradually become supplanted by these yellow flowers.

9.25. Evolution in the Tropics. F. G. BRIEGER (Est São Paulo, Brazil).

The basic mechanism of phylogenetic evolution is likely to be the same in tropical and in north-temperate regions, where most studies so far have been carried out. The course of evolution will have been different, since temperate and tropical areas have had a different geological history since the Tertiary and since ecological conditions are also quite different, causing changes in selective trends. The orchids offer excellent material for such studies, since they are not only one of the youngest families of higher plants, but allow a detailed study of phylogenetic evolution owing to their dispersal, made possible by the smallness and large number of their seeds. Long distance dispersal is most easily studied between continents, and has been followed, almost exclusively by phylogenetic diversification into new species or groups of species or into new genera. In the case of the formation of new genera, this has followed generally the same lines as evolution in the original area, but with strong predominance of terrestrial orchids over epiphytes and with a strong tendency to form saprophytic species in the new area. In the cases of formation of species after dispersal between continents, there

exists a correlation between stronger evolutionary activity in the original area and the frequency of dispersal. Intracontinental dispersal has been studied in the American tropics. In addition to long distance dispersal, followed by allopatric evolution, slow penetration into new areas has occurred followed generally by marginal disruptive evolution. Explosive or sympatric disruptive evolution has also occurred after slow penetration or dispersal into a new area. In general, there seems to exist a correlation between larger evolutionary steps (genus formation) with restricted diversity (fewer species) and between smaller evolutionary steps and high degree of diversification.

9.26. Deviations from Panmixia as a Consequence of Sex-determination in the Marine Copepod, Tisbe reticulata. BRUNO BATTAGLIA (Padua, Italy).

Experiments have been carried out with the purpose of testing if, between the various phenotypes of the polymorphic Copepod *Tisbe reticulata* matings occur at random. Observations concern the following forms: *maculata* (V^m V^m), *violacea* (V^v V^v) and *violacea-maculata* (V^v V^m), controlled by two of the alleles of the *V* series.

Results show that, at least under certain conditions, the observed frequencies of some types of crosses differ from the frequencies expected on the assumption of panmixia. These deviations are mainly due to an excess of backcrosses whereas all crosses of other types are fewer than expected. This situation seems to be conditioned by the selective matings ♀ V^v V^m × ♂ V^m V^m and ♀ V^v V^m × ♂ V^v V^v.

The hypothesis is made that the heterozygous females are sexually more active than the homozygous ones, and the homozygous males more active than the heterozygous ones. This view is supported by other observations on the different fecundities of the three genotypes.

The results of other experiments seem to indicate that the deviations from panmixia depend to a certain extent upon the degree of relationship between the animals which mate.

The problem of the possible bearing of the above situation on the evolution of natural, as well as laboratory populations is being investigated.

The data will be published in full, possibly in *Evolution*.

9.27. Selection Experiments after Inbreeding in Habrobracon. R. E. SCOSSIROLI and R. C. VON BORSTEL (Pavia, Italy, and Oak Ridge, U.S.A.).

In previous experiments, long-winged substrains had been selected from the *small wing, white eye* stock of the parasitic wasp *Habrobracon* after six generations of inbreeding (expected homozygosity = 0.97(1). It was hypothesized that selection for longer wings in the inbred strains was made possible by either (1) maintenance of genetic variance by persistence of heterozygosity, or (2) a high spontaneous mutation rate for quantitative characters. Both long- and short-winged substrains were selected from the same strain of *Habrobracon*, using a family method and a selection pressure of 1.47 s, that is, only those were selected that were beyond 1.47 standard deviations from the mean. Selection under identical conditions was repeated after three generations of inbreeding by back-crossing to the same haploid male of daughters from subsequent generations. It was again possible to select long- and short-winged substrains (expected homozygosity = 0.94). On the other hand, attempts to select these substrains from three replicates after 12 generations were unsuccessful (expected homozygosity = 0.99+). This shows that by prolonged inbreeding, homozygosity can be obtained for characters affecting wing length. Therefore, a high spontaneous mutation rate does not contribute excessively to the genetic heterogeneity for characters affecting wing length.

1. SCOSSIROLI and VON BORSTEL, *Atti Associazione Genetica Italiana*, **7**, 191, 1962.

9.28. Competition Research on Unisexual Polymorphism in Megaselia scalaris. ROBERT SPRINGER (Vienna, Austria).

The newly discovered type of alternative sex determination in the Phoride *Megaselia scalaris* brings about a unisexual polymorphism in the natural population. It has to be assumed that three differing types of males co-exist in nature. In these three types always one of the three non-homologous chromosomes bears the sex realizator. Competition research on these three sex-determining types of males were carried out with artificial populations. Present results showed that the third chromosome always has a strong selective advance. In natural populations, however, the first chromosome mostly seems to be sex-determinating. Further results can be expected in due course.

9.29. Intensities of Selection in Natural Populations. LEIGH VAN VALEN (London, Great Britain).

With respect to any population parameter p, the intensity of selection i_p may be defined as the minimum proportion of the unselected population, per generation, that must die or not reproduce in order to generate the value of p in the selected population. Methods for evaluating phenotypic selection intensities from estimates of initial and final parameters have been developed and applied to new and old data in several phyla of animals.

Selection by mortality on the mean is usually less intense than but not negligible in comparison with that on the variance, indicating possible importance of temporal and spatial heterogeneity in the direction of selection. Intensities by mortality so far observed range from very near zero (blood groups in Englishmen) to 0.55 (tooth proportions in some extinct cave bears) for the mean, and to 0.3 (tooth width in an extinct horse) for the variance. The central tendency for randomly chosen characters is not yet evident. Natural selection by fertility has been largely neglected; the highest intensity known to me is about 0.05 in a hydromedusoid.

Destabilizing selection with an average intensity of about 0.2 is present on tooth width in semi-commensal house mice in Great Britain. Spatial heterogeneity exists for selection intensity on the mean here; the average intensity is about 0.08. The direction of selection on upper tooth dimensions in an extinct horse population is opposite to the direction of its evolution, also suggesting heterogeneity in selection. All the estimated juvenile mortality in this horse population can be considered selective for tooth dimensions.

9.30. Inherited Characteristics of Tribolium Populations. DANIEL J. MCDONALD (Carlisle, U.S.A.).

Several groups of *Tribolium confusum* populations were observed in population cages for more than a year. Weekly samples taken directly from the populations were used to determine the number of live and dead adults, pupae and eggs in the population. This revealed that the numbers of these forms were greater in some groups than in others. In the larger populations, fatalities were estimated to be higher and adult life spans shorter, and, in separate experiments, the population groups were also found to differ in the interaction between fecundity and adult density. The latter response was proposed as a possible cause of various other population differences. Total censuses of the populations revealed variations in the distribution of the life cycle forms within the cage. Dead adults tended to accumulate in corners and pupae preferred regions where the medium had not been recently renewed. On the basis of this information, characteristics and interactions of the populations which may influence both intra- and interspecific competition were proposed. The presence of characteristics leading to density dependent control of population size, was construed as an adaptation to a stable, undisturbed environment. Variations in these characteristics, generated initially by genetic differences and possibly amplified by population interactions, evinced retention by the species of the adaptive variability necessary for the maintenance of population fitness.

9.31. Polymorphisms in the Egg Albumen Proteins of the Domestic Fowl. I. E. LUSH (Edinburgh, Great Britain).

The egg albumen of the domestic fowl is a mixture of several different proteins. Starch gel electrophoresis has been used to analyse the albumen from individual hens at the Poultry Research Centre, Edinburgh, and genetic polymorphisms involving three of these proteins can be demonstrated. Each polymorphic protein occurs in two forms which differ in electrophoretic mobility. The polymorphism of each protein is determined by the segregation of two alleles at a corresponding genetic locus. Data from crosses will be presented. One of the polymorphic proteins, ovalbumin, is electrophoretically heterogeneous even in the albumen of a hen which is homozygous at the corresponding locus (named *Ov*.). Data on the analysis of this heterogeneity by enzymic modification of ovalbumin will be presented.

9.32. Heritability of Developmental Time and Viability of Turkey Embryos in Three Environments. W. A. BECKER and T. P. BOGYO (Pullman, U.S.A.).

The relationship between estimates of genetic variation and environmental change was investigated by subjecting turkey embryos to stress. The eggs from matings of 40 sires with 400 dams were stored for 1, 2 and 3 weeks before incubation. For developmental time, 1 week storage: $\bar{x} = 647.5$ hr, $h_s^2 = 0.31 \pm 0.06$, $h_D^2 = 0.56 \pm 0.08$; 2 weeks storage: $\bar{x} = 652.2$ hr, $h_s^2 = 0.20 \pm 0.07$, $h_D^2 = 0.53 \pm 0.08$: 3

weeks storage: $\bar{x} = 656.5$ hr, $h_s^2 = 0.14 \pm 0.08$, $h_D^2 = 0.63 \pm 0.12$.

The viability of embryos for 1 week of storage was: $\bar{x} = 67.0$ per cent, $h_s^2 = 0.12 \pm 0.03$; 2 weeks: $\bar{x} = 50.9$ per cent, $h_s^2 = 0.08 \pm 0.03$; 3 weeks: $\bar{x} = 29.5$ per cent, $h_s^2 = 0.11 \pm 0.03$. Heritability was highest during the 0-7 day incubation stage ($h_s^2 = 0.08$ to 0.16); the other stage (8-29 day and pipping) had $h_s^2 = 0.00$ to 0.05.

Developmental time was increased by storage while viability decreased. Heritability estimates were not materially changed for these traits by the environmental changes. Developmental time was influenced by maternal effects. Most of the genetic variation of viability of turkey embryos occurred during the 0-7- day stage of incubation.

9.33. The Effects of Temperature on Two Strains of Mice of Different Body Weight and Tail Length. F. COCKREM (Palmerston, New Zealand).

Two strains of mice, one of high body weight and short tail length (line A), and one of low body weight and long tail length (line B), have been developed by selection. As an initial study of possible correlated changes associated with tail length and adaptive ability, the two lines were reared at temperatures of 7°C, 21°C (control) and 32°C from 3 weeks to 6 weeks of age. Tail lengths and body weights were measured at these two ages. Ten males and 10 females of each strain were used for each treatment.

For both strains and sexes of mice, the cold (but not the hot) treatment depressed body weight growth. There were no interactions and the effect of the treatments was proportional in both strains of mice. For the variances of body weight at 6 weeks, the only effect which was consistent for both sexes was a reduction of variance for the A line under the hot environment.

For both lines a higher temperature was associated with a longer tail length, but line B showed a greater increase under the hot and control environment. Under the cold environment, both lines showed a similar increase.

Thus a genotypic difference in tail length was associated with a difference in the degree of response of this character to the three environments. Furthermore, a strain difference in tail length and body weight was not associated with a difference in the ability of the two strains to adapt to the different temperatures.

9.34. An Investigation of Genotype-environment Interaction in the Mouse. PETER HULL (Rochester, U.S.A.).

It is to be expected that the genetic variance between sires, estimated from the performance of their progeny, will be small when the sires are from the F_1 of a cross between two highly inbred lines, and larger when the sires are from the F_2 of a cross of the same two inbred lines. From previous work with poultry it was decided that there was probably a relationship between the magnitude of interaction of a group of genotypes with environment, and the magnitude of the additive genetic variance within the group of genotypes tested. The results of the experiment which is to be described are in agreement with this hypothesis, since it was found that the interaction of sires with environment was greater when the sires were from an F_2 group than when the sires were from an F_1 group. This difference was noted in the case of a metric character (tail length), whose heritability was expected to be comparatively high—in the case of a second character (body weight), whose heritability might be expected to be lower, there was no detectable difference in the size of the genotype-environment interaction component of variance between the two groups of sires.

9.35. Selection Progress Toward an Absolute Limit for Amount of White Hair on Mice. W. H. KYLE and H. D. GOODALE (Lafayette and Williamstown, U.S.A.).

Selection for amount of white hair was initiated in 1931 by one of us (H.D.G.) in a stock derived from one male mouse with a few white hairs on its forehead and four self-colored females. A sample (5 males and 6 females) of the selected stock was transferred to Purdue late in 1959 from which eight generations have been produced. All mice were scored at weaning for percentage of white on the dorsal surface. After the first generation, numbers of offspring ranged from 74 to 210.

The unweighted mean per cent white of the eight generations is 69.5 per cent for males and 64.7 per cent for females. Regression of sex means on generations shows an average gain per generation of 1.73 per cent for males and 0.55 per cent for females. Regressions of offspring on parent are quite variable but indicate a low degree of heritability. There is some indication of a sex-linked effect. Standard deviations within sex and generation are large.

Beginning in the 5th generation, two or more all-white dark-eyed males have been obtained in each generation for a total of eleven. One all-white female was obtained in the 8th generation from an all-white sire. Tests show the absence of albino and the absence of genes dominant to wild type.

The tremendous changes in the mean and variance in this closed population from an initial level of essentially zero are of primary importance. Although the mean of this sample does not approach the absolute limit of selection, a dozen individuals have reached the phenotypic limit.

9.36. Effects of Mating System on Ovulation, Implantation and Litter Size in Mice. T. G. MARTIN, F. E. HARRINGTON and D. L. HILL (Lafayette, U.S.A.).

Four inbred lines of mice were mated in a diallel mating system producing sixteen genetically different groups of offspring. Samples of four females for each trait in each genetic group were drawn to estimate number of ova shed, number of implantation sites and litter size. Embryonic mortality was estimated by number of ova shed minus litter size. Pureline versus crossline comparisons made in the parental generation yielded only one statistically significant difference with crossline and pureline matings having 9.17 and 8.73 implantations, respectively. Averages for the other traits were: litter size 8.33 and 8.38, ova shed 9.88 and 10.25, and embryonic mortality 25.9 and 26.9 per cent for crossline and pureline matings, respectively. In the F_1 generation, crossbred and pureline matings were compared. Crossbreds performed at significantly higher levels than pureline mice in all traits except ovulation rate. Crossbred averages were 11.24 ova, 11.39 implantations, 9.02 offspring and 19.8 per cent embryonic mortality. Pureline averages were 10.29 ova, 8.63 implantations, 7.53 offspring and 29.0 per cent embryonic mortality. Analysis of the F_1 data indicated strongly that specific combining ability was a more important source of variation than general combining ability thus indicating that a large portion of genetic variation is due to dominance and epistasis and a smaller portion due to additive genetic effects. Mating system influenced time of embryonic death with 36, 58 and 100 per cent of embryonic deaths occurring after implantation in pureline, crossline, and crossbred matings, respectively.

9.37. Correlated Responses to Selection for Radiation Resistance in Mice. THOMAS H. RODERICK, (Bar Harbor, U.S.A.).

Selection for survival under daily doses of X-rays was successful in two experiments: (1) under 100r/day, and (2) under 400r/day. Litter size was a correlated response in both experiments, and body weight only in the first experiment.[1]

The correlated response in body weight was only in the low direction, and that response was more pronounced in the males than in the females. For the females, the means and standard errors for body weight were: 39.3 ± 1.0 g for the high line, 39.4 ± 1.1 g for the unselected control line, and 35.6 ± 1.0 g for the low line. For the males: 41.8 ± 0.5 g for the high line, 44.6 ± 0.6 g for the unselected control line, and 29.6 ± 0.8 g for the low line.

The first selection experiment also produced a correlated response in $LD_{50:30}$° $LD_{50:30}$ means and standard errors for the females were: 905 ± 18r for the high selected line, 833 ± 21r for the unselected control line, and 788 ± 21r for the low selected line. Survival under 400r/day was also a correlated response in the direction of selection.

This study suggests that, although the selected trait appears to have responded symmetrically, body weight (a highly correlated character) has responded asymmetrically. These results in turn suggest that the physiology of response in the upward direction is very different from the physiology of response in the lower direction. It is possible that the loci involved in upward selection of this trait are quite different from the loci involved in downward selection.

1. RODERICK, *Genetics* **48**, in press.

9.38. Evidence that Selection for the Maintenance of Developmental Homeostasis may Lead to the Retention of Genetic Variability. H. T. BAND (Vancouver, Canada).

Changes in the lethal + semilethal frequencies in the South Amherst *D. melanogaster* population can be correlated with changes in environmental variables (Band and Ives, 1961). Analysis of homozygotes and random heterozygotes containing second chromosomes from the 1960 population suggest that the gene pool is integrated on two levels: (1) in the maintenance of developmental homeostasis and (2) in the magni-

tude and organization of the genetic load (Band and Ives, 1963). Experiments by Band (1963) indicate that if the gene pool remains coadapted, then genetic changes which occur must be accompanied by the maintenance of developmental homeostasis among the random heterozygotes.

Trends toward more adverse environmental conditions have been observed during 1961 and 1962. The lethal allelism frequency of 1 per cent in 1961 indicates reduction in population size; tests for 1962 are in progress. Genetic variance (σ_g^2) relationships between random heterozygotes and nondrastic (nd) homozygotes in 1960, 1961 and 1962 reveal changes in genetic organization. In 1960 $\sigma^2_{g\ het} < \sigma^2_{g\ hom}$, in 1961 $\sigma^2_{g\ het} > \sigma^2_{g\ hom}$ due to the genetic variance of the nd/nd heterozygotes; in 1962 $\sigma^2_{g\ het} < \sigma^2_{g\ hom}$. Nevertheless, in both 1961 and 1962 heterozygotes containing two, one or no "drastic" second chromosomes possess comparable developmental homeostasis, superior to that of the nd homozygotes in each collection.

These findings provide evidence that the genetic diversity carried in the gene pool enables the population as a whole to shift from one of Wright's adaptive peaks to another. They also indicate that the attainment of new equilibrium gene frequencies is influenced by a variety of factors both external (environmental) and internal (the achievement and maintenance of developmental homeostasis; Band, 1963). Thus, selection for the maintenance of developmental homeostasis may lead to the retention of genotypic variability in the population (Lerner, 1956).

Supported by grants from the National Science Foundation and the National Research Council of Canada.

9.39. The Estimation of Population Fitness. J. A. Beardmore and W. van Delden (Groningen, The Netherlands).

In the simplest terms the fitness of a population is the weighted average of the fitness of the individual genotypes of which it is composed. Two practical difficulties encountered in connection with this simple statement are: first that it is almost impossible in outbreeders to specify all the genotypes concerned (thus preference is given to polymorphisms as objects for study) and secondly even when the genotypes are specified the estimation of the fitness of individual genotypes may have to be made in isolation and hence interactions of all types are ignored. This leads to a situation where empirical observations of suitable populations may be valuable in assessing relative population fitness over both short and long periods. We have attempted to estimate the relative fitness of a number of laboratory populations using as indices of fitness biomass production, longevity, fertility and other characters. We hope to report on the correlation between genetic variance and fitness in a standard environment, correlation of fitness values in different environments and the environment as a component of fitness.

9.40. Synchronous Mutability Changes in Geographically Isolated Populations of Drosophila melanogaster. R. L. Berg (Leningrad, U.S.S.R.).

During 1937-41 high concentrations of yellow mutants and high mutation rates of the yellow locus were detected in five geographically isolated populations of *Drosophila melanogaster*. The studied populations were Uman, Ukraine (1937), Nikita Botanical Gardens, Crimea (1937-38), Dilijan and Erevan, Armenia (1939), Kashira, near Moscow (1940-41).

During 1945-46 we studied the populations of Kutaissi, Georgia (1945), Tiraspol, Moldavia (1946) and Uman (1946). In Uman and Tiraspol high concentrations of yellow mutants were again observed. The rate of occurrence of the yellow mutation proved to be lower than during the previous period of investigations.

During 1957-62 low concentrations of yellow mutants and low mutation rates were seen in nine populations (Piatigorsk and Inosenitsevo, North Caucasus 1957; Uman, 1958, 1962; Nikita Botanical Gardens, 1960, 1962; Dilijan 1960-62; Kashira, 1960, 1962; Alma-Ata, 1961; Tiraspol 1962).

The concentration of yellow mutants among wild males was: in Uman in 1937 *0.246 ± 0.49 per cent* (25 yellow males among 10,159 wild males), in 1946 *0.301 ± 0.090* (11 yellow males among 3652 males), in 1958 there were no yellow mutants among 13,131 males.

The mutability in the Uman population in 1937 was 0.149 ± 0.027 (30 yellow males among 20,144 males) in 1946 in a mutability test among 5394 males we found no yellow mutants and in 1958 among 84,831 males in the progeny of wild flies, only three (0.035 ± 0.020 per cent), i.e. one forty-third, of the percentage observed in 1937.

Such a tremendous change in the occurrence rate of visible mutations was not observed in other loci of the sex-chromosome. A certain decrease in the rate of occurrence of sex-linked lethals was also observed in some populations.

Synchronous mutability changes in geographically isolated populations involving such an extensive area ought probably be considered as caused by some external geophysical or cosmical factor, influencing differently the mutability of different loci. We can only guess at the nature of the factor which may act either directly or through the medium of some bio-ecological changes.

9.41. On the Evolutionary Signification of Heterozygosity in Drosophila Populations. E. BÖSIGER (Gif-sur-Yvette, France).

Natural populations of *Drosophila melanogaster* show a high degree of heterozygosity.

The vigour of males of stocks recently derived from natural populations is higher than that of inbred laboratory stocks. Inbreeding lowers the vigour. Hybridization of two stocks of low vigour produces very vigorous males. This hybrid vigour is maintained for at least sixty generations in populations of at least 200 flies.

The advantage of heterozygous males in intraspecific sexual selection constitutes a mechanism of genetic homeostasis which maintains the high degree of heterozygosity of natural populations. Heterozygous flies produce more eggs and spermatozoa and resist moulds and bacteria better.

A high degree of heterozygosity seems to be important for natural populations of *Drosophila*, which are very reduced in winter. The maintenance of a rich gene pool by heterozygosity avoids rapid changes of the genetical constitution by genetic drift, and enables the species eventually to undergo rapid expansion or an adaptation to changing conditions.

This work on intraspecific sexual selection and on the comparison of the fecundity of homozygous and heterozygous flies leads to a new technique of augmentation of the vigour in inbred lines without loss of the acquired selection profit.

The enrichment of the morphological, physiological and psychological possibilities of a species by the creation of new loci seems to be the major progress of evolution and could be more important than the substitution of alleles at existing loci by mutation.

9.42. The Effect of Inbreeding on the "Fitness" and on the Rate of Attainment of Acclimatization to Temperature in Drosophila subobscura. K. BOWLER and M. J. HOLLINGSWORTH (London, Great Britain).

A further aspect of the hypothesis that inbreeding leads to a loss of "biochemical versatility"[1] has been tested. Males from the B & K inbred lines of *Drosophila subobscura* and from the recriprocal hybrids between these inbred lines were used.[2] The criterion of biochemical versatility has been the rate at which these males can adapt to changes in temperature. Fitness has also been measured by comparing the ability of the inbreds and hybrids to withstand a high lethal temperature (34° C). All flies were fully acclimatized at either 14.5° C or 25° C and then placed at the reverse acclimatization temperature for periods varying from 1 hr to 1 week.

The results have shown that there is no significant difference between survival times at 34° C of the B/K and K/B hybrids. However, both in the 14.5° C and 25° C adapted groups the hybrids survived longer than either of the inbred lines, this being particularly noticable in the 14.5° C adapted groups. Of the inbreds the K males were slightly more "heat-tolerant" than the B males. All flies gained adaptation at 25° C from 14.5° C much more rapidly than they lost this adaptation when taken from 25° C and put at 14.5° C. The hybrids both lost and gained acclimatization to 25° C more quickly than either of the inbred lines.

It is concluded from these results that inbreeding leads to a loss of biochemical versatility and also to a loss of fitness.

1. J. B. S. HALDANE, (**1948**) *Ric. sci. Supp.* **54.**
2. J. MAYNARD SMITH, (**1956**) *J. Genet.* **54,** 261.

9.43. Life Cycle and Mating Activity as Criteria of Heterosis in Heterokaryotypes in Drosophila pavani. DANKO BRACIC and SUSI KOREF-SANTIBAÑEZ (Santiago, Chile).

The study of chromosomal polymorphism due to inversions, found in many species of the genus *Drosophila*, has proven that these constitute an adaptive character, and that frequently the heterozygotes for different gene arrangements (heterokaryotypes) are heterotic. Nevertheless, more experimental data are needed in order to determine the exact mechanisms by which such adaptive superiority is achieved, and why balanced polymorphism is maintained in nature.

The Chilean species, *Drosophila pavani*, is polymorphic for gene arrangements in its chromosomes. Studies of the mechanisms which contribute towards the maintenance of this polymorphism in the populations have shown that the frequency of heterokaryotypes with respect to some gene orders is significantly higher in 100-day-old flies than in larvae or in young 10-day-old adults. This finding suggests that the selective pressures which confer a higher fitness to these heterokaryotypes may act both during the preadult and the adult life stages.

When mating activity was taken as a criterium for the superiority of heterokaryotypes, it was found that, in a very heterogeneous population, the group of males which courted or copulated within the first 30 min of contact with the female, contained a significantly higher proportion of heterozygotes for inversions than the group of males which copulated after this period. As mating activity may be important in fitness, these findings could in part explain the maintenance of balanced polymorphism in the populations.

9.44. Sexual Isolation Between the "Sibling" Species Drosophila pavani and Drosophila gaucha. Susi Koref-Santibañez (Santiago, Chile).

Drosophila pavani and *Drosophila gaucha* are two neotropical "sibling" species of the *mesophragmatica* group of the genus *Drosophila*. They are mostly allopatric in their distribution, although they have been found sympatrically in certain regions. Hybrids have never been found in nature, and in the laboratory, where they can be produced abundantly, they are sterile. Chromosomally the two species differ only in the gene arrangement in their X chromosomes.

The different levels of sexual isolation were studied in order to try to establish the mechanisms by means of which gene exchange between *D. gaucha* and *D. pavani* is prevented. Thus, among others, courtship behaviour, mating ability, sperm viability and early embryonic development were analyzed. Previous work of Fernandez in our laboratory had shown that spermatogenesis in the hybrids was apparently normal.

Courtship behaviour in both species and in their hybrids is very similar, although the hybrid males are slightly more inactive. They copulate, and hybrid females accept *D. pavani*, *D. gaucha*, and hybrid males. Nevertheless, an embryonic development is seen in the eggs of inseminated females. In view of the above-mentioned data, it seems that under laboratory conditions, the main mechanism of hybrid sterility depends on the lack of fusion of the male and female gametes. To support this hypothesis there exists experimental evidence of the rapid inactivation of hybrid sperm within the female vagina. The factors involved in this inactivation are now being analyzed.

9.45. On the Behavior of Lethals in Natural and in Laboratory Populations. A. Brito da Cunha, J. S. de Toledo, S. A. de Toledo, L. E. de Magalhães and C. Pavan (São Paulo, Brazil).

To study the behavior of lethals in nature, 7 wild and 7 radiation induced lethals were introduced in two isolated island populations in 2 experiments. The lethals, which before had an allelic frequency of 6 per 10,000 chromosomes, reached, after the introduction, a mean frequency of 0.105 in one population and of 0.119 in the other in the first experiment and of 0.184 and of 0.205 in the second. The introduced lethal frequencies in the first experiments dropped to 0.012 in one population and to 0.001 in the other after 8 generations, being practically eliminated after 15 generations. In the second experiment the mean frequencies dropped from 0.205 to 0.015 in one island and from 0.184 to 0.008 in the other in 4 generations.

Parallel experiments with the same lethals were carried out in the laboratory using population cages. In these experiments every lethal had an initial frequency of 0.250. After 15 generations the lethals had frequencies between 0.174 and 0.030, 6 out 16 lethals having frequencies equal or above 0.100.

There is a sharp contrast between the behavior of the lethals in nature and in the laboratory. In the laboratory the selection was very slow and the lethals behaved as recessive. In nature the elimination was not uniform. The lethals had deleterious effects in the heterozygotes behaving as incomplete recessives with effects which varied with ecological factors. The period of drastic selection was always correlated with the deterioration of the environmental conditions. Radiation induced and wild lethals behaved similarly.

9.46. Dynamics of Lethal Genes in Natural Populations of D. willistoni. L. E. de Magalhães, A. B. da Cunha, J. S. de Toledo and C. Pavan (São Paulo, Brazil).

The frequencies of lethals released in the populations of the islands of S. João and Queimada Pequena decreased in the 1st to the 4th generations according to the expectation due to elimination through homozygosis and dropped

very quickly in the succeeding generations to almost zero in generation 15. The drastic elimination of such lethals (14 in total, in two different experiments) after so few generations could be explained by changes, due mainly to environmental factors, in the adaptative values of the zygotic combinations carrying lethals.

If this hypothesis were correct, a change in the frequencies of chromosomes bearing lethals in a control island would be expected. We have four samples analysed from a control island, Queimada Grande, very close to the other two. The samples were taken in June and July when the frequencies of lethals should be high and in October and January when the frequencies should be lower. The frequencies found were: 0.41, 0.39, 0.39 and 0.34 per cent respectivelly. No statistically significant difference was found. This shows that there are no corresponding increases of elimination of lethal chromosomes, in the natural population for their own lethal, present in very low frequencies.

This comparison suggest, that the relative frequencies of the genetic components could be very important in their own dynamics.

9.47. A New Type of Hybrid Sterility in Drosophila paulistorum. L. EHRMAN (New York City, U.S.A.).

It has been shown that *Drosophila paulistorum* is a group of at least six races or incipient species, and that, at our time level, reproductive isolation is in the process of formation between these incipient species. Crosses between three (Centro-American, Amazonian, Andean-South Brazilian) of the six races result in the production of fertile female and sterile male hybrids. The male sterility has been found to depend on the genotype of the male's mother. Any female which carries any mixture of the chromosomes of different races deposits eggs giving rise to sterile male zygotes and to fertile female ones. The male sterility is independent of the genotype of the male parent and the genotype of the sons themselves. What happens is evidently that the presence of a foreign chromosome in the female so alters the structure of her eggs, presumably by some modification of the egg cytoplasm, that male individuals developing from these eggs are sterile, and this regardless of the chromosomal complementwhich they come to possess after fertilization. Furthermore, any one foreign chromosome (the species has three pairs of chromosomes) suffices to induce this male sterility.

More recent work involving all six of the *D. paulistorum* races permits the categorizing of six distinctly different manifestations of this new type of hybrid sterility; these further emphasize the unique dichotomy in the genetic causation of the F_1 and of the *backcross* hybrid male sterility exhibited by this species complex.

9.48. Subspeciation in Drosophila melanogaster. G. ELOFF (Bloemfontein, South Africa).

Cultures were made of more than 30 populations of *Drosophila melanogaster* collected over Southern Africa at localities, some of which are almost 1000 miles distant and involving important ecological differences. These populations were bred under similar conditions in the laboratories of the Dept. of Genetics of the Orange Free State (20 C; very dry conditions; banana, agar and yeast moldex food recipe). Of each population the average yield per 10 females was determined. Great differences in number of offspring were observed. These different geographic populations were intercrossed and the number of F_1 per 10 females of each cross counted. The number of offspring ranges from nil to more than the average for each parental population.

Furthermore, it was found that in some cases a population would not cross with Oregon River or Florida population of *Drosophila melanogaster* but would cross with another geographic race, which in its turn does cross with Oregon River. Several of these transitional stages were recorded.

These three aspects of geographic populations, viz. (1) differences in fitness under similar conditions of breeding; (2) differences in average number of F_1 of the different interpopulation crosses; and (3) degrees of succesful crossing between the different geographic populations, strongly suggest considerable genetic differentiation and perhaps subspeciation in progress.

9.49. Isolation by Disruptive Selection. J. B. GIBSON and J. M. THODAY (Cambridge, Great Britain).

Thoday and Gibson[1] exposed a population "Southacre" of *Drosophila melanogaster* to disruptive selection for sternoplural chaeta number using a breeding system that ensured that all selected flies were together in a single 3×1 in. vessel for the limited period during which all mating had to occur. Their population rapidly split into two components between which little if any effective mating occurred.

Selection was discarded in this line when extreme fertility trouble occurred at generation 28. However, isolated high and low chaeta number lines were maintained. A new line was started

from the "Southacre" stock under the same selection régime. Divergence was more rapid in the second experiment than in the first and the two halves of the population became completely distinct by the seventh generation.

Mating preference tests in this new line using chaeta number as a marker have indicated an isolation index of +0.54 (where 0 would represent random mating and +1 complete positive assortative mating). There is therefore evidence of strong mating preferences in the line. It does not however seem sufficiently strong to provide a complete explanation of the lack of hybrids in the line itself. Further evidence suggests that the hybrids that are formed may compete poorly with the non-hybrids.

1. *Nature* **193**, 1962.

9.50. Selection for Xanthine Dehydrogenase Activity Levels in Drosophila melanogaster. EDWARD C. KELLER JR. (Chapel Hill, U.S.A.).

In an attempt to obtain information about the quantitative genetic control of individual proteins, a series of selection and breeding experiments were undertaken using xanthine dehydrogenase levels as the selected phenotype. Various methods of selection have been effective in isolating strains which have very high or very low levels of this enzyme. One high activity line (three or four times the "standard" Oregon-R strain) is of a dominant or overdominant nature and it has not as yet been possible to localize the genetic cause or causes of this high activity. A random sample of about 100 wildtype strains of diverse geographic origin were also assayed for their mean xanthine dehydrogenase activities and many differences in activity levels were found. Analyses of the data revealed that these differences were mainly due to genetic causes. Indeed, several strains contained an autosomal recessive gene which when homozygous limited the xanthine dehydrogenase activity levels to about 25 per cent of the „standard" strain. Subsequent analyses have shown that this gene is located near the center of the left arm of the third chromosome. Therefore, this new mutant is not an allele of the *rosy* or *maroon-like* eye color mutants, which have no detectable amounts of xanthine dehydrogenase.

This research was supported in part by a research grant (GM 08202-03) from the National Institutes of Health.

9.51. Selection for Interpopulation Heterosis. KENICHI KOJIMA (Raleigh, U.S.A.).

The base populations of this study were two divergent long-term cage populations of *D. pseudoobscura*. The trait used was egg production. Within each cage there was no significant additive genetic variance, although there existed substantial non-additive hereditary variances. Their hybrid progenies did not show heterosis, but crossbred half-sib covariance was significant. Reciprocal recurrent selection, a scheme in which two populations are selected for their populational combining ability, was employed in order to increase interpopulational heterosis.

Approximately 40 per cent gain in crossbred progeny performance over the original hybrid was obtained by the 11th cycle of selection, with only slight increase in purebred performance of the two populations. The increase in crossbred performance was accompanied by a reduction of additive genetic variance in crossbred progenies. During the next 9 cycles the crossbred performance more or less plateaued, while significant improvement was observed in purebred performance. Thus, in reference to the average performance of the base populations, the level of heterosis was increased from zero to 40 per cent. With reference to the performance of purebred progenies at various stages of selection, however, the degree of heterosis changed from nil through the highest to a low value of 10 per cent.

9.52. A Chromosomal Analysis of Genetic Variation in Drosophila melanogaster. J. F. KIDWELL, J. W. GOWEN and JANICE STADLER (Ames, U.S.A.).

The marked inversion outcross technique was used to form two lines of *Drosophila* that were isogenic for all four chromosomes. The 81 possible combinations among females were made. Egg production during the sixth, seventh and eighth day and the number of chaeta on the fourth and fifth abdominal segments were measured. Theanalysis was that of a 3^4 factorial experiment, which provided a complete partition into additive, dominance and epistatic genotypic effects. Additive and dominance effects, and some two and three factor interactions, were important. Single, double and triple heterozygotes showed increasing heterosis. The quadruple heterozygote exhibited less heterosis than the triple heterozygote for chromosomes1, 2 and 3.

9.53. On the Influence of the Number of Inbreeding Generations on Viability and Size in Drosophila melanogaster. F. A. LINTS (Louvain, Belgium).

As pointed out by Lerner (1954) attempts to verify the general idea that heterozygotes are better canalized than homozygotes are urgently needed. A wild strain was submitted to inbreeding immediately after capture, and in order to obtain also reciprocal hybrids, each generation was backcrossed to the wild strain Swedish-B. Twelve generations were realized, seven of which were measured for several traits at five temperatures ranging from 30 to 18 C. This study concerns only viability and size. A full report is actually in preparation and will be submitted to *Heredity*.

Viability: In absolute values the viability of hybrids does not vary during the successive generations, but in relative terms, and with relation to the control passes from a superior to an inferior level. There is no relation between the number of inbred generations and the percentage heterosis for viability. The variance of inbreds increases irregularly between the first and the 12th generation; the effects of inbreeding on viability can be more adequately described by the regression viability-temperature which increases regularly with the number of inbred generations.

Size: The coefficient of variation of the size for inbreds and hybrids, considered in the complete array of environments used, is identical. This unexpected fact is due, for the hybrids, to a smaller variance in some of the environments considered, combined to a significant regression in function of temperature, for the inbreds, to a large variance at each temperature and to a quasi absence of temperature-size regression.

Conclusion: The incompatibility of these data with the classical hypotheses of additivity and of heterozygosity *per se* have to be stressed. In view of the actual and earlier results inbreeding degeneration has to be considered at two levels one at which it has its maximal effect, on viability namely, the other at which it is probably less active, namely on various luxuriance and perhaps on fitness traits of the hatched individuals.

9.54. Reproductive Isolation between Incipient Species of Drosophila paulistorum in Brazil. CHANA MALOGOLOWKIN and A. SOLIMA SIMMONS (New York, U.S.A.).

Two races or incipient species of the *D. paulistorum* complex are known to occur in Brazil, the Amazonian in the states of Amazonas and Pará and the Andean-Brazilian in the territory to the south. At Belém, Pará, both races occur sympatrically. The strains of the two races are difficult to cross, and when crossed produce progenies consisting of fertile daughters and sterile sons. A number of so-called "transitional" strains were however found in the states of Bahia and Ceará; they belong to the Andean-Brazilian race, but they can be crossed and produce fertile hybrids with some strains of the Amazonian race. Sexual (ethological) isolation has been studied, by means of the "male choice" method, between various strains of the two races. Females of the transitional strains show a significantly weaker isolation from Belém Amazonian strains than do females which produce sterile hybrids with the latter. The Amazonian females from Belém do not however accept males of the transitional strains any more easily than do males of the other strains of the Andean-Brazilian race.

The results as a whole are consistent with the assumption that sexual isolation is built up, or at least strengthened, by natural selection in territories where two races coexist sympatrically. Territories inhabited by only one race have populations which show relatively weaker isolation when exposed to the challenge of hybridization with other races.

9.55. The Races of Drosophila rubida. W. B. MATHER (Brisbane, Australia).

The species *D. rubida* Mather of the *immigrans* species group occurs in northern Queensland, New Guinea, New Britain and New Ireland and is morphologically uniform. However, it has been shown to be rich in inversion polymorphism by mating males and despermed females from the wild to an inversion free standard strain from Northern Queensland and scoring seven larvae per cross against a photographic giant chromosome map prepared from the standard strain. By these methods eleven simple and three complex inversions have been detected.

Sexual isolation tests between cultures from Cairns (Northern Queensland), Port Moresby, Madang, Lae and Samarai (Eastern New Guinea) and Rabaul (New Britain) have been carried out by confining ten males of one strain with ten females from another and after a food change at five days dissecting the female genital tract for the presence of sperm after a total of ten days. By these methods it has been shown that wherever Rabaul males are involved in the cross the sexual isolation is high (usually less than 50 per cent insemination) and that sometimes when

Rabaul is involved the F_2 does not come through. However, backcrossing of the F_1 female to males from the original parents is successful in these cases.

Cytologically the inversion complex IIRE has only been detected at Rabaul and is homozygous there. Apart from IIRE only simple IID with a frequency of 56 per cent has been detected.

Thus on the available evidence we may distinguish two races of *D. rubida* between which there is considerable sexual isolation and hybrid sterility: *Race A* from northern Queensland and Eastern New Guinea without inversion IIRE and *Race B* from Rabaul with inversion IIRE.

9.56. The Relation between Fitness Components and Equilibrium Frequencies in an Experimental Population of Drosophila melanogaster. RAY MOREE (Pullman, U.S.A.).

In laboratory populations initiated with equal frequencies of the alleles + and e'', the carriers of e''/e'' fell rapidly to a mean stable frequency of about 2.46 per cent. That similar results obtain with several alleles of e and that stabilization is probably a consequence of the superiority of the carriers of $+/e''$, is well known. Several fitness components possibly contributing to these effects were examined. Mass mating tests (but not choice tests) indicated only slight selection against e''/e''. Viability tests, with crowding such that numbers of parents and offspring were approximately equal, gave coefficients of 92, 100 and 46 per cent for the carriers of $+/+$, $+/e''$ and e''/e'', respectively (Moree and King, 1961). Fecundity tests, corrected for inviability, gave coefficients, in the same order, of 88, 100 and 85 per cent (Moree, 1962). Since such components measure sequential events they may be multiplied to obtain partial fitness coefficients of 81, 100 and 41 per cent (same order). These coefficients indicate an equilibrium frequency of $\hat{q}$ $(e'')=24.28$ per cent. If panmixis is provisionally assumed, then $\hat{q}^2$ $(e''/e'')=5.90$ per cent. But this is an overestimate. If the above viability coefficients are included the calculated equilibrium frequency of e''/e'' is reduced to 3.09 per cent, since, at equilibrium, inviability changes genotype, but not gene, frequencies. By t-test, 2.46 and 3.09 per cent differ ($P<0.01$, $N\geqslant 7000$). Thus while heterosis accounts for stabilization, and inviability and infecundity for about 97 per cent of total selection, a small fitness component, probably related to selective mating, is yet to be accounted for.

9.27. "Yellowish" in Drosophila bifasciata, conceivable as an Incipient Species. DAIGORO MORIWAKI and OSAMU KITAGAWA (Tokyo, Japan).

In northern parts of Japan, e.g. Hokkaido, *Drosophila bifasciata* which is known to inhabit cold or mountainous districts in the rest of the country can be collected at level ground even in early summer. At three localities, so far confined to Hokkaido, some mutant flies with yellowish thorax color—designated by "yellowish" (*yh*)—were collected once in a while. Interestingly, they were found always together with wild type flies.

Besides the thorax color, "yellowish" has one —not two—black stripe on the scutum, which expands postlaterally forming a trident-like pattern and it also shows some differences in metric characters from the wild type.

"Yellowish" females can cross with wild type males to some extent, while the reciprocal cross is difficult. Isolation index obtained with the aid of "male-choice" method, amounts to 0.8 on the average. It seems that "yellowish" is considerably isolated sexually from the wild type, and can live sympatrically with it.

According to the test for fertility of the F_1 hybrid flies, the male hybrids are always sterile, notwithstanding slight fertility of the female ones.

On examining salivary gland chromosomes of the hybrid between *yh* and +, no complete paring has been seen in any pair of the homologous chromosomes. Although further careful comparisons are being made, it is tempting to speculate that "yellowish" may be an incipient species.

9.58. A Study of the Genetic Structure of Natural Populations by Means of Polygenic Viability Mutation Rate in Drosophila melanogaster. TERUMI MUKAI (Misima, Japan).

A single male *Pm*/+ from a cross of *Cy*/*Pm* × W160 (Burdick's isogenic wild-type stock) was sampled and multiplied to 104 by crossing to *Cy*/*Pm* females, and each wild-type second chromosome has been maintained through a single male by the cross of *Cy*/*Pm* (5♀♀) × *Pm*/ $+_I$ (1♂) ($i=1, \ldots, 104$) for the purpose of accumulating spontaneous mutations affecting viability.

In generations 10, 15, 20, and 25, the homozygous viabilities of those 104 chromosomes were estimated by *Cy*-method (Wallace, 1956) and their increasing rate of genetic variance and

decreasing rate of average viability in homozygous condition were estimated.

Under the assumptions that the mutations in question are distributed on the chromosome according to Poisson distribution and that all mutations are homozygously deleterious, the average mutation rate was estimated to be at least 0.1 per second chromosome per generation on the basis of counting about 1.8 million flies. This mutation rate is of a surprisingly large magnitude.

When the survival rate of eggs is calculated on the basis of the classical hypothesis and this estimated mutation rate, it becomes about 58 per cent in random mating populations, and is inconsistent with the actual situation. Thus, the result is unfavorable to the classical hypothesis.

9.59. Lethals and the Parameters of a Southern Population of Drosophila willistoni. M. Napp, H. Winge, M. L. Reguly and A. R. Cordeiro (Porto Alegre, Brazil).

Random crossing, mutation pressure and the progressive betterment of gene coadaptation in complex genic balance due to natural selection, builds the genetic structure of natural populations.

Genetic analysis of a natural population inhabiting an isolated, small wood in the grassland of Rio Grande do Sul State, disclosed significantly different frequencies of II chromosomes bearing lethals or semi-lethals, averaging 44.7 ± 1.7 per cent for the period of March 1959 to April 1961 with 895 strains studied. The III chromosome lethals + semilethals frequencies showed no changes and averaged 29.5 ± 2.3 per cent in five samples from March 1958 to December 1959 (389 strains). The total intrapopulation allelism frequency determined is: II=0.0102 III= 0.0029.

With the aid of S. Wright's formulae we calculated that the rate of lethal incorporation for II chrom. is 0.0046 and 0.0049 for the III. The elimination due to random homozygosis: II=0.0007 and 0.0001 for the III. This allows increments of II = 0.0039; III = 0.0048. Nevertheless, taking the same mutation rate [1] the number of lethals, if completely recessives, would be in the II, 2.6—4.6 times, and in the III, 5.6—10.3 times greater than the actually observed, averaging 2.6 and 7.6 times, respectively. Selection coefficients opposed to lethals in heterozygous condition of $\bar{s}$(II)= 0.018 and $\bar{s}$(III)= 0.031 would be necessary to account for the discrepancy. Contrary to others' results the $\bar{s}$ values for semilethals in heterozygous state are smaller than the ones for lethals. Local inbreeding, F(II) = 0.0165, might be another factor of lethal or semilethal elimination. The estimated effective population size: N= 4030 (acc. Wright's formula) conforms with the values of six to ten thousand individuals determined by release experiments of marked flies in the same wood.

---

1. Determined by Dobzhansky *et al.* *Genetics* **37**, 650. 1952,
Work to be reported full in *Evolution.*

Work supported by the Rockefeller Foundation, Conselho Nacional de Pesquisas and the University of Rio Grande do Sul.

9.60. The Persistence of Deleterious Genes in Natural Populations of Drosophila melanogaster. Chozo Oshima (Misima, Japan).

The distribution of viabilities manifested by individual homozygous second chromosomes isolated from the Japanese natural populations of *Drosophila melanogaster* has been in the course of several years repeatedly examined. From the results of allelism tests between lethal genes, some of them were proven to have been retained for several years in the same population. The pre-adult viability of heterozygous flies for single lethal and double lethal chromosomes was estimated and compared with that of normal heterozygotes. A complementary effect between lethal genes was recognized in the viability of double lethal trans- or cis-heterozygotes and it is assumed to be due to interaction between two loci carrying lethal genes in the heterozygous genetic background. The mean relative viability of heterozygous flies for single semi-lethal and double semi-lethal chromosomes was also estimated. The deleterious effect of a single semi-lethal chromosome on heterozygous condition was almost none as compared with that of a single lethal chromosome, but the detrimental effect of double semi-lethal trans-heterozygotes was emphasized. From the above mentioned results, some mechanisms of persistence of lethal genes in a natural population are considered and the difference in their frequency between lethal and semi-lethal chromosomes will be discussed.

9.61. A Comparative Survey of Genetic Variabilities between Second and Third Chromosomes from Korean Populations of Drosophila melanogaster. Yong Kyun Paik (Seoul, Korea).

138 second and 151 third chromosomes collected in mid-July in a suburb of Seoul were

analyzed by *Cy* and *TM3, Ser* balancer chromosomes. The number of lethals and semi-lethals are 13.0 per cent for second and 18.5 per cent for third chromosomes. In a second collection after three months, 160 second and 164 third chromosomes were taken in mid-October and 17.5 per cent of the second and 16.6 per cent of the third chromosomes were found to be lethals and semi-lethals. Another sample was taken in a location at rural Kwangnung in mid-September of the same year, and 16 per cent of 176 second and 20 per cent of 221 third chromosomes sampled were found to be lethals and semi-lethals. Differences in lethal concentration between second and third chromosomes as determined by chi-square are not significant in time and space. However, it is interesting to note that on the average slightly more third chromosomes than second carry lethals. Additional data showed high value of homozygous viability of both second and third chromosomes above semi-lethals and of quasi-normals: on the average, mean viability of chromosomes above semi-lethals appears to be 30 ± 0.3 per cent for second and 31.0 ± 0.5 per cent for third chromosomes. Nearly complete tests showed comparatively high frequency of allelism among the strictly lethals in both second and third chromosomes, showing no significant differences between collections in the same location or between locations (1 to 3 per cent for III and 1 per cent for II chromosomes). Total incidence of visibles was low in each collection, but more than half of the different visibles occurred more than once within the respective populations. Many of the wild males tested, which carried either *bw* or *p*, were further proved to have more than one lethal and/or other deleterious genes either on the *bw* or *p* carrying chromosomes or on the other part of the homologous chromosomes.

As far as we may judge by the present data available, the frequencies and allelism of second and third chromosomes of lethals and other genetic variabilities agree well each other, and a batch of the results falls within the expected range showing smallness of population size. It may be safe to conclude that Asiatic populations of *Drosophila melanogaster* are really different from the other geographical populations so far reported, in respect of population size and genetic structure.

9.62. (D.) Polymorphism in the Water Boatman, Trichocorixella mexicana. W. PETERS (Chicago, U.S.A.).

Dextral and sinistral forms displaying situs inversus occur in a 1 : 1 ratio in the wild population. Phenotypic characteristics are illustrated and a probable mode of inheritance proposed.

9.63. Color Polymorphism in Cage Populations derived from Hybrids between Drosophila l. lebanonensis and Drosophila l. casteeli. S. B. PIPKIN (Panama. Panama).

Founders of four cage populations were F_1 hybrids between a homozygous pale strain of *D. l. lebanonensis*, a species exhibiting a balanced mesonotal color polymorphism in natural populations of the Lebanon, and *D. l. casteeli*, its dark subspecies, lacking a color polymorphism in natural populations of Arizona, U.S.A. The *pale* versus *dark* mesonotal color is dependent upon a single pair of alleles, *S,s*, not associated with a chromosomal inversion.

After twenty-four generations, two populations maintain a *Ss* superiority. An initial *s/S* ratio of 1 declined to 0.9 in the fifth generation and 0.45 in the fourteenth generation, after which there was a rise to 0.7 in one population and 0.65 in the other. Presumably coadaptation of gene complexes resulted in improvement of the selective value of the *Ss* heterozygotes in these two populations from the fourteenth through the twenty-fourth generations. In two other cage populations, the *SS* homozygotes proved superior to *Ss* heterozygotes, although the selective value of the latter improved somewhat after the fourteenth generation.

Mating propensity, known to be low in *D. l. casteeli*, is high in cage populations derived from hybrids between *D. l. lebanonensis* and *D. l. casteeli*, with no differences among the *SS*, *Ss*, and *ss* genotypes. Culturing a high temperatures of from 25° to 28° C of replicate half-gallon bottle populations, derived from the two equilibrium cage populations at the thirteenth generation, resulted in a rapid selection strongly favoring *SS* homozygotes. Length of egg to hatching life cycle in an equilibrium cage population is approximately the same for *Ss*, *SS*, and *ss* genotypes.

9.64. Selection in Experimental Populations of Drosophila melanogaster with Different Genetic Backgrounds. SERGEY POLIVANOV (New York, U.S.A.).

A study was made of the behavior of several mutants of *Drosophila melanogaster* in experimental populations, kept in laboratory population cages. In all experiments the foundation stocks contained mutant and wild type alleles

of certain genes with equal frequencies, 0.5. As expected, natural selection led in all cases to decreases in the relative frequencies of the mutant and to increases of those of the wild type alleles. The selection rates proved, however, to be in some cases very different depending on whether the foundation stocks contained only one or many kinds of wild type chromosomes. The "single-strain" populations were derived from crosses of mutant flies to flies from a highly inbred wild-type stock. The "multiple strain" population's non-mutant chromosomes were derived from many (about a dozen) wild strains of different origin. With the third-chromosome mutants ebony, scarlet and stubble, and apparently also with the second-chromosome brown-75, the selection rates against the mutants were initially much greater in multiple-strain than in single-strain populations; in later generations this difference tends to disappear. With the sex-linked forked and the fourth-chromosome polished, the single strain and the multiple strain populations showed similar behavior within the limits of the experimental errors. The data are, on the whole, consistent with the assumption that the selection rates against certain mutant genes depend not only on the effects of these genes themselves but also on those of the polygenic complexes associated with them in the same chromosome.

9.65. Chromosomal Inversion Polymorphism and Size in Drosophila subobscura. ANTONIO PREVOSTI (Barcelona, Spain).

Data are presented showing that some chromosomal orders of *Drosophila subobscura* present a clear frequency cline north—south. The results of experiments of artificial selection for size carried on in the laboratory are presented. It is found that as a correlated response, a tendency exists to fixation in homozygous condition of complex orders, when selecting for small size. However, selection for larger size increases the frequency of the heterozygosity between the standard and the complex inversion types of the chromosomes. Experiments planned to correlate the chromosomal structure of individuals and its size are in preparation. The preliminary results will be given.

9.66. The Effects of a History of Stabilizing Selection on Sensitivity to Foreign Environments. T. PROUT (Riverside, U.S.A.).

The sensitivity of the time of development to certain environmental variables was investigated in two strains of *Drosophila melanogaster*. One strain, S, had been subjected to 40 generations of stabilizing selection on development time. The other strain, D, had been subjected to 40 generations of disruptive selection for the same trait.

The primary effects of selection (previously published) show a decrease in the within culture variance of the S line and an increase in the D line and further, that the increased variance in the latter was probably due to a loss of buffering.

The present report concerns further studies of the comparative sensitivity (buffering) of the two lines to (1) environmental variables which operate among cultures, (2) temperature variation, and (3) variations in an utritional variable known to effect development time.

The results of these studies indicate that the two lines do not differ in their buffering against these three factors.

It is concluded, therefore, that the genotypes involved in the differentiation of these lines are rather specific in limiting their effects to buffering only against the microenvironmental variation within cultures. If loci for "generalized buffering" exist, apparently they were not segregating in these lines. In general these findings contrast somewhat with studies of the effects of inbreeding on buffering.

9.67. Towards a Synthesis of Population and Physiological Genetics. FORBES W. ROBERTSON (Edinburgh, Great Britain).

The genetic properties of components of fitness, their inter-relations and importance in determining the stability of other characters must be related both to development and ecology. Such information will enable us to specify the conditions, genetic and environmental, which favour particular kinds of adaptive change and thereby bridge the gap between population and physiological genetics. This approach has been applied to the growth of *Drosophila melanogaster* by selecting for body size, development time and egg production on different controlled diets and also by analysing the changes which have accompanied adaptation by a number of populations to novel nutritional conditions. Growth can be divided into an exponential and a later, slower phase and changes in either appear to be genetically independent, within limits. Both contribute to the total variance of adult size, but their genetic properties dif-

fer, especially with respect to the distribution of variation about the mean, the evidence for gene-environment interaction and also whether or not development time and body size are correlated. Selection for either shorter larval period or higher egg production, under novel conditions, led to substantially smaller or larger size, suggesting that the normal stability of the latter is maintained by opposing selection pressures. Populations adapted to different diets have shown consistent changes in body size and components of fitness and thus provide material for the experimental study of evolutionary change. Most of this work is in press and will appear in the *Journal of Genetical Research*.

9.68. Disruptive and Stabilizing Selection on a Mutant Character. W.SCHARLOO, (Leyden,The Netherlands).

Both disruptive and stabilizing selection were practised on the expression of the mutant *cubitus interruptus dominant of Gloor* (ci^{D-G}) in *Drosophila melanogaster*. In this ci^{D} allele the relation between factors affecting expression (i.e. the extent to which the fourth longitudinal wing vein becomes deleted) and their phenotypic effect is almost linear whereas the original ci^{D} shows a strongly nonlinear relation.

A base population (B) was founded by introducing the mutant into the Pacific wild stock. In the stabilizing line (S) animals with expression values nearest the mean were selected as parents of the next generation (4 ♂♂, 4♀♀, among 20 of each sex measured). In the disruptive line (D) the two ♂♂ and ♀♀ with highest and the two ♀♀ and ♂♂ with the lowest values were chosen. Each generation consisted of four bottle cultures. A rotational mating system was used.

The mean expression values did change in a minor degree only, but selection was very effective in both lines with respect to variances. In D the phenotypic variance increased in 6 generations to about 2 times the value in B; in S the phenotypic variance became reduced to half this value. Estimated heritabilities were 0.50, 0.58 and 0.88, in S, B and D respectively.

The independent variance component (based upon the differences between the two wings of one fly) did not change in this period. The common environmental variance component (non-genetic differences between flies) is of minor importance in this character and in the selection response. This is corroborated by the finding that there was no difference between D and S in the response of expression to temperature change.

In generation 10 the phenotypic variance in D showed a further increase to about 4 times the original value, but in S there was no further decrease.

This work was begun at the Institute of Animal Genetics, Edinburgh, during tenure of a N.A.T.O. Science Fellowship awarded to the author by the Netherlands Organization for the Advancement of Pure Research.

9.69. Chromosomal Polymorphism and Position Effect in Drosophila subobscura. D. SPERLICH (Vienna, Austria).

Chromosomal polymorphism is common in natural populations of *Drosophila subobscura*. The frequency and the distribution of various structural types are different in northern and southern populations. The degree of structural heterozygosity and the inversion frequency are higher in Italy than in Austria and again higher in Austria than in Scandinavia. Both values are lower in insular isolation than on the neighbouring continent. The association of linked inversions is not at random although the map distances between the inversions are sometimes large. There are regions in the chromosomes which show accumulation of breaks for natural inversions. An X-ray induced inversion was found to be heterotic with three other natural inversions tested. The distal break of this inversion is identical with that of some natural inversions. Therefore one may assume that position effect plays a great role for the origin of chromosomal polymorphism in *Drosophila subobscura*.

9.70. Mating Propensity of Gene Arrangement Carriers in Drosophila persimilis. ELIOT B. SPIESS and BOZENA LANGER (Pittsburgh, U.S.A.).

Multiple choice matings in 24 hr tests of homokaryotype Whitney and Klamath arrangements of chromosome III demonstrated a relative propensity of 2 :1 for W/W ♂♂ to K/K ♂♂ (Spiess and Langer, 1961). Tests were repeated with varying ratio of W♂♂: K♂♂ in the mating chamber from 9 : 1 to 1 : 9; the ♂ propensity varied randomly from 1.2 to 2.2 W♂♂ per K♂ mating. W/W♀♀ were also mated with more frequently than K/K ♀♀ except at the extremes of the ♂ frequency ratio when ♀♀ mated about

equally. To ascertain which sex was controlling rate of mating, observations were made on ten pairs from uniform cultures in small plexiglass chambers for 1 hr. Pairs were introduced without etherization; mating pairs were withdrawn and time recorded. Cumulative rate curves for 60 to 100 matings give these results: (1) W/W × W/W matings are most rapid, 85 per cent within an hour; (2) K/K × K/K are slowest, 15 per cent within an hour. WT ♀♀ × KL ♂♂ were 20 per cent less rapid than W × W, while KL ♀♀ × WT ♂♂ were about that much faster than K × K. Females control the rate of mating then, and substitution of a WT ♂ of for a KL ♂ increases mating speed. WT ♂♂ court persistently and indiscriminately while KL ♂♂ court very little. KL ♀♀ refuse to mate by extruding the ovipositor or decamping while WT ♀♀ accept mates more frequently. F_2 flies from homokaryotype sib matings maintain about the same rates. Heterokaryotypes (W/K, K/W) mated within cultures had intermediate rate (55 per cent in 1 hr) but when mated to WT as either sex, mating is increased to 80 per cent while to KL it is lowered to 20 per cent. Maternal effect in heterokaryotypes was evidenced by superior mating of both sexes with WT mothers than those with KL mothers when mated to W/W. Therefore these chromosomal rearrangements contain genic complexes controlling mating behavior, especially receptivity of ♀♀ and activity of ♂♂.

9.71. Selection for Rate of Development and Gene Arrangement Frequency in Drosophila persimilis. Luretta D. Spies and Eliot B. Spiess (Pittsburgh, U.S.A.).

It was demonstrated by Spiess (1958) that the third chromosome arrangement Whitney (W/W) surpassed Klamath (K/K) by approximately 3 days in rate of development. To evaluate the rate of development difference as a major component of fitness in controlling frequencies of these arrangements, 6 experimental populations were initiated in which food was changed rapidly (3 "Fast" Cages) to favor fast developing larvae (W/W) or changed slowly (3 "Slow") to allow other karyotypes (W/K and K/K) to eclose. KL decreased in fast and slow cages at about the same rate to about 10 per cent KL equilibrium. A second pair of populations (DF, DS) were initiated in which (1) all emerging individuals were counted each generation and (2) the first 500 (DF) or last 500 (DS) adults to emerge were used to establish each discrete generation. Selection curves were parallel approaching equilibrium at 10-15 per cent KL after 6-8 generations. That WT is superior in other respects is evident from studies on mating behavior (Spiess), but possibilities remained that (1) the rate of development difference was reduced under population cage conditions or (2) selection was not sufficiently intense to effect a change in frequencies. New discrete generation populations were initiated with the same strains (WT = KL = 50 per cent) but with selection intensified 20-fold (DIF, DIS). These populations diverged immediately and KL increased in slow (DIS) to 80 per cent but decreased in fast (DIF) populations to 15 per cent. Selection was reversed after 4-6 generations in subpopulations while continuing the original selection scheme. Reversed selection was effective. Rate of emergence increased under fast but was unaltered in slow selection; progeny per ♀ parent increased from 3 initially to 11 after ten generations in DF and DS populations, indicating improved total fitness. Therefore K/K and W/W differ in rate of development under population cage conditions, but moderate selection does not discriminate between these karyotypes.

9.72. Heritability of Wing Length in Natural Populations of Drosophila melanogaster and D. similans. Abdel-Azim Osman Tantawy (Alexandria, Egypt).

The genetic variance of wing lenght in natural populations of *Drosophila melanogaster* and *D. simulans* has been studied. Flies were captured twice a month from the same locality for sixteen successive months, from October 1961 to December 1962. Heritability of wing length in both species was estimated by the half-sib analysis and all experimental work was carried out at 250°C.

The results are summarized as follows:

1. Wing length in both species measured on the F_2 generation of captured females remained constant from October 1961 up to April 1962 (mild temperature) after which it declined gradually from May 1962 to August 1962 (hottest months of the year) after whichit showed a steady rise again to the end of the experimental period. Thus, the results are in agreement with these reported on populations captured from high altitude and from northern geographical regions, as compared with low altitude and southern regions, respectively.

2. Heritability estimates indicate that populations of *Drosophila melanogaster* possess higher significant values for the h^2 each month than those found in *D. simulans* populations, but the species behaved differently. Populations

of *Drosophila melanogaster* showed a constant value up till April 1961 after which it declined significantly (from 41 per cent to 31 per cent) up to August 1961. Heritability estimates rose once again to about 42 per cent from September 1961 to December 1962, while populations of *D. simulans* showed a rise, though not significantly so, in the heritability estimates (28 to 35 per cent) from October 1961 up to December 1962.

Phenotypic, genetic and environmental monthly correlations between wing and thorax length in both species of *Drosophila* were analyzed and will be discussed.

9.73. Evolution of the Mean Ovariole Number in Experimental Populations of Drosophila melanogaster. GEORGES TEISSIER (Paris, France).

The mean ovariole number of wild type *Drosophila melanogaster* may vary by a factor of two, according to the geographical origin of the studied strains; it is conditioned by a polygenic system, which can be studied by the usual techniques. It seemed useful to observe the evolution of populations maintained for several years in cages of the type we have been using since 1932. Six of these populations have been measured since 1956, six others since 1958. More than 500 counts of the mean ovariole number m of samples of 50 females, hatched from eggs placed on a superabundance of food, have been made during the last four years.

In three populations, constitued by crossing the French strain B U ($m = 29{,}45$) with the Japanese strain O T ($m = 15.67$), the values of m, which were originally 21.12 for the F_1, 25.75 for B_1 and 18.16 for B_2, oscillated during the last three years respectively around 20.93 ± 0.07; 21.52 ± 0.13 and 21.36 ± 0.08. Similar values have been found for the three other populations, constructed from several different French strains, such as B U, and therefore originally more complex.

It appears from these facts, and others which will be discussed, that there occurred in all our populations over the years a selection which adjusted the ovariole number to a value optimal for the conditions of a strong competition for food characteristic of the life of *Drosophila* in population cages. Moreover this value is not the same for all the types of experiments.

9.74. An Experimental Study of Environmental Influences on Population Structure in Drosophila. J. A. THOMSON (Melbourne, Australia).

Laboratory populations of *Drosophila pseudoobscura* initially polymorphic for the *Standard* (ST) and *Chiricahua* (CH) sequences of chromosome III have been studied under a variety of experimental régimes involving differences in temperature, humidity, illumination and food composition. A variable amount of gene flow between interconnected populations held under different environmental conditions was permitted. The degree of genetic differentiation achieved during 30 generations by such island populations in relation to the imposed environmental differences has been investigated by following the frequency of CH, certain physiological properties, sex-ratio and productivity, in addition to comparison of the accumulated recessive lethals of chromosome III and the additive genetic variance of the sub-populations. Evidence has been obtained that increased resistance to desiccation occasioned by larger body size is a major factor contributing to the adaptive significance of the ST/CH polymorphism in at least certain of these populations.

9.75. Cyclic Variation of Bristle Number with Parental Age in Drosophila melanogaster. J. M. WATTIAUX and M. J. HEUTS (Louvain, Belgium).

Emergent flies from successive daily egg batches, laid by single pairs from pupal hatching till death, showed, in 8 out of 40 cases a periodical variation in mean abdominal bristle number. In all these cases Kendall's correlogram takes the form of a sinusoidal function with a constant period whose value lays between 6 and 13 days and whose maxima and minima differ by 3 to 5 bristles, according to the sibships. In the most favourable case 5 periods of 6 days each are evident. Linear trends are not discernible.

Density influences can be ruled out as a cause of the fluctuations on experimental evidence. Separate treatment of males and females yields highly significant correlations between daily values. The heritability coefficients (h^2) calculated from parent males and from parent females are $\pm$ 0.67, viz. $\pm$ 0.35, which are considerably higher than those found for sternopleural chaetae (Scossiroli, 1954).

Cyclical segregation of oligogenes in *Dr. melanogaster* have been described (Heuts, 1956). Pertinent questions as to the nature of the mechanisms involved and as to the apparent restricted occurrence of cycles in quantitative or qualitative characters of offspring with relation to parental age will be discussed in forthcoming publications (*Genetica*).

SECTION 10

DEVELOPMENTAL GENETICS

10.1. Hereditary Ovarian Tumours of Drosophila. R. C. KING (Evanston, U.S.A.).

The hereditary ovarian tumours of *D. melanogaster* demonstrate that a complex genetic system plays a decisive role in restricting the multiplication of the cells destined to become incorporated into an ovarian follicle. Three recessive genes are known which cause in the ovaries of adults uncontrolled cell proliferation which results eventually in female sterility. The genes in question are *female-sterile* (*fes*), *narrow* (*nw*), and *fused* (*fu*). Females homozygous for *fes* or *nw* have ovaries which are completely tumorous at the time of eclosion; whereas freshly eclosed fu females show no tumours. Tumours begin to appear on the second day, however, and eventually the *fu* ovary becomes completely tumorous. With respect to *fu* a study of eight alleles of independent origin has demonstrated that in each case tumors eventually form in the ovaries of homozygotes. However, different *fu* alleles can be distinguished on the basis of the speed with which tumorous chambers appear in the ovary. Dietrich Bodenstein and I have studied the development of ovarian implants which were made using as hosts or donors phenotypically wild type adults or flies homozygous for *fes* or *fu*. Implants from *fes* or *fu* females behave autonomously when left in wild type female hosts for as long as 6 days. Implants from + donors appear cytologically normal after developing in *fes* or *fu* abdomens for times as long as 9 and 12 days, respectively. Thus there is no evidence for diffusible tumorigenic agents as initiating factors in the development of the ovarian tumours characteristic of *fes* or *fu* homozygotes.

10.2. Studies on the Aberrant Function of the Malpighian Tubes in the Drosophila Mutant rosy. ILSE SCHWINCK (Storrs, U.S.A.).

Experimental studies of the ontogeny of the pleiotropic pattern of *rosy* (*ry*) mutants and *maroon-like* (*ma-l*) mutants indicate that the Malpighian tubes perform a central function in the aberrant pteridine and purine metabolism of these mutants. [1] As compared to wild type, the Malpighian tubes of the *rosy* mutant are shorter and cells appear swollen. In all developmental stages some globules and conglomerates of globules, composed of fluorescent pteridines, are found in the lumen of the tubes; the globules are excreted by larvae and flies. The first occurrence of the aberrant metabolites in the lumen can be observed several hours before hatching from the egg. During the major time of metamorphosis no formation of new globules was found. However, in old pupae about 10 hr before emergence, small globules appear in a very regular distribution in the lumen of the tubes, becoming larger approaching emergence. The relation of these findings to the other pleiotropic characters will be discussed.

---

1. HADORN and SCHWINCK, *Z. Vererbungslehre* **87,** 1956; SCHWINCK, *Proc. Xth Internat. Congr. of Entomology*, Montreal, 1956, Vol. 2, 1958; SCHWINCK, *DIS* **34,** 1960; URSPRUNG, *Z. Vererbungslehre* **92,** 1961.

10.3. Studies on the Female-sterile Mutant deep orange of Drosophila melanogaster. JOHN C. LUCCHESI (Berkeley, U.S.A.).

A high percentage of eggs laid by *dor*/*dor* females, mated to *dor*/Y or +/Y males, are unfertilized: 33.7 per cent and 10.7 per cent, respectively (these frequencies were correlated to the fact that +/Y males inseminate females more readily than *dor*/Y males). In addition, about 6 per cent of the eggs from *dor* females are beyond metaphase I when they are fertilized. A disturbance in folic acid metabolism, resulting in abnormal oogenesis (King and Sang, 1959), is suggested as an explanation of the high frequency of unfertilized and belatedly fertilized eggs from inseminated *dor* females, and as a possible cause for the nature of *dor* cytoplasm. Folic acid is structurally and functionally related to the pterins (involved in eye pigment

biosynthesis); therefore, in the hope of restoring normal folic acid metabolism and fertility, *dor* females were made homozygous for *ry*, ry^2 and *bw*; *ry* lacks isoxanthopterin (accumulated by *dor* females: Counce, 1957); *bw* lacks all the pterins; *dor*, *bw* females remained sterile; *dor*, *ry* or ry^2 flies were lethal indicating that the type of interaction sought may have occurred, although in an unexpected direction.

10.4. Genetic and Physiological Studies of Abnormal Abdomen in Drosophila melanogaster. RALPH HILLMAN, and STEPHEN D. BARBOUR (Philadelphia, U.S.A.).

Abnormal abdomen was first described by T. H. Morgan in 1915 as one of the earliest cases in which the environment controlled the penetrance and expressivity of a genetic factor. This original mutation was subsequently lost. In 1953, Hillman [1] reported a recurrence of this abnormality as a result of X-irradiation. The mutation A^{53g} exhibits environmental relationships similar to the original *abnormal abdomen* and is located in the same approximate position as that reported by Morgan. Recent genetic information indicates that A^{53g} acts as a cross-over suppressor of the left end of the X-chromosome. Cytological studies have shown a rearrangement of the banding pattern in the heterozygous A^{53g}/Canton-S salivary chromosomes involving two to five bands in the 3C-3D region of the X-chromosome.

Preliminary chromatographic studies of the amino acid constituents of the mutant stock have yielded a possible explanation of the morphological effects. Since only the adult abdominal hypoderm and tergites are abnormal in A^{53g}, individual prepupae have been squashed on Whatman-1 paper and developed in butanol—acetic acid—water. Results with general and specialized indicators show an apparent increase in the glutamic acid concentration of A^{53g} and a decrease in staining of the spots corresponding to glutamine and proline. Since proline is known to be an important constituent of the hypodermal protein and glutamine is on the metabolic pathway from glutamic acid to glucosamine and chitin, these results are suggestive of an upset in glutamic acid metabolism as a cause of the morphological abnormalities.

1. *DIS* **27,** 56.

10.5. A Cytochemical Analysis of Deoxyribonucleic Acid (DNA) and Protein in Salivary Gland and Gut of the Lethal Mutant lgl of D. melanogaster. ROBERT M. WELCH and KATHLEEN RESCH (Austin, U.S.A.).

Previous research, comparing mature *lgl* with mature wild-type larva, has suggested a differential effect, notably on salivary gland in comparison with gut and on DNA of salivary gland in comparison with protein. An alternative explanation, however, is differential growth in wild-type larva after onset of general retardation in *lgl*. To decide between these two interpretations, DNA and protein in sections of salivary gland, proventriculus, and stomach of mature *lgl* have been compared with corresponding compounds of wild-type larva at a stage where *lgl* DNA is approximately equal to wild-type DNA and where *lgl* salivary gland is approximately equal to wild-type salivary gland. Gland and gut areas have also been determined. Also, wild-type larvae, experimentally altered in DNA or protein metabolism, have been compared with normal. DNA has been determined by Feulgen cytophotometry, protein by naphthol yellow S cytophotometry and photometric interferometry. Results do not indicate increased synthesis of protein over DNA in *lgl* in either salivary gland or gut at stages compared, nor increased growth of gut in comparison with salivary gland. Therefore, they do not support the hypothesis of a differential effect, either chemical or organ, of the mutation in *lgl*, although results on experimentally altered larvae do not exclude a differential effect on DNA, affecting protein synthesis indirectly. In absence of contrary evidence, however, results suggest that differential growth of wild-type larva after a stage of general retardation in *lgl* has produced the appearance of a differential effect in mature *lgl* when compared with mature wild-type larva.

Supported (in part) by PHS Research Grant GM-06492 from the National Institutes of Health, USPHS, and by a grant from the Rockefeller Foundation.

10.6. Gene Action of esc (extra sex comb) in Drosophila melanogaster. CHIYOKO TOKUNAGA (Berkeley, U.S.A.).

When homozygous, the *esc* gene (11 L) initiates the morphological change of the second and third male legs into a first leg in terms of bristle pattern which includes differentiation of an extra

sex comb on each basitarsus. The differentiation of extra sex combs could depend on (a) the primary change of the second or third into a first leg, or (b) the autonomous action of *esc* in the sex-comb area. Which alternative is correct may be decided by producing *esc*/*esc* mosaic patches of tissue at the sex-comb sites on the second and third legs in esc^+/*esc* males.

In order to obtain such mosaics, males having an X chromosome bearing the gene *yellow* (*y*), and second chromosomes—one bearing the esc^+ gene and an insertion of the y^+ locus into the left arm, and the other carrying the *esc* gene—were irradiated during the larval period. Somatic crossing over in a dividing cell yields two daughter cells, one homozygous for the y^+ insertion and for esc^+, and the other homozygous for *esc*. The former results in tissue which is not *yellow* and not *esc*, and the latter in the absence of the y^+ insertion, and the presence of the *y* allele on the X chromosome produces *yellow* bristles which are also homozygous for *esc*.

The differentiation of extra sex combs is interpreted as having been caused by the autonomous action of *esc*.

Research performed under the auspices of the U.S. Atomic Energy Commission.

10.7. Developmental Genetics of the Gene "Sexcombless" in Drosophila melanogaster. ARDHENDU MUKHERJEE (Berkeley, U.S.A.).

The mutant "*sexcombless*" (*sx*, I) affects differentiation of sexcomb teeth on the basitarsus of the male first leg. *Sx* is associated with an overlapping inversion, in the X-chromosome of salivary glands, which involves the region between 11C and 15F. Studies on the morphology and chaetotaxy of the basitarsus of *sx* male first leg reveal that the mean number of sexcomb teeth and transverse rows of bristles per basitarsus is 1.7 and 6.9 respectively. Bristles, that are intermediate between a typical tooth and a macrochaeta, frequently appear in the sexcomb area. Whenever present, the tooth is always oriented at an angle less than that found in the normal male, and may lie anywhere beyond the fourth transverse row. Studies on gynandric mosaics utilizing ring-X reveal that in a small patch of *sx* male tissue in the sexcomb area (in an otherwise *sx*/+ female basitarsus) *sx* inhibits the formation of any tooth and thus behaves autonomously. When *sx* is combined with ey^D, *en* (both differentiate extra sexcomb on the basitarsus of the first leg) or *tra* (*tra*/*tra*—transformed female differentiates sexcomb on the first leg) the mean number of teeth is greatly reduced in every case. This shows that *sx* is epistatic over the action of these genes with respect to the differentiation of sexcomb teeth on the first leg.

10.8. A Gene which Transforms Males and Females into Intersexes. PHILIP E. HILDRETH and JOHN C. LUCCHESI (Berkeley, U.S.A.).

A recessive gene on the third chromosome of *Drosophila melanogaster* has been discovered which when homozygous changes both chromosomal females and males into intersexes. The XX and XY intersexes are extremely similar in appearance, and both exhibit male and female traits. The pigmentation of the tergites is similar to that of the wild-type male. The seventh tergite is present, and at its base is the seventh spiracle, both female characteristics. The ventral part of the eighth abdominal segment has a protuberance similar to the female gonopod while the ninth segment, as in males, bears claspers. The anal plates are situated vertically as in males. Neither the XX nor XY intersex has sex combs; however, on the basitarsus of the forelegs of each, the bristles of the last transverse row are enlarged and rotated toward the area a sex comb would occupy if present. Internally the XX intersex usually has male and female reproductive parts with varying degrees of completeness. Well-developed ovaries are present in some. Frequently a single gonad may be attached to both the male and female systems. The XY intersex usually has a predominantly male internal reproductive system, but both a male and female system are present in some. In all cases examined the gonads are poorly developed.

Research performed under the auspices of the U.S. Atomic Energy Commission.

10.9. Genetics of Gynandromorph Production in Aedes aegypti. K. S. RAI and G. B. CRAIG JR. (Notre Dame, U.S.A.).

About 100 gynandromorphs have been isolated from *Aedes aegypti* (L.) during three years of genetical investigations. Although simple genetic ratios are not apparent, frequency of their occurrence in some strains and in particular crosses indicates an hereditary basis. A line of demarcation between male and female tissue

is always evident. Antero-posterior, oblique, and lateral types occur with equal frequency.

Certain gynanders with female head and male abdomen took a blood meal, then died when the midgut broke open, presumably because the male gut is not adapted to blood feeding. Conversely, gynanders with male head and female body attempted to copulate with females. One bilateral gynander had components of external genitalia from both sexes, one spermatheca (three in females) and two ovaries, one of which contained testicular tissue and mature sperm.

In anterior-posterior gynanders with female head and male abdomen, normal spermatogenesis occurs. Several of these individuals were crossed to normal females, using the forced copulation technique. In crosses using genetic markers on all three linkage groups, the markers were recovered in expected frequencies in the F_1-F_3 generations. Moreover, sex ratio was normal and no additional gynanders were recovered. F_1 larvae had normal chromosome complements. Thus, it seems unlikely that these gynanders are produced through abnormal chromosome segregation.

Crosses with markers have demonstrated that gynanders have different gene complements in their male and female parts. It seems probable that gynanders in *A. aegypti* are produced by fertilization of a binucleate egg or an egg and a polar body by two sperms.

Supported in part by Atomic Energy Commission Research Contract AT (11-1)-38 and NIH Grant No. AI 02753-04.

10.10. The Influence of Temperature and X-rays on the Sex of Carausius morosus Br. L. P. Pijnacker (Groningen, The Netherlands).

The stick insect *Carausius morosus* Br., native to India, reproduces parthenogenetically in Europe. However, ♂♂ may appear spontaneously (0.5 per cent ♂♂ at 16°-20° C) but apparently have no sexual function. Sexually aberrant individuals showing gynandromorphy and/or intersexuality are also found at frequencies of up to 2 per cent. Progeny, if produced, are phenotypically normal.

Eggs treated with higher temperatures gave more aberrant individuals. Incubation at 25°-27°C gave practically 100 per cent abnormals. Temperature shock of 40° or 45°C for two hours also induced abnormals, the percentages depending upon the temperature and the stage treated. Early stages (especially the stage at which the embryonal layers and appendages are developed) were most sensitive with maximum percentages of abnormals being 9 and 32 per cent at 40° and 45° respectively.

Early embryonic stages are also sensitive to the action of X-rays. Eggs receiving doses of 1000r, 2000r, 4000r, 8000r, produced up to 60 per cent abnormals. Only the first two series gave adults. The progeny of these treated individuals showed an increased number of ♂♂ dependent upon the dose and the stage treated. ♀♀ treated in the oldest embryonic stages were most sensitive and gave up to 26 per cent ♂♂ in their progeny.

♂♂ (spontaneously arising in control cultures) have one heterochromatic sex-chromosome compared with the two similar but euchromatic chromosomes possessed by all ♀♀ ($2n = 66$?). Spermatogenesis is abnormal in both meiotic divisions (precocious segregation or division, lagging, non-disjunction, bridges), though normal bivalents are found. Karyological investigations indicate that interspecific hybridization may have taken place in this species and this may account in part for the lability of the sexual phenotype.

10.11. An Embryonic Lethal with Reversed Polarity in Drosophila melanogaster. Alice Louise Bull (Wellesley, U.S.A.).

An inherited disturbance in egg polarity has been observed in three related inbred lines of *Drosophila melanogaster*, the parental line and two others separated 176 and 191 generations before the abnormality was discovered. Normal winged female flies from these stocks, heterozygous for a double inversion second chromosome with markers CyL^4sp^2 and an unmarked second chromosome, produce embryos which develop a second larval abdomen anteriorly and in mirror-image symmetry to the normal abdomen. The reversed posterior end consists of four terminal abdominal segments with normal posterior spiracles. The hindgut and Malpighian tubules are present. The central internal region of the embryo is characterized by an undifferentiated sac connecting the two hindguts.

Although symmetrical bicaudal embryos are rare in an egg collection, other embryos showing partial reversal of symmetry are found. In these one to three terminal segments connect to a normally oriented posterior end of varying length. Still other embryos have normal symmetry, but exhibit disturbances in the formation of the cephalopharyngeal apparatus.

The occurrence of abnormal embryos (approximately 2-13 per cent) is always accompanied

by a larger number of undeveloped eggs. The variation in proportions of undeveloped, abnormal and normal embryos in different collections of eggs from one female or among females suggests a threshold response to an undetermined physiological or environmental factor.

The genetic factor or factors that produce the bicaudal effect appear to be associated with the unmarked second chromosome present in all three $CyL^4sp^2/++$ inbred stocks. The maternal, but not paternal, chromosomes influence the formation of these embryonic abnormalities.

10.12. Phenogenetics of the lozenge Loci in Drosophila melanogaster. HARVEY A. BENDER and M. M. GREEN (Davis, U.S.A.).

The pleiotropic effects of the sex linked *lozenge* (*lz*) mutants include such seemingly unrelated features as: modification of eye color, structure, and size; absence of spermathecae and parovaria; and reduced tarsal claws. Recent studies (Bender and Green, 1962) have, in addition, demonstrated primary ovarian histopathologies in the lz^{34k} and lz^{34k}; *su*(3) lz^{34k} mutants. Such investigations have been extended to include *lozenge* mutants representative of each of the four sub-loci demonstrated in the *lz* pseudoallelic complex, and to flies compounded in all possible permutations with such alleles.

Utilized were studies of fertility, fecundity, egg hatchability and viability, as well as histological and histochemical analyses of the primary and secondary reproductive tissues. The ovarian investigations were implemented with whole mount as well as paraffin sectioned preparations and included transplanted tissues. The histochemical studies detailed nucleic acid, carbohydrate, protein and lipid localizations.

Evidence was obtained linking the imaginal corpus allatum with the primary cause of *lz* ovarian abnormalities.

This work was supported in part by National Institutes of Health Grant No. GM 08697-02 and by Atomic Energy Commission Research Contract AT (11-1)-38.

10.13. A New Approach to the Problem of Sex Determination in Dinophilus. WALTHER TRAUT (Saarbrücken, Germany).

An analysis of egg capsules deposited by *D. gyrociliatus* under constant environmental conditions suggests a special mechanism of sex determination. There is a non random distribution of big female and small male eggs in the egg capsules. The two types of eggs in their production in the ovary depend on each other in a way that resembles genetic segregation. But this segregation must take place before a stage in which meiosis was demonstrated by Nachtsheim (1919) and Shen (1936).

10.14. Pigment and Pleiotropism: Combined Effects of Ww/ww and ff/Ff Gene Substitutions. ELIZABETH S. RUSSELL (Bar Harbor, U.S.A.).

Many different mutant genes in the mouse, including *W* and *f*, combine effects on distribution of pigment in the hair-coat with pathological effects in other tissues. A hyperadditive interaction of *Ww*/*ww* and *ff*/*Ff* genic substitutions in restriction of pigmentation has been demonstrated through quantitative evaluation of percent white-spotting in littermate animals of four genotypes, *Wwff* (40 per cent white), *WwFf* (11 per cent white), *wwff* (14 per cent white) and *wwFf* (solid black). All spotting variation was attributable to these segregating genes, since the animals were otherwise highly congenic with the FL/Re inbred strain. The *ff*/*Ff* substitution has a significant effect on fetal and newborn blood-picture, leading to a microcytic, siderocytic anemia, more severe at 14-16 days gestation than at birth, and completely cured by two weeks postnatal. Although *WW* fetuses and newborn mice are lethally anemic, newborn *WwFf* and *Wwff* individuals have higher erythrocyte counts than do corresponding *wwFf* and *wwff* individuals, and fetal anemia associated with *Ww*/*ww* is very improbable. It is conceivable that severe fetal anemia, resulting from *ff* gene-action, could have resulted secondarily in ventral white-spotting by inhibiting late stages of melanoblast migration. It is highly unlikely, however, that either the ventral while-spotting of *WwFf* mice or the great increase in white area of *Wwff* over *wwff* mice is in any way related to defective fetal blood supply. The results may better be explained by tissue-localized effects of *Ww*/*ww*, and probably also *ff*/*Ff*, gene substitution acting independently in melanoblasts and in hematopoietic cells.

10.15. Interactions at the Agouti Locus. M. M. DICKIE (Bar Harbour, U.S.A.).

The agouti locus in the mouse now has nine

identified alleles. They are: lethal yellow A^{y}, viable yellow A^{vy}, light bellied agouti A^{w}, agouti (wild type) A, intermediate agouti A^{i}, tanoid a^{ta}, black and tan a^{t}, non-agouti a, an extreme non-agouti, a^{e}. Many remutations from non agouti a to black and tan a^{t}, and to light bellied agouti A^{w}, have occurred. This report will summarize the findings about these remutations; describe two of the newer alleles, viable yellow A^{vy} and intermediate agouti A^{i}; report some of the information obtained about the action of these alleles on different backgrounds; the appearance and identification of various allelic combinations, and review briefly some of the biochemical studies and attempts to produce phenocopies of certain alleles.

Certain closely linked genes can modify the expression of some members of this allelic series. The results of crosses of members of the agouti series with these modifying genes may contribute to the understanding of the action of the agouti locus and the interactions of the various members of the agouti series.

The action of the alleles of this series may affect pigmentation of the entire coat or only portions of the coat. Are these true alleles or pseudo-alleles at a complex locus? This review is being presented to open new avenues of discussion and research to try to answer this question.

10.16. The Control of Bristle Pattern by Hairy-Wing in Drosophila melanogaster. FREDERICK JAY GOTTLIEB (Pittsburgh, U.S.A.).

An analysis of the control of developmental patterns exercised by the mutant gene *Hairy-wing* 49c (Hw^{49c}) was undertaken. In homozygotes the thorax is disproportionately wider at the level of the dorsocentrals, there are numerous extra macrochaetae and an increase in the number of achrostichal rows and chaetal density is noted. The heterozygote has normal width, fewer extra macrochaetae and extra achrostichal rows, and no significant increase in chaetal density.

A developmental analysis of the differences in differentiation of Hw^{49c}/Hw^{49c}, Hw^{49c}/Hw^{+}, and Hw^{+}/Hw^{+} tissues was performed by means of genetic mosaics, resulting from X-ray induced somatic crossing-over in heterozygous larvae. Measurements and counts in the dorsocentral region were used.

X-irradiation causes heterozygotes to express themselves in a homozygous-like fashion.

Interactions were observed between tissues of the three genotypes in mosaics. Hw^{49c}/Hw^{49c} tissue acts semi-autonomously in mosaics. Hw^{+}/Hw^{+} tissue acts non-autonomously, occasionally producing extra macrochaetae in the presence of adjacent Hw^{49c}/Hw^{49c} and, more rarely Hw^{49c}/Hw^{+} tissue.

The evidence suggests that Hw^{49c} adds extra width to the thorax on which new prepattern properties can be exhibited. The rest of the thoracic prepattern does not seem to be altered by this mutant, but rather the competence of the hypodermal tissue to respond to these areas of the prepattern appears to be changed.

This work was supported by a Predoctoral Traineeship in Genetics, National Institutes of Health, and is part of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Genetics at the University of California, Berkeley.

10.17. The Developmental Control of Activity and Expression of the Hairless Locus in Drosophila melanogaster. DAVID NASH (Cambridge, Great Britain).

The dominant mutant *Hairless* (*H*) on chromosome III of *Drosophila melanogaster* commonly affects the macrochaetae of the head. Absence of macrochaetae is not accompanied by severe derangement of neighbouring unaffected bristles. Each vacant site may or may not bear a vestigial chitinized disc. Selection for changes in level of mutant expression indicates that one main rule governs the distribution of vestiges and is best interpreted as follows;

(1) In normal development the chaetae at different sites regularly develop at different times.

(2) Within development of a chaeta, later and earlier deficiency of a normal gene product result in presence and absence of vestiges respectively.

(3) By selection the form of the time-course of deficiency of the normal gene product is changed.

(4) Production of the hypothetical substance is under negative feed-back control, which cannot correct for the deficiency caused by the mutant, but is still sufficient to produce a rise of concentration after a critical level of deficiency has been reached.

Two specific modifiers of *hairless* exist. The time-course of their effects can be deduced from similar morphological considerations as above: *Suppressor-Hairless* (Su-H) acts as if it suppresses *Hairless* more strongly as development progresses. *Enhancer Hairless* (E-H) flies are often nearly devoid of chaetae, the majority of sites bearing vestiges. Enhancement of mutant

effect becomes stronger as development progresses.

E-H and *Su-H* are both closely linked to and on the same side of the locus *black* on chromosome II. It is therefore quite possible that these different modifiers are mutants at one and the same locus within the system controlling the level of production at the *Hairless* locus; they act as if they were "constitutive" (*Su-H*) and "super-repressed" (*E-H*) mutants.

10.18. On the Effects of rolled (rl) in Drosophila melanogaster. S. T. LAKOVAARA (Helsinki, Finland).

In homozygous *rolled* flies (chromosome 2, locus 55.1), the following features are observed: wing edges rolled downward, venation of the wings defective, eyes of subnormal size, darker than normal and roughened. In hemizygotes, the eyes are of half the normal size, dark red and rough-surfaced. The wing venation is rudimentary. In hemizygotes, *Df* (2) *rl* gives a stronger effect than *Df*(2)*MS-10*. Heterozygotes are normal.

No change in hemizygotes is found after addition of an extra Y or after loss of this chromosome. However, factors decreasing wing size increase the size of the eye in the combinations tested (*W*,*Bd*^{G}, *vg*).

In both homo- and hemizygotes, the strongest effects are found at 26°C, with a lessening at temperatures above and below this value. In histological sections, developmental defects are observable in homo- and hemizygotes in the ommatids as well as in the brain. Pigment is apparently formed in normal amounts but its location is changed.

In conclusion it may be supposed that: In their effect on the production of a substance important for the development of both the eye and the wing, the genotypes may be arranged in the order rl/- $<$ *rl*/*rl* $<$ *rl*/+. The gene *rl* governs a switch mechanism between the metabolic pathways leading to these two organs. The amount of substance generated in the wing pathway can be reduced by adding factors which have a wing-reducing effect. As a "side effect", larger eyes are obtained, owing to increased flow of the common substance towards the eye pathway. The temperature-dependent changes are due to thermodynamic properties of the enzyme controlled by *rl*.

10.19. Transfer of Phosphorus Isotope from Parents to Offspring in Drosophila melanogaster Meig. I. CSUKÁS-SZATLÓCZKY (Budapest, Hungary).

The distribution of P^{32}, incorporated by *Drosophila melanogaster*, has been examined at various stages of ontogeny, and also the concentration of phosphorus in the gametes of the progeny has been studied. The method of Schmidt and Tannhauser was employed for the separation of fractions. The distribution of the incorporated phosphorus was found to be different in the examined fractions in different phases of the ontogeny.

The amount of phosphorus transferred by the female sex cell was 50 times that transferred by the male cell measured at the end of the stage of third instar larva.

A comparison of the distribution of phosphorus taken up from the nutrient medium with that received by way of inheritance showed sharp differences in the various fractions.

It is worthy to note that the percentage of inorganic phosphorus received from the egg was approximately the same as that taken up from the medium, while inorganic phosphorus originating from the sperm was only in traces present.

As regards turnover, its rate in respect of phosphorus originating from the medium was the double of that for transmitted phosphorus. Within the latter category, the rate of turnover appeared to be much higher in female than in male imagoes.

10.20. Induction of Phenocopies in Cosmarium by RNase and Actinomycin D. ROBERT W. KORN (Kingston, U.S.A.).

The highly specific shape of the unicellular *Cosmarium* is primarily controlled by a series of chromosomal loci. Over 25 different morphological mutants have been obtained to serve as a basis for a study of phenotypic induction. RNase and actinomycin D treated cells produced daughter cells which manifested both shape and symmetry alterations similar to mutant types. These phenocopies differed from mutant lines by persisting for only one cell generation. Actinomycin D produced a higher frequency and a wider range of types than RNase. RNase frequently inhibited all differentiation while Actinomycin D usually affected only one distinct step. Preliminary evidence indicates these changes are induced over the final two hours before cell differentiation. Considerations of time, distribution, and organization of morphogenetic RNA will be presented.

10.21. Progressive Expression of Mating-type Genes in Paramaecium. R. W. SIEGEL (Los Angeles, U.S.A.).

Our data support the hypothesis initially presented by Metz which states that the four mating-type specificities in *P. bursaria* syngen 1 are brought about by the four unique combinations of two independent pairs of complementary ciliary mating-type substances: substances *AB*, *aB*, *ab*, and *Ab* characterize the cell surfaces of mating types I, II, III, and IV, respectively. Conjugation depends upon the complementarity of either the alpha or the beta substances or both. One pair of allelic genes controls the alpha substances and an independently assorting pair of alleles controls the beta substances. Early in the life history of a clone mating does not occur and neither alpha nor beta substances are formed. Later, in adolescence, clones will react with but two of the four standard mating types and it has been shown that now some clones form an alpha substance and others a beta substance. With maturity, each clone forms both an alpha and beta substance. These findings mean that the two genetic loci concerned with mating-type specificity come to expression in a predictive temporal sequence; first neither locus is expressed, then one locus alone is expressed and finally both loci are expressed.

The appearance of each successive life-cycle stage in *P. aurelia* is probably a consequence of the orderly progressive differentiation of nuclei (Siegel, 1961). In *P. bursaria* nuclear differentiations may control the expressions of (or "switch-on"?) the two loci concerned with the development of sexual specificity.

10.22. Slime: a Plasmodioid Variant of Neurospora crassa. STERLING EMERSON (Pasadena, U.S.A.).

The heritable *slime* variant of *Neurospora* grows on an agar surface by plasmodium-like flows which are devoid of cell walls at the advancing margins. Spherical cells with thin cell walls are produced in older parts of such colonies. Growth in liquid culture consists of spherical cells and atypical hyphae, both having flexible cell walls.

Heterokaryons between *slime* and wild type have the wild-type hyphal morphology under all growth conditions. In vegetative isolations from such heterokaryons the *slime* phenotype is invariably associated with nuclei of the *slime* parent.

Three genetic determiners, segregating independently at meiosis, are essential to the production of the *slime* phenotype, but are insufficient for its maintenance. Ascospore isolates carrying these three determiners are often hyphal from the start or, if germinating as *slime*, soon become hyphal. By rigorous vegetative selection from such isolates it has been possible to establish sub-strains which retain the *slime* phenotype permanently.

The unique morphological features of *slime* make it useful in a number of kinds of investigations. The absence of hyphal walls permits easier isolation of cellular constituents than is possible in hyphal forms. *Slime* grows in an extremely thin sheet between an agar surface and a cover glass, permitting observation of living material by phase microscopy under conditions in which cellular structures are well spread and do not overlie one another, and in which light defraction from cell walls is absent.

10.23. Transplantation of Nuclei in Neurospora crassa. JAMES F. WILSON (Salinas, U.S.A.).

Heterokaryons of *N. crassa* were produced artificially by cell-to-cell transfer of protoplasm of genetically marked strains. The injected hyphal sections were excised and cultured separately. The criteria of prototrophic growth and recovery of conidia of the donor genotype were used to prove that functional nuclei had been transplanted. Reciprocal injections were made with 2 pairs of macroconidial and 3 pairs of microconidial strains. Growth curves and conidial ratios of these artificial heterokaryons were compared to the corresponding natural ones. In each pair of heterokaryons produced by reciprocal injection, the growth curve of only one was equivalent to the naturally formed heterokaryon of that pair. The growth curve of the other artificial heterokaryon differed either in rate or in the lag period before growth occurred. There was no apparent relation between conidial ratios of the natural heterokaryons and the artificial heterokaryons with comparable growth curves. Injection of normal nuclei into a morphological temperature-sensitive mutant produced an apparent heterokaryon which grew with normal morphology at 34°C. The apparent stability of *Neurospora* nuclei may make *in vitro* studies possible.

Published *in extenso* in *Amer. Jour. Bot.* (in press).

10.24. Alteration of Frog Zygote Nuclei by Macromolecular Fractions obtained from Adult Liver Cells. HEINRICH URSPRUNG (Baltimore, U.S.A.).

Experiments were carried out to test the hypothesis that differential gene function and cytodifferentiation are based upon complexes formed between chromosomes and specific macromolecules. Macromolecular fractions were prepared from nuclei and cytoplasm of adult *Rana pipiens* liver. Aliquots containing approximately 0.2 mg of protein were injected into fertilized frog eggs. Two of the fractions, the globulin-albumin fraction and the histones, produced cessation of cell division and arrest of further development at the late blastula stage. When nuclei from such arrested blastulae were transplanted into enucleated eggs, the recipient eggs developed to the late blastula stage, but then were arrested also, indicating a persistent nuclear change. Cytological examination of such embryos showed no primary gross chromosomal abnormalities.

The substance responsible for this alteration of nuclear capacities is heat-labile and not dialyzable. Specific activity, expressed as biological activity per milligram of protein, is highest in the fraction that precipitates between 57 and 65 per cent saturation of ammonium sulfate. Removal of digestible RNA by ribonuclease does not decrease the specific activity. DNA has not been detected. It is concluded that the active substance is or contains protein, and it is speculated that its action consists in forming complexes with the DNA portion of the chromosomes in such a way as to impede those nuclear activities that are required for continuation of cell division and gastrulation.

Supported by NSF Grant GB-298.

10.25. "Brown Spots" (bsp), A Genetic Character of Drosophila melanogaster which manifests only after Copulation. ANNA DI PASQUALE and LAURA ZAMBRUNI (Milan, Italy).

"*Brown spots*", a new character arisen spontaneously in a wild stock of *Drosophila melanogaster* in 1959, is due to the presence of one or more recessive factors localized in the 2nd chromosome. It is transmitted by the male as well as by the female, but the phenotypical manifestation, which consists in the formation of browncoloured areas on the pleurae, is limited to the female.

The manifestation shows a clear relation to mating. Females kept apart from males and females isolated from them soon after courtship phases but before copulation, never show spots. A single copulation is sufficient to provoke spotting.

bsp females mated with males of nine different stocks show spots with high frequency; the mating with *y w* males, after which the incidence of spots is particularly low, is a peculiar case.

Mating with sterile males (X/Y^{Lc} and X/O: non-motility of spermatozoa; X/X; *tra/tra*: females transformed into males — no spermatozoa) also determine the appearance of spotting in *bsp* females.

Frequency of spotting increases with the age of the copulating females. No relation is found between aging of the male and spotting.

From these data it is clear that the phenotypical manifestation of "*brown spots*" is strictly dependent on copulation, irrespective of fertilization and presence of spermatozoa in the sperm fluid. The nature of the phenomenon is not yet clear: recent experiments would emphasize an activity of the sperm fluid: mating with males lacking paragonial fluid owing to repeated copulations, does not seem to cause any spotting.

10.26. Gene-environment Interactions and the Penetrance of Melanotic Tumour Genes. JAMES H. SANG and B. BURNET (Edinburgh, Great Britain).

The melanotic tumour phenotype of *Drosophila melanogaster* may be determined by any one of a number of non-allelic, second chromosome, recessive genes. Penetrance of some of these genes depends on modifiers on other chromosomes, usually the third, and also on the environment, particularly the nutritional balance provided to the larvae. The question examined in this report is whether the same environmental manipulations affect tumour penetrance in non-allelic *tu* strains of different origin and, if they do, what this implies with respect to gene action.

Two inbred strains were examined, each of which showed a low incidence of tumours when cultured on live yeast or on complete, chemically defined media. One strain (tu^k) carried a recessive of very low penetrance on the second chromosome (and probably an enhancer — $e\text{-}tu^k$ —on chromosome three); the other also carried a second chromosome *tu* gene but this was hypostatic to a third chromosome recessive

suppressor (*su-tu*). This second *tu* gene was fully penetrant and not an allele of the first. Despite these genetic differences, both strains responded by an increase of tumour penetrance to (a) a sub-optimal balance of dietary pentose nucleotides, (b) cholesterol deficiency and (c) excess l-tryptophan. Their F_1 responded similarly to these abnormal conditions. Only the homozygous *tu*; *su-tu* strain reacted with a high tumour frequency to X-irradiation of embryos, suggesting that X-rays affect only the *su-tu* system.

10.27. Genes, Amino and Nucleic Acids, Progressive Tissues and Cancer. Investigations on Poeciliid Fish, Nicotiana, Drosophila and Vitis. F. ANDERS, A. ANDERS, F. DRAWERT and E. STEITZ (Geilweilerhof/Pfalz, Germany).

Platypoecilus maculatus has a gene causing black spots on its body-surface (Gordon, Kosswig). These spots represent a "progressive tissue" (Anders *et al.*, *Experientia* 1963, in press), because they enlarge in a positive allometric manner, while the number of their constituents, macromelanophores, increases. *P. maculatus* has a low level of free amino acids. In contrast, *Xiphophorus helleri* lacking this gene has a high one. Certain hybrids of these species show a high amino acid level, and, when carrying the spot-gene from *P. maculatus*, exhibit an abnormal increase in the number of macromelanophores thereby producing melanomas. Whether or not tumour formation takes place seems to depend on presence or absence of macromelanophores and on amino acid level. Hybrids of 8 other poeciliid species and subspecies have shown the same dependence.

In certain hybrids of *Nicotiana* producing geneticaltumours, a similar correlation is found. On the basis of this analogy, *N. glauca* for instance may be compared to *P. maculatus* and *N. langsdorffii* to *X. helleri*. In *Drosophila melanogaster* a correlation between amino acid level and tumour formation exists too. In *Vitis* it was found that injection of certain amino acids causes neoplasms identical with galls of *Phylloxera*, when meristematic (=progressive) tissues are present.

Further investigations have shown that high concentrations of free amino acids may accelerate biosynthesis of RNA and that autonomous growth of tumour may be caused by a high level of RNA. A simple genetic concept for cancer formation will be discussed.

10.28. Induced Mutations in Barley causing Transformation of Lodicules. O. P. KAMRA (Halifax, Canada).

During induced mutation experiments in barley, using X-rays, Y-rays, neutrons and chemicals, several single gene mutants with altered sex organs were isolated. In these mutants changes in the structure and function of the lodicules were observed.

In the mutants 1 to 4 lodicules are produced. The normal barley spikelet has two lodicules. The extent of transformation varied greatly from a slight modification in shape and structure to complete alteration in form and function. The two lodicules may fuse to form one highly vascular lodicule or may be transformed into functional pistils or stamens.

The lodicules are considered to have evolved from the petals. From detailed histological and cytogenetical investigation of these mutants a better understanding of gene action in differentiation of sex organs in higher plants, and of some aspects of the evolution of these organs may be expected.

10.29. Flowerstriping in Cyclamen persicum cv. "Harlekin". FRIEDRICH BERGANN (Potsdam-Babelsberg, Germany).

Self-pollinated individuals of the striped-flowering *Cyclamen persicum* cv. "Harlekin" do not breed true. Only a certain part of the offspring is striped-flowering, the rest self-coloured red or pink. The numerical proportionsvary. If a "Harlekin"-bulb is decapitated, the lower part of it will survive and produce numerous adventitious buds from the wound. In the following year most decapitated plants are flowering unicolored, generally with red petioles, more seldom with pink petioles. Occasionally red, pink and striped flowers are found in coexistence on the same plant. It may be concluded, therefore, that flowerstriping in "Harlekin" is not due to a gene but to chimerical constitution.

If we suppose the shoot-apices of "Harlekin" to have periclinal constitution (pink over red, or red over pink), flowerstriping can be interpreted as originating by tissue-rearrangements, namely perforations in L 1, or partial replacements of L 1 by L 2.

Considering the evidence on "Harlekin's" chimerical nature, somatic segregations and tissue-rearrangements must be concluded to have taken place in the early ontogenesis and to have consequently produced mosaicism within the

floral tissues, not only in petioles but even in the archespors of carpels and stamina. In a haphazard manner self-pollination therefore may render possible "intra-individual crossings" and may produce *de novo* a varying number of "ever-segregating" and "ever-rearranging" individuals, in the same way as cv. "Harlekin" is reported to have originated by crossing pink × red. The investigations are being continued.

10.30. Spontaneous Somatic Mutability in the Tomato. W. WILLIAMS (Newcastle upon Tyne, Great Britain).

A case of spontaneous somatic mutability in the tomato causing leaf variegation will be described. The condition occurs in a large number of genotypes but is confined to certain stages of plant development. The histological basis of variegation is a failure of normal development in the palisade parenchyma. In addition there is failure of differentation of the sporogenous tissue in the immediate pre-meiotic stage which results in complete male and female sterility. By a process of elimination the condition may be interpreted on the basis of a highly mutable controller locus acting at a specific stage in development.

10.31. Canalization of Flower Morphogenesis in Wheat. O. H. FRANKEL and ANNE MUNDAY (Canberra, Australia).

In common wheat (*Triticum vulgare*) normal flower morphogenesis is an invariate character. It is controlled by a factor associated with *Q*, which, in turn, controls the "vulgare syndrome". In speltoid mutants, where *Q* is deleted or inactivated, we have obtained, as previously reported, a range of stable genotypes from a virtual absence of flowers to perfectly normal flower morphogenesis. In certain compactoids this extends in the supernormal direction, with extra flowers in the empty glumes.

Flower morphogenesis in vulgare and in the speltoids differs genetically and physiologically. In vulgare flower morphogenesis is conditioned by a single gene; in speltoids it is conditioned by a complex multifactorial system. In vulgare the flower formation is uniformly normal in all experimental environments applied, including drastic heat and radiation shocks; in the speltoid series, frequencies of flower formation are strongly affected by suitable combinations of daylength and temperature applied during the critical period of flower formation.

Thus, in vulgare flower morphogenesis is buffered genetically against mutation and recombination among a multitude of polygenes, and physiologically against environmental shocks. There are striking analogies between canalization systems uncovered in *Drosophila* (Waddington, Rendel), mice (Fraser) and wheat.

It can be readily understood that there can be no more than one canalization factor even in a hexaploid; and it is not surprising that the typical polyploid series have been found in genes conditioning relatively superficial functions. Canalization systems are opportunistic; their transformation in polyploids may shed light on the evolutionary changes in gene function itself.

10.32. Inheritance and Morphogenesis of Capsule Spines in Ricinus communis. HAVA STEIN (Rehovoth, Israel).

At the *S (spiny capsule)* locus, there are 4 alleles: *S*—spiny capsule, s^r—very few spines present, *s*, *sf*—entirely spineless, similar to each other when homozygous hence considered isoalleles. *S* is partially dominant: *S* s^r, *S s*, *S sf* plant bear spines, but their number is much lower than in *S S*. Number of spines decreases from *S* s^r to *S sf*: *S S* > *S* s^r > *S s* > *S sf* In *S s* and *S*-*s*+ plants, spine number varies in a predictable manner within each plant. Number of spines on pods of the first raceme is always much lower than in later racemes. Within each raceme, spine number increases gradually from the first capsule to later ones. This indicated an inverse relation between spine number and growth rate. Growth inhibition induced by continuous removal of leaves resulted in a highly significant increase in spine number, as expected. The control of spine formation on the capsule by a treatment applied to other plant organs may well be mediated by mobile substances.

A spine is a multicellular cylinder crowned by a single extremely elongated cell. Appearance of this cell always precedes, and seems obligatory to, the development of the multicellular organ. Determination of spine formation is tentatively assumed to consist of two steps: Excessive elongation of random epidermal cells on the capsule (perhaps controlled by the above-mentioned substances) and stimulation of cell division in the underlying tissues by the elongated cell (through stress or similar forces). This model affords an interpretation of the entire process of spine morphogenesis through simple events on the cellular level.

10.33. The Developmental Genetics of Leaf Size in Lolium. J. P. Cooper (Aberystwyth, Great Britain).

The developmental genetics of leaf size in *Lolium* has been studied in terms of the rates of leaf initiation, leaf appearance, and expansion of the leaf surface at the following levels: (i) differences between populations, (ii) differences between high and low selection lines, and (iii) the effects of major mutants.

Population differences in leaf size in *L. multiflorum* and *L. perenne* are based largely on variation in cell number rather than in cell size, but show marked interactions with temperature, usually related to climatic origin. At 20 C most populations of *L. perenne* are similar in leaf size, but at 5 the Mediterranean material, with a winter growing season, shows considerably greater rates of expansion of the leaf surface than do the winter-dormant north European populations. These differences are exhibited mainly in the size of the individual leaves; there is little variation in rate of leaf appearance.

Selection for high and low leaf size has been highly effective *within* populations of *L. multiflorum* and *L. perenne*, with realized heritabilities over four generations of 30-40 per cent. A strong negative genetic correlation with rate of leaf appearance has appeared, although the rate of initiation remains unchanged. Response to selection has operated mainly through changes in cell number rather than in cell size.

A number of major genes influencing leaf development have been isolated, including *dwarf* (*d*), *branched culm* (*b*), and *shaving brush* (*sh*), which affect leaf size and rate of leaf appearance differentially. These single gene differences are now being used to elucidate the biochemical processes involved in leaf development.

10.34. Hybrid Growth-Evidence of Differential Physiological Development in Heterotic and Non-heterotic Maize Seedlings. Igor V. Sarkissian (Morgantown, U.S.A.).

Earlier experiments[1] have led to an hypothesis that stimulated photosynthetic carboxylation in young hybrid seedlings of barley and maize is associated with heterosis in plants. Additional evidence supporting this hypothesis is presented from studies of photosynthetic $C^{14}O_2$ fixation by heterotic and non-heterotic hybrid maize seedlings. The patterns of distribution of C^{14} to the intermediary compounds were studied by paper chromatography and autoradiography. Differential shifts in the distribution of C^{14} were observed in the case of the hybrids, suggesting systems undergoing altered patterns of physiological development. Determinations and measurements of the respiration patterns of young seedlings also supported this conclusion.

The significance of enhanced photosynthetic CO_2 fixation and differential physiological development and their relationship to heterotic growth will be discussed.

---

1. Sarkissian and Huffaker, *Proc. Nat. Acad. Sci* (*U.S.A.*) **48**, 735, 1962; and in press.

10.35. The Number of Chloroplasts in Different Cells of Trisomic Sugar Beets. T. Butterfass (Ladenburg, Germany).

It is shown that certain chromosomes (I, II, VIII) of *Beta vulgaris* in the trisomic state increase the number of chloroplasts in spongy parenchyma cells by up to 60 per cent, compared with eudiploids, but leave the numbers in guard cells of stomata unchanged. Other chromosomes (especially III, V, VI) increase the number in the guard cells by up to 30 per cent, but not in the spongy parenchyma cells. The augmentations brought about by chromosomes II (50 per cent) and VIII (60 per cent) turn out to be nearly additive in double trisomics of certain combinations, while in others they are not, thus showing that some system of restriction exists which is responsible for the reduced augmentation of only about 45 per cent from diploid to triploid level compared with 130 per cent by the summed up effects of the nine chromosomes of the set. The mean numbers of chloroplasts are independent of cell size as well as of chloroplast size. In adult epidermal and spongy parenchyma cells the numbers mostly show a bimodal frequency distribution. Endomitotic polyploidy, lacking in guard cells, is considered one of the causes for the existence of a second peak. The usefulness of the plastid number as a character in differentiation studies is emphasized.

10.36. Influence of the D-locus on Phenylalanine Metabolism in Mice. Harold Rauch and Martha T. Yost (Amherst, U.S.A.).

Using an inbred strain of mice segregating alleles *D* (full pigmentation) and d^1 (dilute-lethal), we have followed the development from birth through weaning of two liver enzymes

involved in phenylalanine metabolism. In *DD* and *Dd*^{1} the activity of phenylalanine alpha-ketoglutarate transaminase rises rapidly after birth to a peak at 14 days and then falls off about 50 per cent during the third week to the adult level; the activity in d^1d^1 increases similarly only until 7 days at which low level it remains until toward the end of the third week when it falls slightly to the normal adult level. The activity of phenylalanine hydroxylase increases at the same rate in all three genotypes until 17 days; following this d^1d^1 remains the same while *DD* and *Dd*^{1} continue to increase reaching the high adult levels at about four weeks. The low hydroxylase activity in dilute-lethals is due to the presence of an inhibitor rather than to a failure in enzyme formation. The inhibitor, associated with the microsome fraction in brain, kidney, heart, etc., as well as liver can be solubilized with deoxycholate. Evidence suggests the inhibitor may be an enzyme. Reduced transaminase and hydroxylase activities can account for significantly higher serum phenylalanine levels in dilute-lethals (e.g. d^1d^1 ten times *DD* at 14 days). Excess circulating phenylalanine may be associated with the myelin degeneration observed in brains of dilute-lethals.

Supported by U.S. Public Health Service Grant GM 05921.

10.37. Effect of Somatotropin on the Weight Gain of Yellow Dwarf (A^y a dw dw) Mice. GEORGE L. WOLFF and BARBARA RESNICK (Philadelphia, U.S.A.).

Possible involvement of somatotropin (STH) in the increased fat deposition by *yellow* (A^{y-}) mice was investigated with *yellow dwarf* (A^y *a dw dw*) and non-*yellow dwarf* (*aa dw dw*) mice known to be deficient in STH. A^y *a dwdw* and *aa dwdw* mice of both sexes were injected with 0.5 USP unit bovine STH daily for 29 days beginning at about 8 weeks of age. Weekly weights were recorded for injected and control mice for over six months. During the treatment period, weight increase was about the same for both genotypes. After injections were stopped, the A^y *a dwdw* mice continued gaining weight while the *aa dwdw* mice stopped gaining weight immediately. By 9 months of age the A^y *a dwdw* males had reached a mean plateau weight of about 50 g and females about 45 g. These are similar to plateau weights of normal *yellow* ($A^y a$ Dw^-) mice.—Results from current experiments with 0.05 USP unit Bovine STH indicate that weight gain of *yellow* and non-*yellow dwarf* mice is increased to the same degree during the injection period and is proportional to the STH dosage. Apparently the *percentage* increase in weight of *yellow* as compared with non-*yellow* mice is independent of the STH level in the body. Since excess weight gain of normal *yellow* (A^y *a* Dw^-) mice does not usually begin until after about 8 weeks of age, a metabolic process which depends on a certain maturation of the animal may be involved. Administration of STH to $A^y a$ *dwdw* mice may merely allow maturation to proceed to the stage at which excess fat deposition begins.

Supported in part by USPHS Grants RG-6275 and GM 10112-01 and American Cancer Society Grant IN-49.

10.38. The Effect of Hormonally induced Changes During Development and During Adult Life of Drosophila. K. C. SONDHI (Baltimore, U.S.A.).

The present experiments describe the effect of ring gland transplantations in *Drosophila melanogaster*. The experiments were performed on *Samarkand* inbred line, which was inbred for over 200 generations and was structurally homozygous for all the chromosomes. Three mature ring glands were transplanted into the body cavities of host larvae. Two synchronous control populations were maintained, one consisting of individuals which were allowed to develop normally and the other of individuals in which only Ringer's solution was injected. The times of emergence of flies were recorded. To minimize the nutritional differences affecting body weight, the flies were weighed on a torsion balance immediately after eclosion.

The results of these experiments showed that the transplantation of extra ring glands increases the mean body weight of *Drosophila* over 35 per cent compared to controls. The response of females to hormone secreting ring glands was found to be much greater than in males. The effect of altering the hormonal balance on reproductive ability and on the adult life-span is under investigation.

10.39. Growth Equations and Late Morphogenesis of Quantitative Characters. G. FÁBIÁN, J. ERNHAFT and M. VARGA (Gödöllö, Hungary).

The basic growth equations were used pre-

viously in the phaen analysis of some quantitative characters by Brody and others. This method, naturally with its limitations, still has many really useful aspects and so the authors, analysing during some years different species, races, hybrid forms, back cross generations, are inclined to think that this effort had given some results in the "kinetic" point of view of the epigenesis of quantitative characters.

In any case one must take into consideration that the so-called "allometrical ground plan" in Needham's sense shows little if any change in respect of the different quantitative varieties. This fact precludes not the possibility of the different manifestation of the adult quantitative characters. In realizing the final state the quantitative characters are developing in many ways: different startpoints, different velocity of growth in the subsequent development phases, accelerations and compensations. It is not difficult to perceive sometimes the "transit-heterosis" state. This has practical importance.

The differences of reciprocal hybrids during the postembryonal development are also the results of kinetic changes and describing them in this sense is much more accessible than in the end state.

From the linear transformations of the growth curves one can conclude about the degree of the homoestasis by means of the deviations from linear regressions for each separate genotype and the so-called intraspecific allometry is available in the phaen-analysis of quantitative characters.

10.40. Contribution to the Teratogenic Effect of Thalidomide in Golden Hamsters and Mice. H. STENGEL, K. H. DEGENHARDT, and G. BADTKE. (Frankfurt/Main, Germany).

Gross analysis of a control group of 124 newly-born golden hamsters has not shown any malformations. However, after processing the new-born animals for skeletal study (alizarin, glycerin) the examination disclosed several malformations: 1× a slight reduction of the maxilla, 2× a bent radius and ulna on both sides (right > left), and 19× defects of the centra of the vertebrae, especially in the cervical region. For the treatment, thalidomide was suspended in 1 per cent carboxymethylcellulose. Daily doses of 10 mg or 100 mg thalidomide/100g body weight were administered female golden hamsters by oral intubation for a period of eight months. Gross analysis of 119 offspring in the 10 mg series showed no malformations. The skeletal system, however, revealed various malformations: 2× a slight reduction of the maxilla and 15× defects of the centra of the vertebrae, especially in the cervical region. Furthermore, in more than 50 per cent of the animals microphthalmia of various degrees occurred, and in some cases other eye abnormalities. Of the group treated with 100 mg none of 127 new-borns was found to have external malformations. Again a number of skeletal malformations were found: 1× a reduction of the mandibula, 30× defects of the centra of the vertrebrae, and 4× chondrodysplastic changes in the long bones of the upper limbs (right > left). The eyes are being examined histologically.

Analogous experiments have also been done with mice of the inbred strain C57 BL/6 Hannover. In the control group of 149, 16 abnormalities of the vertebrae have been found. Two animals had an extreme microphthalmia. Following treatment with 10 mg, 117 new-borns could be analysed. 21 showed abnormalities of the vertebral column, 5 defects of the jaws, 2 cleft palate, 3 extreme microphthalmia and 1 a kidney dysplasia on the right. Of the 118 animals from 25 litters treated with 100 mg thalidomide 100 g b.w. 109 skeletal systems have been analysed, 10× abnormalities of the vertebral column occurred, 5× defects of the jaws, 7× microphthalmia, 2× cleft palate and 1× aplasia of the abdominal organs. Histological investigations are being carried out.

10.41. An Experimental Contribution to the Determination of Origins of Complex Abnormalities of the Anterior Chamber of the Eye. K. H. DEGENHARDT and G. BADTKE. (Frankfurt a/M, Germany).

Complex abnormalities of the anterior part of the eye known in human beings as "Peter's malformation of the cornea, congenital leucoma and staphyloma of the cornea", differ chiefly in the various strengths of the single symptoms. Features in common are opacity of the cornea and adhesion between the opaque area of the cornea and the iris or the persistent pupillary membrane. Changes in the structure of the lens consist of different degrees of cataract, leading up to aphakia. Microphthalmia and coloboma are also observed. The pathogenesis remains as yet unclear. Peters thought that disturbance due to the separation of the lens from the ectoderm was the cause; but up to now it has not been possible to prove this using embryological materials. The alternative theory that the cause is disturbance by the development of the mesenchyma has also not yet been proved.

Distinct disturbances due to the separation of

the lens from the ectoderm have been observed in the experimental investigations on the eyes of new-born rabbits and mice by one of the authors (K. H. Degenhardt) using the techniques of oxygen deficiency and X-irradiation damage at particular stages of embryonic development.

In these cases ruptures of the cornea, anterior synechia, aplasia of the Decemet membrane in the region of synechia, disintegration of the fibres of the lens, aphakia, coloboma, microphthalmia and other known derangements were observed. These malformations of the cornea, iris and lens are to be considered as the beginning of a teratological series of which the above mentioned derangements of the human eye are the most severe. If the animals had been allowed to live longer secondary reparative changes would have caused a scarred closure of the rupture of the cornea and special changes of the iris and the lens, as have been observed in human eyes. This hypothesis is supported by the condition of a rabbit eye, in which the rupture of the cornea was already scarred. In human beings the long duration of intrauterine development prevents the observation of the early stages of this special category of eye defects.

We think disturbances of inductive relationships between the optic vesicle and the ectoderm, initiated experimentally by oxygen deficiency or by X-irradiation, are responsible.

10.42. Response to a Teratogen of Mice Heterozygous for Anophthalmia. SIDNEY L. BECK (Ann Arbor, U.S.A.).

Normal-eyed female ZRDCT-N [1] and C57BL/10 mice were mated with their own strain or Anophthalmic (ZRDCT-An) males. Pregnant mice were injected on the 7th, 8th and 9th days *post coitum* with 0.25 cc. of 0.3 per cent trypan blue [2], sacrificed during the 18th day of gestation and examined for total implantation sites. Living fetuses were examined for eye defects. Unilateral and bilateral anophthalmia and various degrees of microphthalmia on one or both sides were found.

The ZRDCT-N × ZRDCT-An matings produced many more defective progeny than the ZRDCT-N × ZRDCT-N ($P < 0.001$); mortality was higher among the pure line matings ($P < 0.001$). These findings suggest that the presence of the anophthalmic mutant (*ey*) in the heterozygous state brings an animal close to a threshold for defective eye development in a cross of two closely related lines.

Crosses of C57BL/10 × HL-ZRDCT-An responded with a lower frequency of eye defects (not significant) and mortality ($P < 0.001$) than C57BL/10 · C57BL/10. Here the most obvious phenomenon among the hybrids was heterosis, suggesting that differences in polygenic modifiers between the two lines were stronger than, or could mask the deleterious effect of, the single dose of *ey*.

In all cases the C57BL/10 crosses were affected less than the corresponding ZRDCT-N crosses; among all but C57BL/10 · C57BL/10 there were no spontaneously occurring eye defects. In this latter group the teratogen doubled the spontaneous frequency. In all crosses spontaneous fetal mortality was far lower ($P < 0.001$) than mortality in stressed litters.

1. BECK, *Am. Zool.* **1**, 436, 1961.
2. See BARBER, *Am. J. Ophtalmol.* **44**, 94, 1957

10.43. The Effect of Cortisone on the Penetrance of an Eye Mutant in the House Mouse. MURIEL J. WATNEY and JAMES R. MILLER (Vancouver, Canada).

The mutation *elo* which has recently occurred in the C3H/MI mouse strain results in the condition "eye-lids open at birth" (the mouse is normally born with its eye-lids fused). This mutant appears to be a simple autosomal recessive with about 65 per cent penetrance in homozygotes. In an attempt to increase this penetrance, cortisone was administered to pregnant females for several days prior to the closure of the lids *in utero* which occurs during the 17th day. Rather than increase the penetrance, the cortisone treatment resulted in a significant decrease (to 4 per cent) in the frequency of the defect. On the basis of investigations on the effect of cortisone on palate closure in the mouse [1], it is suggested that the cortisone modifies the action of the *elo* gene through mucopolysaccharides.

1. WALKER and FRASER, *J. Embryol. Exp. Morph.* 5, 201, 1957.

10.44. A New Gene Causing Cerebral Degeneration in the Mouse. M. S. DEOL and GILLIAN M. TRUSLOVE (London, Great Britain).

A new recessive gene (symbol *cb*) causing cerebral degeneration in the mouse has been dis-

covered. The degeneration produces an *ex vacuo* hydrocephalus which is clearly visible in the living animal. The time of onset of the anomalies varies, but classification is usually possible at birth. The most susceptible parts of the brain are the cerebral hemispheres and the olfactory lobes. In later stages the epithelium of the nose and the trachea also degenerates. The involvement of the skull is believed to be secondary. The homozygotes generally die before reaching maturity, but if they live on they are always sterile.

10.45. (D.) Hereditary Cerebral Degeneration in the Mouse. GILLIAN M. TRUSLOVE and M. S. DEOL (London, Great Britain).

A demonstration showing the extent of the fibre degeneration in the cerebral hemispheres caused by a new recessive gene (*cerebral degeneration*, symbol *cb*) in the mouse. A series of drawings based on sections through normal and *cb*/*cb* brains with degeneration and hydrocephalus. Photographs to show the distribution of the cells in the nasal epithelium in different regions of the nose. High power photographs showing the normal and abnormal mucous membrane and also differences in the bone structure of the cranium.

10.46. Cellular Changes in the Central Nervous System of Dilute-Lethal Mice. DIANE E. KELTON (Amherst, U.S.A.).

Previous study of the effect of the *dilute-lethal* gene (d^l) on the central nervous system showed that the sequence and degree of myelinization did not differ among *DD*, Dd^l and d^ld^l mice from 4 to 25 days of age. Death of d^ld^l mice occurs at 18 to 20 days of age. The Marchi method showed increasing amounts of degenerating myelin in the vestibulospinal, spinocerebellar and tectospinal systems of d^ld^l mice with increasing age. The degenerating myelin appeared within a day or two of the onset of myelinization. Current studies indicate that although myelin disintegrates partially or completely in scattered foci there is little or no destruction of axis cylinders, nor is an axonal reaction of nerve cells observed. Little cellular change is seen at 10 days of age. By 20 days of age in the areas where the myelin sheaths have disappeared the number of identifiable interfascicular oligodendrocytes is drastically reduced. Those seen are small and pyknotic. In adjacent areas where myelin is intact the oligodendrocytes are normal in size and shape and are lined up in rows along the nerve fibers. An astrocytic reaction is not apparent although some cells with two or more nuclei were seen. Microgliacytes are plentiful. Perivascular cuffing by macrophages is not a prominent feature, but is occasionally present.

Supported by U.S. Public Health Service Grant GM 05921-05.

10.47. A Developmental Analysis of Strong's Luxoid Mutant. PAULINUS F. FORSTHOEFEL (Detroit, U.S.A.).

Deficient growth of the facial processes and anterior components of the chondrocranium first detected at 12 days of gestation restricts the forward growth of the brain resulting in excessive upward expansion of the telencephalon and mesencephalon. The pressure exerted by the upward pushing brain inhibits hair follicle development and ossification of the frontal and parietal bones over the dorsal part of the brain. Tension exerted on the tissues about the eyes by the outward-pushing mesencephalon results in defective formation of the eyelids. Excessive growth of the preaxial margins of the footplates can be detected at $11\frac{1}{2}$ days in the forelimb and at 12 days in the hind limb. The pattern of blastematal condensations in the limbs and pelvis combines excesses with deficiencies, resulting in polydactyly, radial reduction and duplication, reductions of the pubis and tibia. Deficient skeletal elements lag in chondrification and ossification and show weak alkaline phosphatase and ascorbic acid activity. The posterior belly wall and the anterior half of the genital phallus show deficient growth. Epidermis never covers the belly between the phallus and the root of the umbilical cord. Hair follicles (guard, awl, auchene, zigzag) form at the normal times on the dorsum, but in early stages appear to be retarded. The failure of hair to persist on the dorsum for several weeks after birth is probably due to a weakness in their structure. The primary effect of the *Strong luxoid* mutation is on growth in localized body regions.

Research supported by U.S. Public Health Service Grant C-3613.

10.48. Lethal Micromelia in the White Pekin Duck. WILLIAM J. ASH (Cornell, U.S.A.).

Routine inspection of unhatched pedigreed eggs uncovered a lethal syndrome in the White Pekin duck. Affected embryos appear smaller than their normal sibs. Most conspicuous is the marked shortening of the extremities. Shortening of the upper mandible is observed in some but not all birds. Feathering is greatly retarded, and many embryos appear naked. Feathers which develop often take on a "wormy" appearance. Subcutaneous edema is conspicuous in the cervical region.

Affected embryos survive to terminal stages of incubation. Some pip their shells, but none has ever hatched.

Histological examination of the long bones reveals irregular cartilagineous architecture at the epiphyses. The orderly transformation of young discoidal chondrocytes into mature ovoid ones is absent. Ossification does occur, but it is limited presumably by the aberrant cartilagineous model which precedes it.

Matings of phenotypically normal carriers indicate that this syndrome is an autosomal recessive obligate lethal mutation.

10.49. Dominant Renal Adenomas in the Rat. JEANNE MOSSIGE and REIDAR EKER (Montebello, Norway).

The renal adenomas which occurred in an inbred strain of Wistar rats at this laboratory have proved to be due to a dominant gene with complete penetrance. No homozygote has been found.

The earliest macroscopically detectible tumors have been found at about 3 months, but they may appear first at the age of one year or later. Their development has been followed from early stages in serial sections. They begin as small, cystic tubules and develop into cystic, cysto-papillary or solid tumors and are almost always bilateral. Most of them are well limited without cellular atypia, but many exhibit atypical nuclei and invasive growth in the kidney. Only a few, however, have metastasized, to the liver and lungs, proving malignancy. In humans they would be diagnosed as renal carcinoma.

They develop earlier in males than in females and usually appear in the left kidney earlier than in the right. Various experimental series are being run to test factors which might influence the development of the tumors.

The pathology of the tumors has been described by Eker in 1954 (1) and a preliminary report on the genetic aspect has been published (2).

---

1. *Acta Path. et Microbiol. Scand.* **34,** 554.
2. EKER and MOSSIGE, *Nature* **189,** 858, 1961.

10.50. Periodical Hypotrichosis in Mice. N. KOBOZIEFF and N. A. POMRIASKINSKY-KOBOZIEFF (Maisons Alfort, France)

Periodical hypotrichosis in mice is a recessive mutation with incomplete penetrance. The phenotypical manifestation takes place between the 16th and 21st day after birth and may reappear at more or less frequent intervals.

The genetical analysis of the normal descendants of two abnormal parents (penetrance 30 per cent), shows that they behave genetically as abnormal animals.

When crossed to abnormal, penetrance among the offspring is lower (24 per cent), when crossed *inter se* it is only 20 per cent, and in the F_2 it drops to 10 per cent

The phenotype is variable, there are 6 classes:
- — little loss of hairs, hair sparse on the whole or part of the body (Type I),
- — loss of hair in the scapular region (Type II),
- — loss of hair on the whole thoracic region (Type III),
- — loss of hair as far as the sacral region, hairs remain only on the head and the base of the tail (Type V),
- — loss of hair on the thighs and later than normally (Type VI).

The *gestation factor* is important: the highest frequency of abnormal is seen in the first litter. It is lower in the second and inexistent in the others litters.

The *seasonal factor* is also interfering: during the whole year we observe litters comprising normal and abnormal subjects but it has been noticed that 54 per cent of the litters containing abnormal mice appear in July-August; whereas in the same period we have found 28 per cent of the litters to consist exclusively of normals. This shows clearly that the highest frequency of litters with abnormal subjects is observed in July-August.

10.51. Response of Polycythemic WW^v Anemic Mice to Erythropoietin. MARGARET W. THOMPSON, ELIZABETH S. RUSSELL, and ELEANOR C. MCFARLAND (Edmonton, Canada).

The hematopoietic defect in W/W^v anemic mice

has been studied by comparison of the response of $W^{v}W^{v}$ and normal *ww* mice to erythropoietic stimulation, as measured by Fe^{59} incorporation in erythrocytes. Adult $W^{v}W^{v}$ mice and normal littermate controls were plethorized by transfusion with C57BL/6 erythrocytes to hematocrit levels of 60 per cent or higher, in order to repress erythropoiesis and inhibit formation of endogenous erythropoietin. Purified exogenous erythropoietin, made available through the kindness of Drs. G. Keighley and P. H. Lowy of the California Institute of Technology, was then administered to half the animals of each genotype. The response of animals of the two genotypes to this stimulus was compared by measurement of Fe^{59} incorporation in erythrocytes 24 hours and 72 hours after Fe^{59} injection. Plethorized animals of both genotypes, without erythropoietin, exhibited very low levels of Fe^{59} uptake. Erythropoietin increased the rate of incorporation of Fe^{59} in mice of both genotypes, but the increase in rate was much greater in the normal than in the $W^{v}W^{v}$ mice. It is apparent that $W^{v}W^{v}$ mice are not incapable of responding to erythropoietic stimulation, as previous investigations had suggested, but that they differ from normal mice in degree of response. This new finding implies that the *W*-genes affect the rate and end-point of the response to erythropoietin, but do not inhibit its onset.

This investigation was supported by a Grant (He-05748) from the National Heart Institute, U.S. Public Health Service.

10.52. Analysis of Gene Action and Characterization of a New Hematological Abnormality, Hemolytic Anemia. SELDON E. BERNSTEIN (Bar Harbor, U.S.A.).

This paper describes a new mutation in the house mouse called *hemolytic anemia* (symbol *ha*). Evidence is presented indicating that the hemolytic condition results from the action of an autosomal recessive gene at a locus as yet not located in any of the 20 known linkage groups. This blood dyscrasia is characterized as being a severe neonatal hypochromic microcytic anemia, resulting neither from a maternal-fetal incompatibility nor from the presence of an abnormal hemoglobin. Erythroblastosis, jaundice, splenomegaly, and cardiac hypertrophy are prominent features. Homozygotes of *hemolytic anemia* (*haha*) resemble in nearly every detail *jaundiced* homozygotes (*jaja*). Genetic evidence will be presented, however, which will show that the two conditions are caused by unrelated genes. An analysis of the action of hemolytic anemia genes in terms of a pedigree of causes will follow.

10.53. Effect of Folic Acid Inhibitor upon Morphogenesis of the Wings of vestigial Heterozygotes in Drosophila. J. DAVID (Lyon, France).

When added to an axenic medium, aminopterin slows larval growth a little, but the adult morphogenesis of the wild strains remains normal. When *vestigial* (*vg*, 2rd chromosome heterozygotes are reared with the abovementioned medium, the wings of the flies are not normal and present some notches on the border. This result appears to signify an imperfect dominance of the wild type gene over its *vestigial* allele and suggests that, in the heterozygous condition, *vg* alters the folic acid metabolism in the imaginal disc of the wing.

10.54. Variability of Sex Ratio and Sex-determining Mechanism in Asellus. G. MONTALENTI and G. VITAGLIANO TADINI (Rome, Italy).

In *Asellus aquaticus* sex ratio shows a great statistically significant variability between the progeny of different pairs, the total ratio of the population is approximately 1 : 1. In the population of the river Sarno (Naples) the percentage of males in different sibships varies from 0 per cent (absolute thelygeny) to 95 per cent (high androgeny) with higher frequencies toward 50 per cent ("normal" s.r.). The distribution is of the gaussian type. Sex is genetically determined and so is this variability; intersexuality or hermaphroditism do not occur with appreciable frequency; parthenogenesis does not occur. In previous papers the authors have put forward, as a first approximation, the hypothesis that sex in *Asellus* is under control of a series of polygenes, with the assumption of a clear-cut threshold effect between maleness and femaleness. Further experiments however failed to bring evidence in favour of the loose recombination of a number of genes. A modification of the former hypothesis is proposed, which requires a limited number of genes and assumes that they are more or less closely linked. Three pairs of genes (or gene blocks) would be involved, two (A, B) closely linked, a third (Z) more loosely linked with the former two. Two female genotypes would

exist, with none or one dominants; males would belong to three genotypes, with two, three or four dominants. The variability of sex ratio and the results of different crosses are thus explained by a relatively simple oligogenic hypothesis, without introduction of modifying genes, cytoplasmic factors, etc., as has been implied by other authors in other species. The selective value of this situation versus a clear cut 1 : 1 mechanism is investigated and discussed.

10.55. (D) Genetic Analysis of the True Slime Mould, Physarum polycephalum. JENNIFER DEE (London, Great Britain).

The true slime mould (Myxomycete) *Physarum polycephalum* undergoes simple morphogenetic changes during its life-cycle and passes through a uninucleate phase and an acellular multinucleate phase, either of which can be indefinitely prolonged in culture. It should be a highly suitable organism in which to combine genetical and biochemical approaches to the problems of morphogenesis. Biochemical investigations are becoming advanced, but very little genetic analysis has been done. The demonstration illustrates an attempt to develop genetic analysis of *P. polycephalum*.

Simple techniques are shown for culturing the organism through its life-cycle in two-membered culture. Stages of the life-cycle are illustrated by living cultures or photographs. The uninucleate phase ("amoebae") can be handled by the usual techniques of microbial genetics including plating, which is demonstrated. The mating-type system, methods of isolation of genetic markers and the results of a cross showing recombination between genetic markers are also described.

SECTION 11

IMMUNOGENETICS

11.1. Am Immunochemical Study of the NADP linked Glutamic Dehydrogenase and Mutant Forms of the Protein in Neurospora crassa. D. B. ROBERTS (Cambridge, Great Britain).

Sera containing antibodies against the normal NADP-linked glutamic dehydrogenase of *Neurospora crassa*, have been prepared in rabbits. These sera have been used to study the immunological relationships between the normal and mutant forms of the enzyme. The survey has been carried out on double diffusion plates and on immunoelectrophoretic plates. The line of the enzyme–antibody complex in the gel has been identified by a stain which acts specifically on this complex.

A detailed comparison has been made between the wild type enzyme and the protein from one of the mutants, *am-3*. The *am-3* protein shows activity if incubated with the substrate glutamate. At a concentration of 33 mM of substrate the *am-3* activity is about one-third of wild type activity and at a concentration of 133 mM is slightly greater than wild type activity. At a concentration of 33 mM of substrate small quantities of serum appear to enhance the activity of the *am-3* protein, larger quantities inhibit the activity slightly. At 133 mM concentration of substrate the *am-3* activity is greatly inhibited if incubated with the serum before incubation with the substrate. If the *am-3* protein is incubated with the substrate before the serum or with both together, the serum has less of an inhibitory effect. At both substrate concentrations the serum inhibits wild type activity and there is no substrate protection.

These results suggest a different relationship between antibody–enzyme in wild type to that in *am-3*, and also a difference in the enzyme activity–antibody relationship of the *am-3* protein at different substrate concentrations.

11.2. The Role of Cytoplasm and Nucleus in the Determination of Serotype in Paramecium. JOHN R. PREER, JR., MARY BRAY, and SADAAKI KOIZUMI (Philadelphia, U.S.A.).

Sixteen years ago T. M. Sonneborn demonstrated that in *Paramecium* the cytoplasm at nuclear reorganization is important in determining the expression of genes for serotype. Although it has been assumed that a system of cytoplasmic inheritance is involved, an alternative possibility is that different cytoplasms induce stable macronuclear states in the developing macronuclei. Dryl recently[1] showed that serotypes change more readily at nuclear reorganization than at vegetative reproduction. On the hypothesis of macronuclear determination, conditions inducing change in macronuclear anlagen should not cause developing macronuclear fragments produced at macronuclear regeneration to change so readily.

We have studied the frequency of change from serotype B to A in macronuclear regeneration induced in exconjugants of stock 51, *Paramecium aurelia*. Five hours at 38° C followed by 31° induced both macronuclear regeneration and serotype change. Four lines were obtained from each of several hundred exconjugants. 24 exconjugants produced both changing and stable lines, as well as macronuclear regenerating and normally reorganizing lines. In 22 of the 24 groups of four the macronuclear regenerates were stable B, while 75 per cent ($\frac{38}{51}$) of the normally reorganizing lines changed to A. These results support the notion of nuclear control. Possibly, however, secondary factors associated with macronuclear regeneration favor serotype B more than A, and the results, while suggesting nuclear control, do not really prove it. Attempts are being made to extend the study to the A to B change which is induced by low temperature.

---

1. S. DRYL, *J. Protozool.* **6** suppl.), 25, 1959.

11.3. Blood Group Investigations of Two Marine Animals. J. E. CUSHING, D. VANN, D. BORAKER (Santa Barbara, U.S.A.).

Current investigations involving the blood group antigens of two marine forms are reported. The first concerns the use of *Dolichos biflorus* lectin in detecting an antigen in the California

bonito with a specificity related to human A_1. The second concerns the exchange of blood samples between individual sipunculids (*Dendrostomum zostericolum*) as a technique for the investigation of potential immunological incompatibilities involving hemerythrocytes and other hemocytes of this species.

11.4. Immunogenetic Relationships of Trout. JAMES E. WRIGHT, ROBERT SKLENARIK, and SUZANNE M. JAMES (University Park, Pennsylvania, U.S.A.).

Antisera were prepared in rabbits and chickens against erythrocytes of individuals of four trout species—brook (*Salvelinus fontinalis*), lake (*Salvelinus namaycush*), brown (*Salmo trutta*), and rainbow (*Salmo gairdneri*). Agglutination tests show cross reactions of any of these sera with erythrocytes of any of the trout species. Comparative studies of dilutions of each serum provided evidence of extensive antigenic homology between the two *Salvelinus* species. Also, marked similarities in the cellular antigens of rainbows and the two *Salvelinus* species were found. In contrast, brown trout apparently lacked practically all antigens common to any of the other three species.

Absorptions of each serum by erythrocytes from individuals of each species provided antisera specific to each species; cross-match agglutination tests confirmed the antigenic relatedness of brook, lake, and rainbow trout and the lack of homology between brown trout and any of the other three species, even rainbow trout, the other *Salmo* species.

Although genetic blood group systems previously identified in brown and in rainbow trout are species-specific, these results suggest that blood groups may be identified that are common to rainbow, brook and lake. Absorptions of anti-brook trout sera with cells of individual rainbows produced reagents that identify several blood types in rainbows, but these reagents failed to differentiate among brook trout. Absorptions of anti-lake or anti-brook sera have produced reagents that identify a major blood group system in lake trout; these reagents also agglutinate erythrocytes of some individuals among brook trout.

11.5. Sex-linked "Histocompatibility Reactions" in the Salamander Triturus (diemictylus) viridescens. ALEXANDER WOLSKY, C. EDWARD QUINN, JOSE SQUADRONI S.J. and MARIA DE Issekutzi WOLSKY (New York, U.S.A.).

In these salamanders female skin grafts are better tolerated than male ones (Pizzarello and Wolsky, Squadroni and Wolsky). This was interpreted by assuming the existence of histocompatibility genes on the sex chromosomes (although no sex chromosome dimorphism is known in the species). Recent work, however, indicates that the phenomenon may be due, at least partly, to histological and histochemical rather than immunological differences between the sexes.

Male and female skin grafts behave differently even on the same host (Squadroni and Wolsky). Female grafts, when alone on a host, remain histologically normal, whereas male grafts quickly lose their structure; especially the breakdown of the epidermis and the skin glands is characteristic (Pizzarello). A double graft elicits typical lymphocyte accumulation around both grafts but the female skin structures are not attacked immediately and appear normal at a time when the male graft has been resorbed (Squadroni). Studies with the Ouchterlony technique, involving rabbit sera immunized against *Triturus* skin homogenates, indicate no difference in the antigenicity of the male and female skin (Quinn).

The situation in the female *Triturus* skin seems thus comparable to that found in the cheek pouch skin of the golden hamster by Billingham and co-workers, except that in the case of *Triturus* one must assume a double barrier in the path of histocompatibility reactions (both to and from the site of the antigenic stimulus) whereas in the hamster only the latter seems to exist.

11.6. Different Degree of Compatibility between Several Stocks of Drosophila melanogaster, tested with Ovary Grafting. CARLA HALFER (Milan, Italy).

Since it is generally known that grafting of organs in *Drosophila* is successful, I have tried to analyse the degree of compatibility between genotypically different stocks of *Drosophila melanogaster*, using the method of ovary grafting. The compatibility between grafted ovary and recipient has been measured as percentage of flies where the grafted ovary was able to deliver viable eggs, flies which allowed the ovary to develop without becoming functional, and flies which rejected the graft. As recipients, two different wild tumorous stocks have been used (tu A_2 and tu B_3), one tumourless (Varese); as donors: Chieti—vermilion, vermilion and yellow white. The first data show a different

behaviour between recipients and implanted ovaries in the different combinations. The more significant differences have been obtained in the survival frequencies; the highest percentage found is 55.1 per cent in the grafts of Chieti—v in tu A_2, while the lowest percentage was of 1.0 per cent grafting yellow white in tu B_3. As regards both development and functionality, the differences are less significant, even if there is significance when the recipient stock is tu B_3. The grafts generally are more successful in stock tu A_2 than in tu B_3 and in Varese. Such a different behaviour leads to consider the differences between the two stocks as a result of behaviour of lymph gland and the number of cells swimming free in the haemolymph. In fact, in tu A_2 the number of free cells is high, very low in tu B_3 and in Varese.

11.7. Blood Groups of the Domestic Mink. JAN RAPACZ and RICHARD M. SHACKELFORD (Krakow, Poland, and Madison, U.S.A.).

Immunogenetic investigations in the domestic mink (*Mustela vison*) started in 1961 by Rapacz and Shackelford at the Fur Animal Research Laboratory, University of Wisconsin, have been reported in three papers.[1] At least seven blood factors have been recognized by using reagents from hetero- and iso-immune sera. All reagents functioned as agglutinins and saline agglutination tests were employed for discovery of all antigenic factors except the anti-globulin test for D specificity. Thirteen sera of the 790 tested showed normal agglutinins; three anti-A, six anti-B_2, one unidentified and three nonspecific auto-agglutinins (these three mink, mother and sons, died).

A total of 1599 individuals were tested against anti-A, B, B_2 and 428 of them with anti-C also; each mink reacted with one or more of these reagents. Pedigree analysis of 668 offspring from 171 litters resulting from planned matings indicate that these four specificities are determined by triple alleles. Using the reagents anti-A, B and C, six phenotypes corresponding to the six possible genotypes were identified in this system. Blood factors A, B and C correspond with genes A^a, A^b and A^c, respectively. The B_2 specificity is produced by gene A^b as well as by gene A^c, and consistently accompanies factors B and C in the mink; a single specificity similar to B has been found in the Stone marten (*Martes foina*). Reagents in the A system, especially anti-B_2, show clear dosage effects. There is slight evidence that the mink is subject to hemolytic disease—this problem is under investigation.

1. *Nature*, **196**, 4861; *Immunogenetics Letter* **3** (No. 1), 55-61; *Nature* (in press).

11.8. Full-thickness Skin Grafting in Pigs. L. N. BAKER and E. ANDRESEN (Ames, U.S.A.).

Results of full-thickness skin grafting in pigs have demonstrated the anticipated existence of a histocompatibility mechanism. This grafting technique has been introduced in connection with blood-typing to detect animals which are either monozygotic twins or erythrocyte mozaics (natural chimeras). The dorsal surface of the ear provides a suitable graft bed while satisfactory grafts can be taken from the dorsal or ventral side of the ear. A circular cutting tool and a scalpel were used to prepare the graft bed and to excise the graft. An adhesive patch held the graft in place 4 to 6 days. Fifty-six pure-bred pigs from outbred Duroc and Hampshire stock were used. Forty of these were paired as littermates of the same sex and same blood type as detected with twenty blood-typing reagents. Each paired pig received three grafts: an auto-graft; a homograft from its pair-mate; and a homograft from another littermate of the same sex but slightly different blood type (not available to 4 pairs). All autografts were accepted. Four of the homografts exchanged between littermates of the same blood type were lost by accident; the remaining 36 were sloughed after 37.0 ± 2.5 days. Nine homografts taken from littermates of a different blood type were lost by accident; the remaining 23 were sloughed after 32.7 ± 3.8 days. Although the difference in rejection time is not statistically significant, it suggests an association of blood type with rejection mechanism. Thus, the combined techniques have proved satisfactory for routine examinations.

This work has received assistance from Contract AT(11-1)-707 from the U.S. Atomic Energy Commission.

11.9. Gene Interaction and the A-O Blood-group System in Pigs. BENJAMIN A. RASMUSEN (Urbana, U.S.A.).

Anti-pig-A antibodies are present in normal sera of certain cattle, sheep, and pigs, and anti-

pig-O antibodies are present in normal sera of certain cattle, goats, and pigs. Reagents prepared from these sources were used in hemolytic tests to type the red cells of 961 Duroc, Yorkshire, and Duroc-Yorkshire crossbred pigs and their sires and dams. Three phenotypes, A, O, and — ("dash", *i.e.* A-negative, O-negative), were observed. Blood-typing results were in agreement with the hypothesis that the allele for A is dominant to that for O, and that the expression of A and O phenotypes is prevented by a homozygous recessive genotype at another locus, resulting in —. Five matings of — — gave 22 — offspring; 33 matings of O O gave 213 O and 3 —; 23 matings of O — gave 18 A, 70 O and 56 —; 27 matings of A — gave 66 A, 29 O and 66 —; 36 matings of A O gave 147 A, 98 O and 30 —; and 23 matings of A A gave 96 A, 21 O and 26 —. Red cells of some pigs reacted only weakly with available A or O reagents, and the normal serum of an occasional pig appeared to react most often with red cells of — pigs, suggesting additional complexities of A-O genotypes in pigs.

This work was supported in part by a research grant, GM-08752, from the National Institutes of Health, Public Health Service.

11.10. Studies of Equine Blood Groups. CLYDE STORMONT, YOSHIKO SUZUKI, and E. A. RHODE (Davis, U.S.A.).

Studies on equine blood groups have been underway in our laboratory for the past several years. Although there have been no published reports of the data, some of the progress may be mentioned here. Sixteen specifically different equine blood-typing reagents have been produced, and the blood factors corresponding to these reagents have been named A_1, A_2, B, C, D, E, F, G, H, J, K, L, M, N, O and X. Our main interest is that of determining the genetic systems in which these reagents act. It is found that reagents for blood factors A_1, A_2, B and C react in a system named A in which five heritable combinations (phenogroups) A_1, A_2C, B, A_2BC and —, (the negative alternative) are established. A second system, named D, involves blood factors D, M and X, and the phenogroups DX, MX and —. Blood factors F and G appear to be independent of each other and of all the other blood factors. Consequently, they have been assigned to respective systems F and G. Likewise, H appears to be independent of all the others with the possible exception of E. Although the remaining blood factors J, K, L, N and O are excluded as members of the systems A, D, F, G and H, their possible relationships with respect to one another have not been fully ascertained. Thus, it would appear that genes at a minimum of six loci are involved in the control of equine blood groups.

11.11. Genetic Polymorphism in the Transferrings of the Horse. MIKAEL BRAEND (Oslo, Norway).

Plasma samples from 220 horses belonging to the Norwegian Döla breed have been subjected to starch gel electrophoresis. Nine different transferrin patterns were found. They could be explained by a theory of five different alleles at a single locus. These alleles were named Tf^D, Tf^F, Tf^H, Tf^O and Tf^R. The Tf^R and Tf^F alleles were most common, the Tf^D gene was very rare. Results from studies of sire families, dam-offspring pairs and gene frequency analyses were in agreement with the genetic theory.

In the Fjording breed 44 horses were investigated. Three transferrin alleles only were recognized in this breed, but two genotypes were found which had not been seen in the Döla horse. The gene frequencies in the Fjording material were markedly different from those found in the Döla breed. Most common was the Tf^F allele, but the Tf^D and Tf^R alleles were seen quite frequently too.

In horses of foreign origin additional transferrin patterns have been detected. Most important is the occurrence of a sixth transferrin allele, Tf^M. In total, 14 different transferrin genotypes have been found in this study.

11.12. The Inheritance of Bloodgroups in the B and C Blood Group Systems in Cattle. J. BOUW (Wageningen, The Netherlands).

Investigations on blood groups in cattle have demonstrated that these bloodgroups can be classified into at least 11 genetic systems.

Within the systems the blood groups are composed of one or more serologically determinable antigenic factors.

In the so-called B- and C blood group systems numbers of at least 240 and 50 blood groups, composed of various antigenic factors, controlled by these systems, have been established.

Concerning the inheritance of these blood groups mainly 2 explanations have been advanced:

1. The possibility of closely linked genes each controlling one single antigenic factor.
2. The assumption of series of multiple alleles of which each member produces one antigenic complex.

The data on blood groups in cattle as collected in the U.S. and in Europe have contributed largely to the second assumption as the blood groups were usually found to be composed of serologically closely related antigenic factors.

The blood grouping work in cattle, as performed by now in more than 20 laboratories all over the world, has as a result of this been based upon this assumption of multiple alleles of which each is controlling one antigenic complex.

During the last decade several authors have drawn attention to irregularities by taking this assumption into consideration.

Recent studies in Holland including over 8000 parentage cases are indicating that the assumption of non-crossover units each controlling a part of the blood groups on the loci controlling the B- and C-groups are presenting a more satisfactory explanation for the inheritance of the blood groups in these systems.

11.13. Possible Somatic Recombination in Twin Cattle with Erythrocyte Mosaicism. W. H. Stone, Janis Friedman, Audrey Fregin and Joan Caulton (Madison, U.S.A.).

About 90 per cent of dizygotic cattle twins contain a mixture of two antigenically distinct kinds of blood cells derived from genetically different tissues. This condition is known as erythrocyte mosaicism (chimerism) and presumably results from an anastomosis of embryonic membranes *in utero* followed by a reciprocal exchange of primordial erythropoietic tissues. Chimeric twins are immunologically tolerant to erythrocyte and to transplantation antigens of their co-twins. Recent observations indicate that the proportion of the two kinds of cells in co-twins is not constant, suggesting that a twin may lose tolerance for the antigens of the co-twin. In 6 sets of twins tested over a period of years there was evidence of a shift in the proportion of the two kinds of cells to a predominant type. In addition, one surviving twin of a set showed a shift toward a "recombinant" cell type containing blood group factors that were previously unique to each of the two original types. This recombinant type represents over 95 per cent of the erythrocytes and may have been associated with an abrogation of tolerance. We have sub-lethally irradiated one member of each of 4 pairs of chimeric twins, the other serving as a control, to determine if there are changes in the proportion of the two cell types and if "recombinant" types appear. At 10 weeks post-irradiation, only 3 of the irradiated twins have survived, but there were no definite changes in their bloods.

11.14. Production and Effects of Antileukemic Globulins from Animals pretreated with Normal Human Blood. B. Sekla (Prague, Czechoslovakia).

Immunogenetic conception of the origin of malignant cells through somatic mutation and thus acquisition of distinctive antigenic properties of these cells led to trials at production of specific immune sera by biological means; the principle of acquired immunological tolerance (or lowered reactivity) towards antigens applied during early ontogenesis and in large amounts, has been exploited for this aim.

Pretreatment of new-born sheep was made with large doses of whole human blood mixed from many normal donors. Later, the pretreated and control sheep were immunized with human leukemic cells, every patient having his own experimental and control group of animals.

In vitro tests have shown that immune sera from pretreated animals do not agglutinate erythrocytes of normal persons as well as those of the patient in question in any considerable measure. Furthermore, these sera have *in vitro* a relatively specific cytotoxic effect against the leukemic cells in question.

First trials *in vivo* have shown that relatively large doses of immune globulins, produced by ammonium sulfate fractionation of immune sera, did not alter the red blood cell count of the patient. Indications of specific effect upon the white blood cell count which have been obtained, must be considered cautiously in connection with previous other therapy.

11.15. Independence of Genetic Variants of Egg White Proteins and Blood Group Alleles of the A and B Systems of the Chicken. G. R. J. Law (Johnston, U.S.A.).

Egg whites obtained from hens of six inbred lines of chickens were subjected to starch-gel electrophoresis. Variations were observed in three regions corresponding to those reported by Lush [1] and by Baker and Manwell [2]. No one line of chickens exhibited segregation of each of the genes controlling the variants at all

three loci. The genetic potential of sires with regard to egg white protein types was inferred by pedigree analyses. Blood types of all birds were determined by saline agglutination tests employing specific blood-typing reagents made by iso-immunization within each inbred line. Linkage analyses of egg white protein types and blood types were made on data from two generations and part of a third generation. No evidence was found for close linkage between the A and B blood groups and the three loci controlling the egg white protein variants.

1. *Nature* **189**, 981, 1961.
2. *Brit. Poultry Sci.* 3, 161, 1962.

11.16. Effects of B Locus Genotype on Immunological Tolerance in Fowl. L. W. SCHIERMAN and A. W. NORDSKOG (Ames, U.S.A.).

Similarity of the *B* blood group locus in chickens with the *H*-2 locus in mice has previously been established. Techniques employed to study immunological tolerance were similar to those of workers using birds of unknown histocompatibility relationships except that *B* genotype was determined by blood typing. Of 70 B compatible skin transplants exchanged between closely related chicks at 17 days of age only three were rejected prior to 34 days post-grafting. All of 47 transplants between *B* incompatible birds were rejected early; median survival time (MST) being 6.6 ±0.4 days. Survival time of 47 transplants between *B* incompatible birds injected at hatching with erythrocytes of the same *B* incompatibility did not differ significantly (MST 6.8 +0.5 days) although the bird's ability to produce *B* hemagglutinins at 3 months of age was markedly reduced. Neonatal injection of donor-type leukocytes was relatively effective in inducing tolerance to later *B* incompatible transplants. In a subsequent experiment injection of erythrocytes at hatching and leukocytes at 17 days induced a greater degree of tolerance to *B* incompatible transplants, exchanged on the 27th day, than did injection of leukocytes at 17 days only. Of interest was the fact that heterozygous *B* incompatible transplants remained intact significantly longer than homozygous *B* incompatible transplants; presumably an effect of differences in concentration of foreign *B* antigens at the cell surface. Partial tolerance existed in some cases such that a homozygous incompatible (e.g. B^1/B^1) transplant was rejected early while a heterozygous incompatible (e.g. B^1/B^2) transplant on the same individual (eg. B^2/B^2) showed no apparent rejection during the bird's lifetime.

11.17. Genetics of Rabbit 7S γ-globulins. S. DRAY, J. E. COLBERG, G. O. YOUNG, L. GERALD (Bethesda, U.S.A.), and A. NISONOFF (Urbana, U.S.A.).

Isoantibodies identify several rabbit γ-globulin allotypic specificities which are heritable. Analysis of progeny confirm the hypothesis that the A1, A2 and A3 allotypic specificities are determined by three autosomal allelic genes at the *a* locus; the A4 and A5 specificities, by two autosomal allelic genes at the *b* locus. Progeny tests show that the *a* and *b* loci are not closely linked. An allotypic specificity *P* is determined at a third locus. Using I^{131}-labeled γ-globulins, molecules with Aa1, Ab4 and Ab5 specificities were estimated quantitatively by repeated precipitations with antibody. In the Ab4 or Ab5 homozygotes or the Ab4-Ab5 heterozygote, 80-90 per cent of the γ-globulin-I^{131} molecules have Ab4 or Ab5; 10-20 per cent of the γ-globulin molecules have neither Ab4 nor Ab5. In the Ab4-Ab5 heterozygote, the quantities of γ-globulin molecules precipitable by anti-Ab4 (64 per cent) or anti-Ab5 (27 per cent) are independent of the order of precipitation indicating that allelic specificities Ab4 and Ab5 are not found on the same γ-globulin molecules. However, cellular studies of lymph nodes, using anti-A4 and anti-A5 conjugated with fluorescein isothiocyanate or lissamine rhodamine B, show that allelic specificities Ab4 and Ab5 are found within the same cell. Aa1, Ab5 and Aa3, Ab4 double homozygous rabbits were mated and γ-globulin from a doubly heterozygous offspring tested: 68 per cent of the molecules with Aa1 also had Ab4; 16 per cent of the molecules with Aa1 also had Ab5 specificity. If, as is probable, non-allelic specificities are present on different polypeptide chains, the data are consistent with random assortment of non-allelic chains in molecules of the offspring.

11.18. Immunogenetics of Rabbit γ-globulin. A. S. KELUS and P. G. H. GELL (Birmingham, Great Britain).

There are known six (A1, A2, A3, A4, A5 and A6) allotypes of rabbit γ-globulin distinguishable serologically.

The inheritance of all six allotypes has been studied in nearly 100 matings with several

hundreds of offspring and, in some cases, through several generations. The allotypes appear to be determined by six genes equally divided between two loci (a and b) giving a maximum of nine possible and not closely linked "gene pairs" on chromosomes.

The phenotypic distribution of the allotypic determinants on the γ-globulin molecule and its component parts will also be reported.

11.19. Non H-2 Antigens of Mice. D. Bernard Amos (Durham, U.S.A.).

Mice of one H-2 genotype hyperimmunized with tumor from another H-2 genotype produce antibodies detectable as hemagglutinins and cytotoxins. Most of the antibodies are directed at H-2 antigens, but in certain combinations antibodies reacting with other antigens are present. An antibody produced in C57BL against C_3H/St tumor 6C_3HED reacts with cells carrying H-2 antigens C, K, H, A, but also with cells from 129 mice which carry none of these. The antigen on 129 was tentatively called alpha. Since the antigen is a single factor controlled by a gene H-5a present in C_3H/St and 129 but not in C_3H/He or C57BL, the designation was changed to H-5A.

Another antigen, H-6A (first designated delta), is present in a different C_3H subline, C_3H/He, but is not in C_3H/St. Both antigens are hemagglutinogens. H-5A is present in quantity on kidney, testes and lung; H-6A in testes, spleen, lung, brain and gut. A strongly cross reactive antigen is present in feces independently of the H-6 genotype of the host.

Both antisera react with other antigens not related to the hemagglutinogens. One cytotoxin has been found in antibody to C_3H/St and a mixture of several cytotoxins in antibody against C_3H/He. These antigens appear to segregate independently of H-2, H-5 and H-6 and are being studied in reciprocal crosses between C_3H/He and C_3H/St. Strains co-isogenic for the various factors are being established for a study of the relationship between graft rejection and a variety of cytotoxic or hemagglutinating antibodies.

11.20. Developmental Genetics of H-2 Antigens of the Mouse. G. Hoecker, O. Pizarro and P. Rubinstein (Santiago, Chile).

H-2 antigens of the mouse offer to be good materials for the study of gene action at the cellular level. The *H-2* locus determines a complex system of tissue and red cell antigens which are inherited *en bloc*. The *H-2* locus exhibits pseudoallelism, some antigens crossing-over in about 1 per cent of the gametes.

H-2 antigens seem to be present in very small amounts—basal—in embryos and in newborn mice. After birth, they increase and reach adult concentration in abont 5 to 6 days. During this period different antigens from this system increase their concentrations at the same rate and reach maximum concentrations at the same time. The phenotypic expression of H-2 genes is strongly modified by the cellular environment during development, both in quantity and quality. As a result, different tissues vary in their antigenic contents and some lack them altogether. Besides these main variations in phenotypic expression, subtler changes were found: (1) H-2 antigens from tissues having the same amounts of antigens vary greatly in their ability to induce and sustain antibody production and in the types of antibodies induced preferentially. (2) Differences in solubility of H-2 antigens in saline were also found in some strains of mice but not in others.

11.21. An H-2-associated Serum Protein Variant in the Mouse. D. C. Shreffler (Ann Arbor, U.S.A.).

A 20- to 25-fold difference in the concentration of a specific serum α-globulin has been detected among inbred mouse strains by immunodiffusion with rabbit antisera. Studies of the inheritance of this system and evidence suggesting association with the histocompatibility-2 locus have been reported. A pair of additively acting alleles at an autosomal locus determine, in homozygous condition, the low concentration serum serological phenotype (Ss-L), or the high type (Ss-H); the heterozygote has an intermediate phenotype (Ss-HL). The Ss components of Ss-H and Ss-L sera have the same apparent electrophoretic mobility, precipitation behavior, and pH and heat stability. No differences in antigenic specificity have been detected, even with specific rabbit anti-Ss serum. The Ss protein is a euglobulin, probably of high molecular weight, and has little or no lipid or carbohydrate content. Tests for esterase, phosphatase, and other enzymatic activities have been negative. Present evidence strongly suggests control of the Ss trait by the *H-2* locus. All seven Ss-L lines tested have the $H\text{-}2^k$ allele; eleven Ss-H lines surveyed have various H-2 alleles, but none has $H\text{-}2^k$. Offspring from backcrosses of (C57BL/10

C3H)F_1 × C3H and (DBA/2 × C3H)F_1 x C3H have been classified for serum type by immunodiffusion and H-2 type by hemagglutination. No recombination has been observed among 363 individuals typed, indicating at the 95 per cent confidence level a recombination frequency not greater than 1 per cent. Absorption, inhibition and blocking tests reveal no antigenic relationship between Ss protein and H-2 antigens.

1. *Genetics* **46,** 898, 1961; *Genetics* **48** (in press), 1963.

11.22. Gamma-globulin Isoantigens (Allotypes) in the House Mouse. LEONARD A. HERZENBERG, ROBERT I. MISHELL and LEONORE A. HERZENBERG (Bethesda, U.S.A.).

Although genetic differences in gamma-globulins have been recognized for some time in the human and the rabbit, it was not until 1961 that a genetically determined isoantigen in this class of serum proteins was found in the house mouse.[1] We have reported a second such antigen, designated it Gg-2 and shown it to be determined by a single segregating genetic factor.[2] We have also shown that this antigen is on antibody molecules of diverse specificities.

Data to be presented will show that Gg-2 and an antigen (very likely identical to the one reported by Kelus and Moor-Jankowski) which we label Gg-1 are controlled by a pair of allelic genes, designated Gg^1 and Gg^2. Appropriate crosses with linkage testing stocks have been set up to establish the linkage relations of the Gg locus to known markers in the mouse.

Whether these antigens are found only on separate molecules of gamma-globulin in heterozygotes and the submolecular location of the antigenic determinants are questions under study.

1. A. KELUS and J. K. MOOR-JANKOWSKI, *Nature* **191,** 1405, 1961.
2. J. WUNDERLICH and L.A. HERZENBERG, *Records of the Gen. Soc. of America*, **31,** 126, 1962.

11.23. A Genetic Approach to the Mechanism of Partial Immunological Tolerance. A. LENGEROVÁ, V. MATOUŠEK, and M. VOJTÍŠKOVÁ (Prague, Czechoslovakia).

The clonal selection hypothesis of immunity presumes the existence of multiple clones of globulin-producing cells, each genetically predetermined to form one (or a small number of) specific antibody. The cells of a particular clone can also develop specific non-reactivity (immunological tolerance) if their contact with the respective antigen (or antigenic determinant) takes place within a critical period of their maturation. Tolerance of an antigenic complex is not an all-or-nothing phenomenon occurring in various degrees between complete reactivity and non-reactivity. The hypothesis was being tested that complete tolerance is the issue of a series of independent events; the likelihood of their occurring simultaneously thus greatly depends upon the total number of clones involved in the formation of antibodies against the given complex.

Assuming the existence of n antigenic differences between two inbred mouse strains (A and CBA), then the F_2 individuals represent a distribution of antigenic differences from 0 to n with respect to each parental strain. Under the hypothesis of n independently segregating histocompatibility loci and further hypothesis concerning the probability of induction of tolerance of k antigens from the inducing complex (F_1 hybrid spleen cells), the distribution of survival times of both parental skin grafts has been calculated and compared with that obtained in the experiment. The consequences and limitations of the model are discussed.

11.24. Transplantation of Mammary Glands from Two Strains of Mice to F_1 Hybrids of these Strains. JAMES S. THOMPSON and FLAVIA RICHARDSON (Edmonton, Canada, and Bar Harbor, U.S.A.).

A technique has been developed for the transplantation of mammary glands from C3H/HeJ and C57BL/6 female mice to dorsal and nipple line sites in female F_1 hybrids of these two strains. The effect of site of transplant and heterochronicity upon the grafts has been investigated. The site of transplant apparently had little effect, except that glands in the nipple line were sometimes suckled, whereas those in the dorsal area were not suckled even though they lactated. The percentage of successful grafts varied with the age of both host and donor. With young mice, four to six weeks of age, as both donors and hosts, 80 to 100 per cent of the grafts persisted in most series. As age of either host or donor increased, the percentage of grafts accepted declined, but there was considerable variation in this respect from series to series.

The effect of pregnancy and of estrogen stimulation upon the functioning and the histological appearance of the glands has been investigated. Some of the transplants reacted normally to pregnancy, with lactation and, on occasion, suckling being observed. Histologically these glands had the typical appearance of lactating glands. The effect of estrogen stimulation will be discussed.

11.25. A Specific Alteration of Histocompatibility Expression in the Progeny of a Homograft Tolerant Male. RONALD D. GUTMANN (Bethesda U.S.A.) and J. BRADLEY AUST (Minneapolis, U.S.A.).

The following results are from experiments designed to study the effects of continual exposure of maturating male germplasm in the mouse to living cells of a foreign genotype. An adult C_3H male was rendered tolerant to A strain tissue by the neonatal intravenous injection of A splenic cells. Breeding this $C_3H_{tolerant A}$ male with (A × C_3H)F_1 hybrid females produced a backcross population of mice which accepted an (A × C_3H)F_1 hybrid mammary adenocarcinoma with an incidence of 91.3 per cent and an 18th transplant generation A strain mammary adenocarcinoma in 30.4 per cent of the cases compared with 7.7 and 0 per cent respectively in the backcross control group. Other control groups for the tumors were composed of A, C_3H, (A × C_3H)F_1, and (A × C_3H)F_2 hybrid litters which accepted the tissue in accordance with the expectations of the genetical theory of transplantation based on susceptibility controlled by multiple dominant histocompatibility genes. Subsequent litters of backcross mice sired by the $C_3H_{tolerant A}$ male were tested with normal A, (A × C_3H)F_1, and (C_{57}BL × C_3H)F_1 hybrid skin and accepted the A and (A × C_3H)F_1 hybrid skin in increased incidence when compared with controls, but rejected the (C_{57}BL × C_3H)F_1 hybrid tissue showing that this change in histocompatibility relationship has a donor-host specificity and is demonstrable using normal as well as neoplastic tissue.

11.26. Mutations of Histocompatibility Genes in the Irradiated Mouse. DONALD W. BAILEY and HENRY I. KOHN (San Francisco, U.S.A.).

Tail-skin grafts were exchanged among (BALB/c ♂ × C57BL/6♀) F_1 or the reciprocal type hybrid mice, referred to here as CBF_1 and BCF_1, respectively. Their fathers received spermatogonial X-irradiation (522 rads) or were not irradiated. Of 2641 F_1 mice skin-graft tested, 30 were mutants. Of these, 26 showed a gain in graft specificity (dominant), 3 showed a loss in specificity (recessive), and only 1 showed a loss and a gain in specificity (co-dominant). The higher frequency of gains might be explained thus: if the great majority of histocompatibility genes or subgenes in the two parent strains were identical, then by the laws of chance the gain would be the most likely type of mutation detected by our methods.

Irradiation did not significantly affect the mutation rate. The relative frequency of mutants was: treated groups, 0.009 (BCF_1) and 0.012 (CBF_1); untreated group, 0.014 (CBF_1). It was noted, however, that many mutants occurred as clusters within families, and thus presumably arose in earlier generations. The high spontaneous mutation rate may therefore have masked the effects of irradiation. Nevertheless most mutants had different specificities and thus must have arisen independently.

All observed histo-incompatibilities that were adequately tested were shown to be inherited.

11.27. Heritability of the Natural Immunity. GYÖRGY SZEMERE (Szeged, Hungary).

Searching the genetic circumstances of the natural immunity, the author has pointed out that rats belonging to different races (brown, laboratory white and Wistar) differ from each other regarding their immunobiological titres—namely the complement titre and bactericidal properties—of their sera. Sera of brown rats had the highest and that of laboratory white rats the lowest complement titre and bactericidal activity. If Wistar and brown rats were crossed, the complement titre and bactericidal power of the hybrids' sera showed intermediate values when compared with the titres of the parents.

It has been also pointed out that similarly to the complement titre and bactericidal activity, the sera of brown rats contained the greatest amount of properdin, while sera of laboratory white rats the smallest one. It was supposed, therefore, that the properdin level, and other factors which play a role in the formation of the natural immunity, are different not only between species, but between races too.

Crossing of brown and laboratory white rats showed a surprising result. While the average properdin content of brown rats' sera was 36.6 U./ml and that of laboratory white

ones 18.3 U./ml, the sera of the hybrid animals contained 48.3 U./ml of properdin. These results suggest that immunobiological titres are determined by genetic factors, and heterosis effects may occur in them, though it seems to be evident by the results of the author's other investigations that environmental factors (such as exposure to cold) can change the degree of immunity within a comparatively short period.

11.28. Histocompatibility and Linkage Relationships of Loci Determining Isoantigens of the Rat. JOY PALM (Philadelphia, U.S.A.).

Histocompatibility and linkage relationships of genes determining cellular antigens in three inbred rat strains (Lewis, N.B. and W.I.F.) are being investigated. Allelic genes determine two red cell antigens, 1 of Lewis and 3 of B.N.[1] The histocompatibility function of the locus was indicated by the rapid (under 12 days) rejection of Lewis or B.N. grafts on F_2 hybrids which always occurred when donor and host were incompatible for these antigens. W.I.F. strain rats lacked antigens 1 and 3 but did possess an antigen with the B specificity. The segregation ratios in backcross tests indicated that the genes determining antigens 1 and 3 were allelic with the gene for the B antigen, but were transmitted independently of sex and the non-antigenic loci for albinism and hooding. Tests for linkage with other mutant genes are in progress. The C-D antigenic locus, defined by Owen (*loc. cit.*), was not associated with histocompatibility reactions even with the exchange of tissue grafts between inbred rats differing only for these antigens. The locus was not obviously sex-linked or linked to loci controlling albinism, chocolate coat color and hooding, or to the locus determining antigens 1 and 3. A search is being made for evidence (either by linkage relationships or serological cross-reactivity of specific antisera) indicating an homology of the antigenic loci with those of the mouse.

1. PALM, *N. Y. Acad.* **97,** 1962
2. BOGDEN and APTEKMAN, *loc. cit.*

11.29. (D.) Genetics of the Anaphylactoid Reaction in Rats. H. KALMUS, J. M. HARRIS and G. B. WEST (London, Great Britain).

A single injection of dextran or egg white produces hyperemia, pruritis and oedema in the face, ears and paws of most laboratory rats. This reaction resembles an anaphylactic reaction, but no prior sensitization is necessary. Rats which do not react in the described way even after repeated injections were found in some Wistar albino colonies and pure strains of reactors and non reactors were bred. The difference appears to be caused by a recessive autosomal gene dx which in the homozygous state prevents the reaction; Dx dx and Dx Dx animals are reactors. The gene was outbred into other strains and segregates independently of the fur colour genes c, b and a. In 14 colonies of different origin non-reactivity occurred in 3 Wistar strains at frequencies of 17.5, 23.4 and 100 per cent. Two of these colonies were maintained by brother sister matings and the third as a closed population.

J. M. HARRIS, H. KALMUS and G. B. WEST, Genetical control of the anaphylactoid reaction in rats. *Genetical Research* 1963 (in press).

11.30. New Crossreacting Hetero- and Iso-antigenic Systems in Serum of Mammals including Man. J. MOOR-JANKOWSKI and K. S. BROWN (Bethesda, U.S.A.).

On the basis of our previous results on genetically controlled variations in serum isoantigens, a working hypothesis was established for experimental determination of antigenic markers in animal sera. Experiments were performed in (1) closely related species, e.g. monkeys and man, and in (2) inbred strains of mice.

(1) Numerous selective antisera produced recognized polymorphisms of several serum components in isologous, and in closely related species. A system of notation was developed to define the antigen antibody relationships between the antigen donor, the antibody producer, and the reacting propositus: I, isogenic iso-antibodies (ii). II, isogenic heteroantibodies (ih); III, heterogenic isoantibodies (hi) (still hypothetical); IV, heterogenic isospecific heteroantibodies (hih); and V, heterogenic heteroantibodies (hh). The abbreviations are used to classify the following experimental results.

Individual human isoprecipitating antisera recognized polymorphisms in serum lipoproteins and gamma-globulins of (ii) man and of (ih) *Papio cynocephalus*, and yet undefined serum polymorphism in (ih) *Macacus rhesus*.

Individual isoprecipitating antisera of *Papio cynocephalus* recognized polymorphisms in serum lipoproteins and gamma-globulins of

(ii) *Papio cynocephalus*, and yet undefined serum polymorphism in (ih) man.

Individual heteroprecipitating *Papio cynocephalus* anti-human sera recognized polymorphism in serum gamma-globulins and other fractions of (hih) man, and yet undefined serum polymorphism in (hh) *Macacus rhesus*.

(2) Isogenic strain isoantibodies (i_Si) in mice recognized the antigen gamma-B^A present in BALB/c, C3H/He and "Champagne-Glaxo" but not in C57Bl, and the antigen gamma-C^A present in C57Bl but not in BALB/c.

Genetic transmission of the above systems and their postulated role in maternal-foetal incompatibilities in primates including man, and in mice, are being investigated.

11.31. Immunological Studies of Genetical Markers in Tissue Cell Populations in Cultures. J. Moor-Jankowski (Bethesda, U.S.A.).

Investigation of the genetics of tissue cells in cultures depends on demonstration of hereditary characters. Immunological tools for this purpose are: (I) Mixed agglutination (Coombs), based on specific agglutination, by the same antibody, of tissue cells and indicator cells sharing the same antigen; and (II) Double diffusion in agar gel (Ouchterlony), consisting of the diffusion of antibody and of antigen (here, cell homogenate) in a concentration gradient towards the reaction area, where they meet in proportions optimal for precipitation.

In present tests, antisera for (I) were made against erythrocytes, and antisera for (II) against tissue cells.

The investigated characters of tissue cells were species specificity and blood group specificity. Diachronic studies were performed on consecutive cell generations.

The cells tested were derived from (1) primary animal and human explants, (2) human diploid cell strains, and (3) long established aneuploid human and animal cell lines.

Species specificity was ascertained, and no diachronic changes were observed by (I) and (II) in (1), (2), and (3); selective advantage of some species of (3) was demonstrated. Blood group antigens A, B, or H, and M and Tja were found by (I) in human (1) and (2); no diachronic changes occurred in diploid cells observed from a few, up to 20 passages. Findings on blood group antigens in (3) were not consistent. Partial or complete loss of blood group phenotype in aneuploid cells is evident.

The well studied human diploid strain WI-38 is being used to investigate antigenic markers and their changes after viral-induced transformation to aneuploidy.

11.32. Experimental Studies on the Prevention of Rh Haemolytic Disease. C. A. Clarke and R. Finn (Liverpool, Great Britain).

The results are described of experiments involving the injection of Rh positive blood into 96 Rh negative men and designed to find out whether or not the production of immune anti-D can be prevented.

Giving 10-20 ml of anti-D sera containing high titres of complete antibody half an hour after the Rh positive blood it was found that only about 50 per cent of the injected cells had been cleared within 48 hr and immune anti-D production was *enhanced* as compared with controls who received only the Rh positive blood.

Using 35-50 ml of plasma containing predominantly incomplete antibodies we found that only 3 of 21 "treated" men developed immune antibodies after three or four stimuli as compared to 11 of 21 control men, the difference being statistically significant ($P = 0.02$).

Examination of these results and those of other experiments which are described suggests that about 95 per cent of the injected cells have to be cleared from the circulation within 24 hr if immune antibody production is to be prevented. The anti-D antibody most likely to be effective in this should have no saline activity and as high an incomplete titre as possible.

Preliminary work with anti-D gamma-globulin given intramuscularly has shown that in appropriate dose it is even more effective in rapidly clearing Rh positive cells than the most powerful plasma used.

The next step proposed is to give Rh positive foetal cells to Rh negative infertile women, half of whom will act as controls and half will be given gamma-globulin. It is hoped that the results of this experiment will be available.

11.33. Anti-RH Inhibition by RNA Derivatives and Amino Acids. Emanuel Hackel (East Lansing, U.S.A.).

Earlier studies from this laboratory have shown that several ribonucleic acid derivatives and some amino acids are capable of inhibiting specifically the antibodies of the Rh series. This inhibition is presumed to be the result of partial neutralization of the antibody by the inhibitor

and, in turn, leads to the tentative conclusion that the inhibitor, at least in some way, resembles the antigen.

Recent experiments have shown that mixtures of some RNA derivatives and amino acids have a greater inhibitory effect on the Rh antibodies than equivalent amounts of either substance alone. Adenosine monophosphate, uridine monophosphate, and cytidine monophosphate were the nucleotides used in this investigation. The amino acids used were histidine, arginine, lysine, phenylalanine, and serine. A number of quantitative relationships between inhibitors and antibodies have been determined. These further support the idea of the complexity of the Rh antigens and therefore the complexity of the genetic material governing Rh. Furthermore, while it is not possible to postulate a definitive chemical composition for the Rh antigens on the basis of these studies, they do offer an important insight into the nature of Rh and into the ultimate unravelling of this problem.

SECTION 12

PLASMATIC INHERITANCE

12.1. Chemical Characterization of Metagons in Paramecium. I. GIBSON (Edinburgh, Great Britain).

Metagons are gene-determined factors in mate-killer paramecia. They control the maintenance and multiplication of visible μ-particles in the cytoplasm of these paramecia and are thought to consist of RNA. Metagons have now been extracted from cells and re-infected into other cells. Results will be described with phenol extracts and other techniques for the purification and characterization of the metagons.

12.2. Mutations affecting Mating Type Differentiation in Paramecium. STEPHAN R. TAUB (Cambridge, U.S.A.).

The mating type of an individual *Paramecium aurelia* is controlled by its macronucleus. Two paramecia of identical genotype, however, can differ in mating type. The nuclear differences responsible for mating type differences become established soon after the origin of the new macronucleus. In certain strains (including those of syngen 7), cytoplasm transmitted by the previous sexual generation directs the differentiation of the new macronucleus to control permanently one of two mating type potentialities. The mating type-determining state of the cytoplasm is, in turn, controlled by the differentiated macronucleus and its specificity usually is such as to maintain the same mating type from one sexual generation to the next (Sonneborn, 1954).

In syngen 7, mutations affecting these processes have occurred at two unlinked loci (*n* and *mt*). Paramecia carrying the *N* and the *mt* ^{XIII,XIV} genes can express either mating type XIII or XIV, the type actually expressed depending on the state of the cytoplasm received from the previous generation. Cells homozygous for either the *n*, the *mt* ^{XIII} or the mt_2 gene express type XIII regardless of the state of the inherited cytoplasm. In addition, cells either heterozygous or homozygous for *mt* ^{XIII} and cells homozygous for mt_2 carry XIV-determining cytoplasm regardless of their mating type.

The possible role of the wild type *mt* ^{XIII,XIV} and *N* alleles will be discussed in terms of a mechanism which assumes that the *mt* locus controls an essential reaction in the synthesis of the XIV mating type substance, that this locus is repressible and that it does not function in cells expressing type XIII.

This research was suppor ted by grants from the U.S. Public Health Service and the National Science Foundation.

12.3. Transmission and Segregation of a Cytoplasmic Factor for Streptomycin Resistance in Diploid Chlamydomonas reinhardi. NICHOLAS W. GILLHAM (New Haven, U.S.A.).

Resistance to 500 μg per ml of streptomycin is controlled by a nonchromosomal factor (*sr-2*) which exhibits a uniparental pattern of inheritance via the mating type plus (mt^+) parent. When resistant mt^+ cells are crossed to sensitive cells (*ss*) of the minus mating type (mt^-), the resulting tetrads segregate four *sr-2*: zero *ss* progeny. The remaining 10 per cent or so of the tetrads are exceptional and in these tetrads the *sr-2* factor is transmitted to the meiotic progeny by the mt^- parent. A study of these exceptions has shown that the meiotic products segregate pure clones of resistant or sensitive cells or mixed clones containing both types of cells. Therefore, segregation of resistance and sensitivity may occur during either the meiotic or postmeiotic mitotic divisions.

Recently, a method for obtaining diploids in *Chlamydomonas reinhardi* has been devised by Ebersold (unpublished). Cells are allowed to mate as in an ordinary cross, but through the use of appropriate selective markers it is possible to isolate clones of cells that appear to behave like diploids. In diploids made between resistant and sensitive cells the uniparental pattern of inheritance of the *sr-2* factor appears to break down and the factor is transmitted efficiently by either parent. In the resulting diploid clones segregation of resistance and sensitivity seems to occur within the first few mitotic divisions.

12.4. Interactions between a Cytoplasmic Factor and Nuclear Genes in Neurospora crassa. SELMA SILAGI (New York, U.S.A.).

Mitchell and Mitchell showed that the slow-growing aspect of the phenotype of poky, a maternally inherited condition, could be suppressed by a nuclear gene, f.

As a result of crosses between poky and several other strains of *Neurospora it* has been found that the f locus is closely linked to inositol on linkage group V, with a centromere distance of approximately 35 units. It has also been observed that the f gene manifests itself in the poky strain not only by increasing the growth rate but also by shortening the lag period associated with ascospore germination.

Additional attempts have been made to see if other genes might modify the growth rate of poky strains, including crosses with pe^{m} fl. Poky strains containing fluffy, and therefore aconidial, were found to have a relatively short lag phase, resembling in this respect the behavior of hyphal fragments of "cured" growing mycelium. From this and other evidence which will be presented it appears that the formation and/or germination of conidia or ascospores are a prerequisite for the full expression of the poky phenotype.

12.5. A Gene-induced Cytoplasmic Mutation in Yeast. M. GRENSON (Brussels, Belgium).

Following the application of X-rays, a mutant of *Saccharomyces cerevisiae* was isolated (D77) which simultaneously requires glutamic acid and is respiratory deficient.

When crossed with normal strains, D77 give a mixture of normal and respiratory deficient diploid clones, all glutamic-acid-sufficient. It behaves thus as a suppressive petite. Tetrad analysis of normal diploids show a 2 : 2 segregation of both deficiencies, which are linked without exception.

However, the respiratory deficiency behaves, at the same time, as an extrachromosomal character, since it does not complement a neutral cytoplasmic petite.

Two nuclear petites, N2 and N4, were shown to be complemented by D77.

A hundred glutamic-acid-sufficient revertants isolated from D77 remained respiratory deficient without exception.

The above may be understood if we assume that the glutamic deficiency block is responsible for the induction of a cytoplasmic petite mutation.

This is further supported by the observation that the cytoplasmic petite mutation can be avoided in the ascospores carrying the glutamic acid deficiency when germination takes place on glycerol, lactate or acetate, instead of on glucose, as the carbon source. Under these conditions, the clones arising from the two glutamic-acid-less spores exhibit normal respiration as do the two glutamic-acid-sufficient clones.

12.6. The Inheritance of a Killer Character in Yeast (Saccharomyces cereviseae). M. MAKOWER and E. A. BEVAN (Oxford, Great Britain).

In certain strains of yeast three phenotypes have been observed: "killer", "sensitive", and "neutral". When killer and sensitive cells are grown together in the same broth or agar medium a high proportion of the latter are killed. No killing takes place when neutral are grown with either killer or sensitive cells.

Crosses between different killer and neutral strains followed by Ascus analyses of over 800 tetrads reveal that the difference between killer and neutral cells is controlled by two loci N/n and K/k, showing 36 per cent recombination. The presence of *N* determines the neutral phenotype; its recessive *n*, the killer phenotype. *K* is epistatic to *N*. Thus, all neutral strains are Nk, but either of three genotypes, NK, nK or nk, may determine the killer phenotype.

Sensitive cultures are never recovered following crosses of sensitive by killer or neutral. But the results of such crosses show that sensitive cells may possess any of the above four possible nuclear genotypes all lacking a cytoplasmic component which is present in both killer and neutral cells.

Mutant sensitive strains have been recovered at a frequency of 0.26 per cent following treatment of killers with ethyl-methane-sulphonate. These mutants retain the nuclear genotype of the original strain. No mutation of sensitive to either killer or neutral has yet been observed. These observations provide evidence for the conclusion that sensitives differ from killers and neutrals in lacking a cytoplasmic component.

12.7. The Physiological Basis of the Killer Character in Yeast. E. A. BEVAN and M. MAKOWER (Oxford, Great Britain).

A high percentage of sensitive cells are killed when incubated in cell-free filtrates of buffered

medium in which killer cells have been grown. The percentage killing depends on the period of incubation of both (1) the killer cells prior to filtration, and (2) the sensitive cells following filtration. So far, up to 39 per cent of the sensitive cells have been killed when incubated for 3 hr in a filtrate derived from a 48 hr growth of killer cells. A filtrate obtained from homogenized killer cells resuspended in fresh medium also shows killer activity.

The particular nature of the killing factor is suggested by three types of observation: (1) the retention of activity after dyalasis of the cell-free filtrate for 24 hr in distilled water, (2) the concentration of activity following its ultracentrifugation at 40,000 rev/min for 1 hr, and (3) the appearance of particles approx. 350Å in size when a concentrated filtrate is examined under the electron microscope.

Killer cells have been recovered from an 18 hr growth of sensitive cells (nk genotype) in cell-free killer medium. They are presumed to arise by infection since, by appropriate crosses, they have been shown to possess the same nuclear genotype (nk) as the original sensitives. Killer or neutral cells have not yet been recovered following incubation of sensitive cells in cell-free neutral medium.

12.8. Male Sterility in Castanea. RICHARD A. JAYNES (New Haven, U.S.A.).

Male sterility was observed in *Castanea sativa* Mill., *C. mollissima* Bl., and several *Castanea* hybrids. In most cases the event(s) leading to sterility occurred before meiosis. The arrangement and early growth of individual flowers on catkins was normal; however, staminate primordia failed to develop anthers. The number and fertility of pistillate flowers of the male sterile trees compared to male fertile trees was not diminished. Sterility observed in these trees may be genic, chromosomal, or the result of a gene-cytoplasm interaction. Somatic chromosome counts of one male sterile tree and progeny (germinating nuts) of six other male sterile trees revealed normal complements, $2n=24$, in all but one tree. Neither abnormal chromosome number nor meiotic irregularities appear to be the predominant cause of male sterility among the trees examined. Data obtained indicate that male sterility may be cytoplasmically controlled in a few trees. Final proof will depend on completing enough successive backcrosses to accomplish genome substitution in male steriles by their male fertile parent.

12.9. Genic-Cytoplasmic Interactions in Peanuts (Arachis hypogaea L.). A. ASHRI and E. GOLDIN (Rehovot, Israel).

There are two geotropically distinct growth habits in peanuts—runner (spreading) and bunch (erect). Hitherto their control was attributed to duplicate genes. Reciprocal crosses were made between Virginia Beit-Dagan 4(V.4) and Georgia 2, N.C.2, Dixie Giant, Line 123 and Red Virginia, all bunch. In all crosses, when V.4 was female parent, the F_1 hybrids were runners. Their F_2 progenies segregated (pooled data) 3178 runner: 2511 bunch (fit to 9:7 ratio $P=0.70$-0.50). When the other varieties were female parents and V.4 male, the F_1 plants were bunch. Their F_2 progenies segregated (pooled data) 433 runners: 672 bunch (fit to 6 : 10 ratio $P=0.30$-0.20). The backcrosses of [(runner F_1 hybrids) · (their male parent)] gave 10 runners: 15 bunch. Reciprocal crosses between the last mentioned five varieties and also with Virginia Improved (bunch) produced only bunch F_1 and F_2 plants. Conclusions are: I. There are two types of cytoplasm—one found only in V.4 and another found in all the other tested varieties; II. There are two genes, to be termed Hb_1 and Hb_2, which interact differently with each other in each cytoplasm and with each cytoplasm. It appears that V.4 is $Hb_1Hb_1hb_2hb_2$ while all others are $hb_1hb_1Hb_2Hb_2$. In V.4 cytoplasm Hb_1-Hb_2 produce runners, other genotypes give bunch. In the cytoplasm of the others Hb_1-Hb_2 and hb_1h_1b-Hb_2Hb_2 produce bunch, other genotypes give runners. Implications of this genic-cytoplasmic interaction and evolutionary aspects will be discussed.

12.10. Cytoplasmic Effects in Nicotiana. D. R. CAMERON (Berkeley, U.S.A.).

The observation that there were differences in male-sterility in reciprocal species hybrids and failure to obtain certain hybrids in one direction among species of section Tomentosae, led to a study of various associations of genomes with plasmones of related and unrelated species. Within the Tomentosae, morphological effects ranged from no detectable deviations from normal to those in which flowers had no visible corolla and stamens were absent or vestigial. Some results indicated that failure to obtain certain hybrids was not attributable to the cytoplasm of the ovule parent. When *N. tabacum* genomes were incorporated in cytoplasms of several species, the flower morphology was altered depending on the species involved.

N. tabacum in *sylvestris* cytoplasm and *sylvestris* in *tabacum* gave typical *tabacum* or *sylvestris* plants, indicating that the *sylvestris* parent had provided the cytoplasm in the original hybrid from which *N. tabacum* was derived by amphiploidy. Association of *tabacum* with cytoplasm of certain Australian species and *bigelovii* resulted in the development of split corollas and complete pollen sterility. When *plumbaginifolia* cytoplasm was involved, corollas were reduced in size and there were only scanty amounts of pollen formed. In the combination, *tabacum* chromosomes with *rustica* cytoplasm, plants were normal *tabacum* in appearance and had abundant germinable pollen. They were fully female fertile but, when selfed, set only an occasional small capsule with a greatly reduced number of seeds in each. When pollen of this type was applied to stigmas of *tabacum*, virtually all pollinated flowers abscissed.

12.11. Nucleo-cytoplasmic Interactions. Luis B. Mazoti and Ruderico S. Velásquez (Llavallol, Argentina).

A pure line of *Zea mays* (Selfing[20]) was used as male recurrent parent during 14 back-crosses X *Euchlaena mexicana*. Among both lines of the same homozygous genotype, which were different only in their cytoplasm, were found the following differential characters:

(a) *Significative of mean differences in per cent of pollen sterility.* In cytoplasm of *Euchlaena*, $\bar{x} = 52.1$ per cent, in cytoplasm of *Zea*, $\bar{x} = 8.8$ per cent; $n_1 + n_2 = 480$ anthers, $P < 1$ per cent. The environment affects the percentages, but the signification is permanent.

(b) *Correlation in the percentages of pollen sterility between neighbor anthers.* In cytoplasm of *Zea*, $r = 0.575$; in cytoplasm of *Euchlaena*, $r = 0.948$; $n_1 + n_2 = 480$ anthers. This results are indicating that the initial factor responsible of pollen sterility is independent from meiosis.

(c) *Significative of variance differences in the nucleolus diameter.* In cytoplasm of *Euchlaena* $V = 2.653$ μ, in cytoplasm of *Zea* $V = 0.751$ μ, $n_1 + n_2 = 86$ and $P < 1$ per cent. This results would indicate that the variance in nucleolus diameter could be an index of the nucleo-cytoplasmatic harmony.

(d) *Significative of mean differences of the Knobs volume.* The Knobs volume in *Zea* cytoplasm is $\bar{x} = 17.41$ μ³, in cytoplasm of *Euchlaena* $\bar{x} = 26.62$ μ³, $n_1 + n_2 = 960$, $P < 1$ per cent. This results could indicate that the cytoplasm had modificated the chromosomic structure.

When, after 14 back-crosses of (*Euchlaena* × *Zea*) × *Zea*[14], we obtain autofecundations, the pollen sterility decreased in a significant measure: $\bar{x} = 20.8$ per cent in the back-cross to $\bar{x} = 16$ per cent in selfing, $n_1 + n_2 = 960$ (anthers), $P < 1$ per cent. When this genome of *Zea*, which was modified by the cytoplasm of *Euchlaena*, was transported again to *Zea*'s cytoplasm, an increase of the per cent of pollen sterility is produced from $\bar{x} = 8.92$ per cent with the original genome) to $\bar{x} = 13.75$ per cent (with the recuperated genome), $n_1 + n_2 = 1161$, $P < 1$ per cent. It wasn't changed in one year selfing. This results would indicate that the variation produced by *Euchlaena's* cytoplasm on *Zea*'s genotype, are inheritable and favorable to *Euchlaena*'s cytoplasm and unfavorable (and irreversible in S[1]) to *Zea*'s cytoplasm.

The percentage of pollen sterility was estimated in every "item" in base of 100 counted pollen grains of one anther.

12.12. Morphology, Cytology, and Biochemistry of Male-sterile Lines of Maize. Patricia Sarvella and C. O. Grogan (Mississippi, U.S.A.).

Normal, cytoplasmic male-sterile, and restored versions of five lines of maize were studied morphologically, cytologically, and biochemically. Measurements were taken from meiosis to anthesis (about 15 days after meiosis). At anthesis only slight variations occurred in ear location, ear height, tassel length, and number of internodes to the ear. Some internodes and sheaths above the ear, and tassel culms and sheaths were shortened in the male-sterile plants. Internode-sheath ratios showed large differences between the versions. Stalk lengths above the ear were correspondingly affected which sometimes was reflected in the total stalk length. Restored plants usually were shorter than normal which could be attributed in some cases to fewer internodes above the ear rather than shorter internodes. Variations of the different versions in different environments showed a cytoplasmic-environmental interaction. Pre-anthesis plants showed that shortening started in the male-sterile versions between 10 and 14 days after meiosis. At ten days after meiosis it could even be the longest of the three versions. The exact time of the shortening depended on the line, on the internode location in relation to the ear, and when elongation started. Pollen degeneration in all lines occurred about 5 days

after meiosis, which was before the shortening effect was evident. Amino acid content of the leaves and tassels at various stages were analyzed by means of paper chromatography. Certain amino acids varied quantitatively and qualitatively, depending upon the stage of development of the plants.

12.13. A New Interpretation of Cytoplasmic Inheritance in Streptocarpus (Gesneriaceae). HANS MARQUARDT (Freiburg, Germany).

In *Escherichia coli* and yeasts regulator systems (i.e. the interaction of structural genes, operator-regions and regulator genes, producing a repressor-protein) have been found. Oehlkers analysed in numerous papers the cytoplasmic inheritance in the formation of the androeceum and gynaeceum in the flowers of *Streptocarpus* hybrids (Sympetalae, Gesneriaceae). His results can be interpreted on the assumption, that in the genome there are androeceum-determining and gynaeceum-determining genes with their corresponding operator regions. Instead of a regulator gene, regulator units may be present in the cytoplasm. The repressor-protein produced by the regulator-units of the plasmone of various *Streptocarpus* species either reacts with the operator-region of one of the two structural genes, thereby blocking the normal development of the androeceum and the gynaeceum; or else inactivating substances in the cell metabolism are formed, which affect (inactivate) the repressor-protein. On the basis of these assumptions it is possible to interpret the observed variable anomalies in the development of the androeceum, especially in the *Str. wendlandii* hybrids. The hypothesis is supported by a temperature experiment conducted by Oehlkers (1956) and by the observed switching of sex-expression in the androeceum of suitable hybrids during flowering time. Further experimental tests of the hypothesis are envisaged.

12.14. Alternative Cytoplasmic and Nuclear Hereditary Elements in Petunia. RAFAEL FRANKEL (Bet Dagan, Israel).

Asexual transmission of cytoplasmic male sterility in *Petunia hybrida* through heteroplastic grafts induced two types of male sterility in the progeny of fertile graft components: One of these was maternally inherited, as was the original trait in the male sterile graft symbiont, whereas the other one exhibited mendelian segregation. The progeny of the donor plants for the graft components served as controls.

The very fact of the control on and the mode of the transmission through grafts indicated that the cytoplasmic hereditary elements have attributes of both plasmids and viruses. This, and the induction in one case of an apparent chromosomal integration of the trait, after its induction in the progeny of the fertile graft symbionts, resemble an episome-like behaviour.

12.15. Plasmon Analysis of Species of Solanum. PAUL GRUN, and MARY AUBERTIN (University Park, Pennsylvania, U.S.A.).

Plasmon factor In^S is sensitive to dominant genes of the *In* series, the expression of the gene-plasmon interaction being a failure of pollen dehiscence. To date five different *In* genes have been located any one of which can lead to indehiscence when it is combined with plasmon factor In^S. Separate accessions of *Solanum chacoense*, which have the In^S plasmon factor, are different in the number of alternative *In* genes to which they are sensitive. The geographical distribution of the individual *In* genes appears to be rather wide, for plants sharing the same *In* genes have been located in populations from Ecuador, Colombia, and Peru. No single distinctive abnormality in cytological development has yet been identified as the cause of indehiscence, for indehiscent plants even within single progenies vary in their anther contents. Some have anthers containing normal pollen, some shrunken pollen, some a mixture of normal and shrunken pollen, and some degenerate bubble-surfaced sporocytes. It seems, therefore, that the gene-plasmon interaction may operate through failure of normal anther development rather than failure of pollen development. Abnormality of anther development can, as a result of specific epistatic interactions, lead to other pollen or sporocyte irregularities.

This research was supported by a grant from the National Science Foundation.

12.16. Extranuclear Inheritance of Induced Plastid Mutations in Arabidopsis thaliana. G. RÖBBELEN (Göttingen, Germany).

Plastid development towards an active, normal green chloroplast is controlled by intra- as well as extranuclear factors. The mutations of both block these processes at many different points.

Towards the various types of "chlorophyll mutants" the contribution of gene mutations is well recognized in general. In *Arabidopsis thaliana* we made a detailed analysis of many of these Mendelian factors considering mutation rate, phenotypical manifestation in different genetic backgrounds, and effects on plastid development.

Objections are constantly raised by other authors as to the second genetic component of plastid development, i.e. the "plastom" as being the sum of the autonomous heritable elements within the plastid (Renner). In the course of the mutation experiments reported above, however, we got some strong evidences in favour of the existence of this plastom.

Just as has been found for other plants, plastom mutations in *Arabidopsis* are to be induced by a homozygous recessive nuclear gene. Plastids so mutated remain in that state independent of following changes in the nuclear gene contents. Moreover, plastom mutations have been produced for the first time by X-raying of egg cells or zygotes as well as by treatment of seeds with some alkylating agents (ethyl-methane-sulfonate, di-ethyl-sulfate, etc.) The extranuclear constitution of the resulting somatic variegation in all three cases above is supported by the following evidences:

1. In light- and electron microscope true "mixed cells" (Correns) with both normal and mutated plastids have been found. The appearance of the mutated plastids observed does not seem to be principally different from that one following after an equivalent gene mutation.

2. The pattern of variegation within a leaf is correlated to the margin of a cell descendence. In mixed cells of our standard variety of *Arabidopsis* the rate of sorting out of both plastid types is rather high; this effect, however, varies with different residual genotypes. Using gene dependent "chlorophyll mutants" for induction of plastom mutations we found the resulting variegated regions to be larger, pointing to a smaller competitive superiority of the initial plastids in these mutants.

3. Wherever in plant development the mutated plastids reach the egg cell, a clear proof can be made on their continuity by means of cytological investigations as well as by demonstration of non-Mendelian inheritance.

12.17. Reciprocal Crosses by Raphanus sativus L. P. Raicu and I. Popovici (Bucharest, Rumania).

In our researches reciprocal crosses between seven sorts of radishes of various forms, size and colour of the roots, have been effectuated, obtaining a total of 12 hybrids.

The study of reciprocal hybrids effectuated by us during the 1959-1961 period showed the following:

1. The number of plants of maternal type was considerably higher than those of paternal type. This phenomenon was noticed in F_1 and F_2, manifesting itself as regards the habit in general, as well as the observed characters in particular of form and colour of roots. For instance out of the 135 plants from the four reciprocal hybrids of F_1 between various sorts of the form, a total of 97 plants (71.8 per cent) were of maternal type, 29 plants (21.5 per cent) were of intermediate type and only 9 plants (6.7 per cent) were of paternal type. In F_2 out of a total of 639 hybrid plants, 435 plants (61.1 per cent) were of maternal type, 189 plants (29.5 per cent) were of paternal type and 15 plants (2.4 per cent) were of intermediate type.

2. The variation of hybrids was generally very high, appearing after the segregation in a large scale of colours and intermediate forms, between parents. At the same time new characters appeared, such as: lilac and violet colours of the roots, or a longish-global form in the higher part of the roots. These new characters appeared in a great number of individuals (from the total of 12 hybrids, 10 hybrids).

3. The appearance of the hetorosis phenomenon is in great measure influenced by the way in which the crossing is effectuated. The biometrical measure of plants showed that sometimes great differences exist as regards the vitality between direct hybrids and the reciprocal ones. In some cases the direct hybrids manifested heterosis such as: Eiszapfen ♀ × Red with white end ♂, Tara Bîrsei ♀ × Red with white end ♂, and Saxa ♀ · Round white ♂, while the reciprocal hybrids presented a biological depression.

4. The effectuated study by us showed the outstanding importance of the way of crossing on the segregation of hybrids and their vitality.

The predominant maternal type of the reciprocal hybrids and the great difference as regards their vitality could be explained by taking in consideration the cytoplasmic heredity and the metabolic influence of the maternal organism on the hybrid seeds development.

12.18. Hereditary Infections and Plasmatic Inheritance in Drosophila. D. F. Poulson (New Haven, U.S.A.) and B. Sakaguchi (Misima, Japan).

The demonstration that several striking in-

stances of presumed cytoplasmic inheritance in *Drosophila* are the consequences of hereditary infection raises the question of the nature of all non-nuclear inheritance in that genus.

The maternally transmitted condition referred to as "sex-ratio", SR, in *D. willistoni* and *D. nebulosa* was shown to be the result of the presence in the particular strains of hereditarily transmitted spirochetes closely resembling those of the genus *Treponema*. This was subsequently demonstrated to be true in the cases of SR in other related species, *equinoxialis* (Malogolowkin) and *paulistorum* (Malogolowkin and Poulson), of the neotropical *willistoni* group. On the other hand, no direct evidences of spirochetes could be found in the SR strain of the palearctic species *bifasciata*. Success in transfer of the SR condition from *D. willistoni* into *D. melanogaster* and *D. pseudoobscura* provided unequivocal proof that the spirochetes are the etiological agents of SR. Expression and stability of the transferred SR have been shown to be dependent on a number of factors of which host genotype, spirochete strain, level of infection, and environmental conditions, such as temperature, are important.

Introduction of SR spirochetes from *D. willistoni* into *D. bifasciata* has been carried out with the result that a "sex-ratio" condition has been artificially established showing many of the features associated with naturally occurring "sex-ratio" in *D. bifasciata*. The data suggest that the SR condition in that species, although of such long standing as to have lost its infective character, had its origin in a similar way from an infection which has now become wholly stabilized. Other evidence indicates that hereditary infections are widespread in nature and are the probable sources of most cases of plasmatic inheritance. Our data will appear in a series of papers being publishedin *Geneti cs.*

12.19. A Case of Episome-like Transmission in Drosophila melanogaster (Melanotic Tumours). C. Barigozzi, A. M. Kravina and M. Sari Gorla (Milan, Italy).

Melanotic tumours in *Drosophila* prove to be a chromosomally inherited trait, when tested with mendelian crosses; nonetheless, there are different reasons to believe that an additional extrachromosomal mechanism is at work, which collaborates with the former one. The chromosomal mechanisms seems to be the most efficient, while the extrachromosomal one seems to be rather weak.

The relationship between both mechanisms has been studied by means of ovary transplantations and of injection of cell-free extracts. Especially the last technique proved to be useful, consisting in injecting extract of tumourous flies tu A_2 (recessive abdominal and thoracic tumours) into tumourless ones. The results point to a transmission of a factor which—originally located in the cytoplasm and capable to migrate from cell to cell—becomes eventually integrated in the germ-plasm, and is transmitted with equal efficiency by the egg and by the sperm.

Using the dominant mutant Freckled, lethal in double dose, and apparently located in the 2nd chromosome, it proved possible to obtain again a transmission to the progeny of non Freckled individuals, injected with Freckled extract. Transmission through the sperm has been also demonstrated.

Moreover, Freckled can be transmitted also in a way which recalls paramutation in maize, since from heterozygotes *Freckled*, one recovers a proportion of cychromosomes "infected" with Freckled.

The results obtained so far show a mode of transmisson which can be understandable in the best way interpreting the involved factor as a unit similar to an episome, the integration of which within the nucleus and the cytoplasm is demonstrated, while the integration within the chromosome still requires further work to be elucidated.

12.20. Action of FUDR on the Multiplication of Virus of Drosophila. N. Plus (Gif sur Yvette, France).

FUDR delays the multiplication of σ virus, when injected in the flies together with the virus. This inhibition is not reversed by thymidine. This nucleoside, by itself, acts as an inhibitor of σ multiplication and its action is synergic of FUDR action. The inhibition of virus production by either FUDR or by thymidine is reversed by addition of an equal quantity of deoxycytidine. FUDR seems, with respect to the virus multiplication, to act as an analogue of thymidine, blocking deoxycytidine formation. These facts seem to indicate that σ virus contains DNA. This conclusion is enhanced by the finding that both FU and RNAase are ineffective on σ virus multiplication. It has been so far impossible to analyse the virus nucleic acid directly.

12.21. Genetic Recombination with Drosophila σ Virus. A. OHANESSIAN (Gif-Sur-Yvette, France).

The hereditary virus of *Drosophila* (σ virus) shows numerous genetic variations. Several genetic factors which are described, are controling aspects of the relationships between the σ virus and its host. It has been possible to estimate the mutation frequency of two of them.

Genetic recombination has been demonstrated with σ virus. The recombination takes place when *Drosophila* eggs are mixedly infected with two genetically different viral particles. One resulted from the infection of an oocyte in a non stabilized female and the other is brought by a spermatozoon of a stabilized male.

Genetic recombination probably occurs at the beginning of *Drosophila* ontogenesis. The recombinants are detected in the resulting adult fly. In the course of these experiments some partial heterozygous particles are found. They are heterozygous for one of the viral genes only. This phenomenon has been observed several times.

SECTION 13

PLANT GENETICS AND BREEDING

13.1. Studies on the Interspecific Hybrid Lupinus rothmaleri Klink. x lupinus luteus L. T. KAZIMIERSKI (Poznań, Poland).

Lupinus rothmaleri possesses several qualities, e.g. small-seedness, delicacy and others which it would be very advisable to combine with the properties of *L. luteus*. For this purpose attempts were made to cross these two species. Hybrid seed was obtained when *L. rothmaleri* was the seed parent, and *L. luteus* the pollen parent. Out of 174 *L. rothmaleri* flowers pollinated by *L. luteus* 5 pods yielding 14 seeds were set. On the other hand, out of 505 *L. luteus* flowers pollinated by *L. rothmaleri* 134 pods were set but they contained no well-developed seeds.

Hybrids of the first generation possessed intermediate morphological features and physiological properties. They were in the blooming period since April 20 to October 20, but set only 4, i.e. 0.4 per cent one-seeded pods.

Backcrossed hybrid flowers failed to produce pods and shanked off after 12 to 20 days. Also parent flowers mated with the hybrids wilted after 10 to 14 days.

Analyses of pollen viability performed in a mixture of acetocarmine with glycerin revealed around 20 per cent (19.6) of viable pollen in the hybrids. Cytoembryological studies of hybrid ovules proved that the sporogenesis and gametogenesis were normal only in 1.8 per cent of ovules; in the remaining ones macrospores and embryo sacs disintegrated. The high sterility of hybrids was mainly due to aberrations in the processes of macrosporogenesis and macrogametogenesis.

13.2. Meiosis in Triploid and Allotetraploid Hybrids between Beta vulgaris L. and Species of the Section Patellares Tran. in connection with Transmission of Genes. HELEN SAVITSKY (Salinas, U.S.A.).

Hybrids between *B. vulgaris* (sugarbeet) and diseases-resistant *Patellares* species (*B. patellaris* Moq., *B. procumbens* Chr. Sm., *B. webbiana* Moq.) are usually inviable and sterile; viable semi-fertile triploid and allotetraploid hybrids are obtained. Hybrids between *B. vulgaris* and *Patellares* species are segmental hybrids. Sterile diploid hybrids show 0 to 5 bivalents ($n=9$). Triploid and tetraploid hybrids form complex associations. Triploids form tri- and quadrivalents, while allotetraploids form quadrivalents, pentavalents, hexavalents, and higher valency associations. Configuration of some multivalents and excess of chiasmata over the limits possible for *vulgaris-patellaris* associations indicate the occurrence of translocations and structural changes of chromosomes. Each multivalent association involves 1 or more chromosomes of *Patellares* species, or translocated chromosomes of parental species. First backcross generation ($F_1 \times B.$ *vulgaris*) resembles sugarbeet, but carries some characters of wild species. Segments of chromosomes and some chromosomes of *Patellares* species (in trisome plants) are transferred to b_1 generation. Outline of meiosis indicated the possibility of genes' transmission in *Vulgaris-Patellares* hybrids.

13.3. The Allopolyploid Hybrid between Fragaria vesca and F. moschata. J. R. ELLIS (London, Great Britain).

Many attempts have been made to hybridize the diploid *Fragaria vesca* with the hexaploid *F. moschata*, the object being in most instances to combine the everbearing habit of alpine forms of *F. vesca* with the highly esteemed musk flavoured fruit of *F. moschata*. A small number of sterile hybrids have been reported and these have been either tetraploids, pentaploids or heptaploids, the latter two types having originated from unreduced gametes in the diploid and hexaploid species respectively. No hybrids have as yet been reported to have originated through non-reduction in both parents, an event which would give rise directly to the allo-octoploid hybrid.

From a series of colchicine treatments and hybridizations, a moderately fertile allo-octoploid hybrid line has been established. Pentaploid hybrids obtained from crosses between autotetraploid *F. vesca* and *F. moschata*, were colchicine-treated to give decaploid hybrids,

which on subsequent hybridization with *F. moschata* gave allo-octoploid hybrids. The sequence of colchicine treatments and hybridizations being:

F. vesca (2x) → *F. vesca* (4x) x *F. moschata* (6x) → 5x hybrid → 10x hybrid *F. moschata* (6x) x 10x hybrid → 8x hybrid.

The allo-octoploid hybrids closely resembled the hexaploid parent in vegetative characters and in being dioecious. In view of this morphological similarity, it is conceivable that the controversial mid-nineteenth-century variety "Belle Bordelaise", could have been a similar allo-octoploid hybrid, having originated through chance non-reduction in both parents. In such circumstances this octoploid derivative could have provided an opportunity for *F. moschata* to enter into the evolutionary history of some present-day varieties of *F. grandiflora*.

13.4. Natural and Experimental Festulolium Hybrids and Their Derivatives. F. WIT (Wageningen, The Netherlands).

Lolium perenne, *L. multiflorum* and *Festuca pratensis* are important forage grasses. In addition to their valuable qualities, they show, however, some imperfections which are difficult to eliminate by conventional breeding methods. Intergeneric hybridization might be useful, either through the transfer of single desirable characteristics or through the production of stable and fertile alloploids.

A study of some natural hybrid swarms from *L. perenne* ($2n=14$) × *F. pratensis* ($2n=14$) showed that the predominating plants were triploids (73-94 per cent, and belonged to two distinct groups). Both are supposed to have partly originated by fertilization of unreduced egg cells of the male sterile diploid hybrid. They may be represented by the genome formulae LLF and LFF respectively.

As unreduced gametes are rare, these triploids must exhibit high competitive abilities. They are, however, unstable and partly sterile. Corresponding hexaploids might represent stable and fertile plants.

The development of successful alloploids requires efficient methods to provide for the widest possible range of genomic and genetic recombinations. Results of breeding experiments suggest a method starting with the production of hexaploids LLFFFF. Such plants can easily be obtained either by colchicining seeds from the cross *Lolium* 2x × *F. pratentsis* 4x, or by crossing *Lolium* 4x and *Festuca* 8x. By backcrossing to diploid *Lolium* large numbers of tetraploids LLFF are obtained, which by a second backcross produce triploid seeds LLF. By colchicine treatment these give rise to hexaploids LLLLFF.

The triploids LLF are reasonably fertile, they may be the proper plants for starting introgression breeding.

13.5. Breeding Spineless Gooseberries using Ribes nigrum as Donor Parent. R. L. KNIGHT and ELIZABETH KEEP (Maidstone, Great Britain).

Seedlings and mature plants of black currant (*Ribes nigrum*) are entirely devoid of spines. Sterile diploid hybrids (BG) between black currant (BB) and gooseberry (*R. grossularia*, GG) and the fertile colchiploids (BBGG) derived from them bear weak nodal and internodal spines when juvenile but are free from both types of spine on their mature growth.

Crosses between diploid gooseberry, as female, and these alloploids failed, except for one cross from which sterile diploids were produced. The reciprocal crosses set few seeds most of which failed to germinate, but three triploid (BGG) and one approximately triploid plant were obtained. The spine development of these four plants was similar to that of self-bred progenies from BBGG allotetraploids.

Crosses of the triploid (BGG), as female, with diploid gooseberries (GG) failed, or in one case gave a single very weak distorted seedling. Crosses between diploid gooseberries, as females, and the triploid (BGG) set poorly. From over 2000 flowers pollinated, 62 plants were raised, all of which closely resembled gooseberries in their vegetative growth and all appeared to be diploid. Root tip counts of eight of these confirmed them to be $2n=16$. Some plants with much reduced juvenile spines, and no mature spines were present. These have not yet flowered but they should show adequate fertility for crossing with a range of gooseberry varieties for the immediate selection of commercial spineless types.

13.6. Amphihaploids and Amphidiploids obtained by Hybridization between Aurea Forms of Nicotiana rustica L. and N. tabacum L. IGOR BOLSUNOV (Fürstenfeld, Austria).

Interspecific hybridization of *N. rustica* var. *aurea* × *N. Tabacum* var. *aurea* is extremely difficult. By operating crossings of different cultivars and strains belonging to the aurea

types of *N. rustica* with such of *N. tabacum*, the author succeeded in finding genotypes which permit relatively easy crossing. With the help of acenaphten treatment of F_1 subjects a great number of amphidiploids with partial fertility could be obtained, offering thus the possibility of observing many amphihaploid and amphidiploid plants under normal field conditions. On this occasion the inheritance of morphological and some most important physiological and agronomical characters was studied. It was interesting to note the following particularities: disappearance of the "aurea" character in F_1, increased resistance of F_1 hybrids to some diseases and unfavorable environmental conditions, a clearly expressed heterosis with amphihaploids and diminution of this phenomenon with amphidiploids.

Testing of smoking qualities of fermented leaves of amphidiploids *N. rustica* · *N. tabacum* gave positive results, especially with regard to the use of such raw material as cigar filler.

13.7. Incompatibility Among Species of Darwinia. Barbara G. Briggs (Sydney, Australia).

Interspecific crosses in *Darwinia* (Myrtaceae) have shown several cases of non-reciprocal incompatibility acting at about the time of fertilization. The twelve eastern Australian species and subspecies may be divided into two groups, within which the species are interfertile. In one group the flowers are small and regularly self-pollinate. The members of the other group are also self-compatible but show considerable outbreeding. Crosses between the groups are fertile when an inbreeder is used as the female parent. In the reciprocal cross, best studied in the example *D. fascicularis* ♀ x *D. biflora* ♂, pollen of an inbreeder germinates on the stigma and the growth of pollen tubes to the ovary is unimpaired, but failure apparently occurs just before fertilization. The combination of incompatibility and breeding behaviour prevents or greatly restricts the formation of natural hybrids between some combinations of species. The genetical and physiological basis of the incompatibility reaction is unknown.

13.8. Incompatibility in Betula verrucosa Ehrh. and Betula pubescens Ehrh. Max Hagman (Maisala, Finland).

Self-incompatibility is found in both *Betula verrucosa* and *B. pubescens*. The incompatibility inhibition occurs in the style and is expressed through retarded growth of the pollen tube. Interspecific incompatibility of the same type occurs when the two species are crossed. However, the incompatibility reaction is more pronounced when *B. verrucosa* is used as father. In the reciprocal cross many of the combinations hitherto made show a certain percent of *B. pubescens* pollen tubes with good growth in the style of *B. verrucosa*. This agrees with earlier results that more filled seed is obtained when the species cross is made in the direction *B. verrucosa* ♀ · *B. pubescens* ♂.

In the 25 combinations made with 5 trees of *B. verrucosa* from the same stand there are 7 combinations indicating cross-incompatibility. In 20 individuals from the distribution limit of *B. verrucosa* in Northern Lapland and from isolated islands in the southwestern Finnish archipelago there was no tendency toward increased self-compatibility.

Low temperature slows down the incompatibility reaction so that fertilization may occur after selfing. The same effect may be reached through pollination at a late stage of development of the female flower. The knowledge of these facts may help the forest tree breeder carrying out inbreeding as a breeding programme for birch.

There are serologically detectable differences between the pollen of the two species. Individual differences which could be used as a guide for selecting compatible combinations are under investigation.

13.9. Radiation-induced Chromosomal Aberrations which Simulate Point Mutation of Incompatibility Alleles. James L. Brewbaker (Honolulu, U.S.A.).

Self-incompatible flowering plants of the gametophytic or *Nicotiana* type mutate with comparative ease to self-fertile (SF) forms. Mutants were sought in petunia following premeiotic X-irradiations among 40×10^6 pollen grains. A 16-fold increase in mutation was obtained at 1000 r. Over 300 mutants were studied cytogenetically. Most mutants were self-fertile, and most of these reacted in crosses as if the pollen activity alone had been lost ("pollen-part" mutations). With few exceptions, these plants carried supernumerary centric fragments on which the S gene was located. Fragments varied greatly in size, and could be shown to pair with trisomes bearing the S locus. Subsequent fragment loss was held to account for self-

incompatible offspring (called "revertibles" by some authors) among the mutants. Exceptional SF mutants included several tetraploids, and rare deletions. Colchicine-induced tetraploids were self-incompatible only when homozygous for S alleles (5 tested); all heterozygous tetraploids, triploids, and S-gene trisomes were SF. The following conclusions are drawn: Incompatibility mutations, in petunia at least, rarely if ever involve intragenic change; aberrations which lead to loss of pollen activity include duplication (largely through centric fragments) of the locus and deletion. The data do not support theories of bipartite S gene structure or of revertible mutations; they do encourage consideration of the possibility that point mutations are not expressed due to the probable multiple-stranded structure of the chromosomal gene.

13.10. Pollen-Style Incompatibility in Oenothera. ADOLPH HECHT (Pullman, U.S.A.).

Self-incompatible races of *Oenothera organensis*, *O. rhombipetala* and *O. caespitosa* are being studied by the author and his students in an attempt to discover the mechanisms of the incompatibility reaction. Unlike most other plants that have a gametophytically determined incompatibility, the pollen of these species is inhibited at the time of its germination on the stigma, and few if any pollen tubes in an incompatible mating even reach the style. When the cut ends of styles were reunited in a moist chamber and their stigmas pollinated with compatible pollen, the cut surfaces resulted in only a slight inhibition to the growth of pollen tubes from the upper (scion) portion into the lower (stock) portion of the style. When stylar tissues incompatible with the pollen were used as the stock portion of the graft, there was an appreciable inhibition of the pollen tubes, but some of them did grow several centimeters into this otherwise incompatible tissue. Compatible pollen tubes passed readily from the cut end of a style into a stigma lobe which was also compatible with the pollen, but when a stigma incompatible with the pollen was used in this position below a cut style, the pollen tubes were completely stopped at the stigmatic surface. These experiments show that the incompatibility reaction in these species is considerably stronger in the stigma than in the style.

Work supported by National Science Foundation Grant G-14056.

13.11. A Theory of the Control of Complex Unilateral Incompatibility in Lycopersicon hirsutum. FRANKLIN W. MARTIN (Mayaguez, Puerto Rico).

Unilateral incompatibility is the ability of two hermaphroditic plants to cross in only one of the reciprocal combinations. The type considered here is similar to self-incompatibility in that both are due to inhibition of pollen-tube growth in the style. In *Lycopersicon hirsutum* and *L. esculentum* this phenomenon has 4 levels of expression. The various compatibilities occur in a step-wise sequence suggesting a single form of behavior with various levels of manifestation. Measurements of inhibited pollen tubes indicated two kinds of variability among 5 accessions of plants tested: variability in strength of an inhibiting reaction in the style and of a stimulating reaction in the pollen. The presence of different balances of stimulating and inhibiting substances could account for this hierarchy of incompatibility reactions. This theory is consistent with knowledge of the occurrence of such substances. Evidence for the occurrence of different balances of such substances has come from 4 other sources. First, measurements of pollen tube growth within styles showed variances associated with degree of heterozygosity of a single, partially imbred parent. Secondly, segregation for degree of inhibition of pollen-tubes occurred among plants of a single family. Thirdly, crossing among self-incompatible plants from different geographical sources sometimes destroyed the balance resulting in pseudo-compatibility. Finally, the inheritance of unilateral compatibility was conditioned by many minor genes in hybrids. The body of evidence suggests polygenic control of the quantity of growth substances, modified by environmental factors.

13.12. Changes in Pollen Proteins during Pollen Tube Growth from the Incompatibility Point of View. JAROSLAV TUPÝ (Prague, Czechoslovakia).

Proteins of the pollen with different S alleles are serologically distinguishable (Lewis, 1952; Linskens, 1960; Mäkinen, Lewis, 1962). This fact supports the antigen-antibody hypothesis of the incompatibility reaction. The germination and the growth of pollen tubes in incompatible styles is normal during first hours with the gametophytic system of incompatibility. In *Nicotiana alata* the incompatible tubes often attain the length of as much as 4 cm without any inhibition. That is why we can expect changes in

pollen proteins during the pollen tubes growth.

Pollen proteins of *Nicotiana alata* were separated into at least 14 fractions by means of gel filtration and chromatography on Sephadex and DEAE-cellulose. After 15 hr artificial cultivation under aseptic conditions new protein fraction was found. More than one half of the total proteins of pollen tubes have been released into the medium. The same fractions, but in relatively different quantities, have been found both in the proteins of medium and in those of pollen tubes. The new fraction occurred mainly in the medium. After the administration of L-proline-^{14}C the highest specific activity in this fraction was determined.

It is possibler, therefore, that the proper specific proteins which are responsible for incompatibility reaction in species with a gametophytic system of incompatibility are synthesized during the pollen tube growth.

The corresponding data will be published in full in *Biologia Plantarum, Praha.*

13.13. Pollination with Pollen Mixture to Produce Interspecific Hybrids of Plum and Cherry (Prunus), K. K. Enikeyev (Moscow, U.S.S.R.).

Soviet horticulturists use the method of pollination with pollen mixture in interspecific crosses.

This method was worked out by I. V. Michurin in order to overcome incompatibility of different fruit species. I. V. Michurin based this method on the regularities of selective fertilization and complex physiological interaction between the components of pollen mixture and the pistil of a maternal form.

The author has used two variations of the pollination with pollen mixture and got the following results:

The first variation consists of the pollination of one species with the pollen mixture of several varieties of another species which is almost or entirely unable to cross with the former, it secures the selective fertilization by means of the most biologically adapted pollen grains of one of the varieties of a paternal form.

As a result of pollinating *Cerasus Besseyi Bail* with pollen mixture of several European varieties of apricot (*Armeniaca vulgaris* L.) quite viable and fertile hybrids have been produced.

The hybridization of several *Prunus* species with different chromosome numbers in F_1 results in the development of low-fertile hybrids of an intermediate type. Such are *P. spinosa* L. × *P. domestica* L. hybrids ($2n = 40$) and *P. cerasus* L. × *P. avium* L. hybrids ($2n = 24$).

When these low-fertile hybrids (F_1) were pollinated with the pollen mixture of several varieties of one of parental species, F_2 consisted of quite fertile and commercially valuable plants with the characteristics of both species.

The second variation consists of the addition of small amounts of own or alien pollen related to maternal form to the pollen of an uncrossable species. This variation is based on the fact that in the process of fertilization own or alien pollen favourably affects the pistil, creating conditions which stimulate the growth of pollen tubes and the fertilization with the pollen of an uncrossable or hardly crossable species.

The analysis of morphological characteristics and chromosome numbers in F_1 hybrids shows that preferable fertilization with own or closely related pollen takes place in these crosses, though in some cases this method helped to cross two hardly crossable species, for example, *Cerasus Besseyi* Bail × *Prunus cerasifera* Ehrh, ($2n = 16$); *C. Besseyi* × *P. spinosa*. ($2n = 24$.)

13.14. Transfer of Self-Compatibility from Solanum verrucosum to Diploid S. tuberosum. L. A. Dionne and K. M. Graham (Birmingham, Great Britain, and Fredericton, Canada).

The development of homozygous diploid lines of *Solanum tuberosum* has been complicated by the extreme selfincompatibility of the diploid forms of the series *Tuberosa*. Attempts to isolate mutations for self-compatibility from the diploid *Tuberosa* have not given much encouragement to this means of solving the problem. However, it has proved to be relatively easy to transfer the self-compatibility of *Solanum verrucosum*, a Mexican diploid species in the series *Demissa*, to *S. tuberosum*. By this means it has been possible to establish breeding lines that combine a high level of self-compatibility with earliness and good resistance to *Phytophthora infestans*.

13.15. Aneuploids in Some Artificially induced Polyploids of Cultivated Plants. Mechtild Rommel (Zaragoza, Spain).

When investigating chromosome numbers of artificially induced autotetraploid barley stocks (*Hordeum vulgare*), autotetraploid sugarbeet populations (*Beta vulgaris*) and wheat–rye derivatives (*Triticum durum* × *Secale cereale*)

many plants were found not possessing the euploid chromosome number. While in diploids loss of a chromosome seems to be lethal many polyploid plants with off-numbers will survive. But gain or loss of one or more chromosomes or even the presence of a fragment apparently disturbs plant development thus making these plants unsuitable to grow as commercial crop plants.

In autotetraploid barley ($4x = 28$) euploid plants produced progenies with 38-57 per cent euploid plants only. Among poor seeds aneuploids were much more frequent than among well-developed ones. Aneuploid plants showed great variation in morphological appearance ranging from very poor to almost normal growth. Fertility of aneuploids was much more reduced than fertility of euploids.

In populations of autotetraploid sugarbeets ($4x = 36$) aneuploidy occurred in about 42-44 per cent of all plants investigated. Growth of leaves and roots varied from very poor to almost normal. Weight of aneuploid roots seldom reached that of euploid ones.

In some strains of wheat-rye hybrids ($6x = 42$) aneuploids were present but in lesser number.

The occurrence of aneuploidy in autotetraploids of homozygous and heterozygous plants as well as in newly established amphipolyploids enlightens the fact that production and/or survival of off-number gametes and zygotes must be primarily conditioned by induction of polyploidy. Their presence necessarily leads to reduce fertility in polyploids. In addition the number of aneuploids in a plant progeny apparently is depending upon the genotype used and upon environmental conditions.

13.16. The Relation between Fertility and Cytological Properties in Autotetraploid Rye. KNUT AASTVEIT (Vollebekk, Norway).

Twelve years ago six autotetraploid populations of winter rye were produced by colchicin treatment. The original tetraploid populations have since been kept isolated. At the same time new populations have been established by intercrossing and selection. In 1960, 1961 and 1962 a number of populations were compared in respect to fertility (seed set). Highly significant differences were found in several cases. It is concluded that the fertility of autotetraploid rye is a heritable character, which can be influenced by selection.

Populations differing significantly in fertility are now under cytological investigation. As far as our results go, the population differences in fertility can be related to the frequency differences of aneuploids. In 1962 aneuploid plants had a reduction in fertility of appr. 18 per cent, as compared to euploids. The fertility of the euploid plants of the most thoroughly examined tetraploid population had a fertility which was only 3.3 per cent below that of the corresponding diploid population.

Meiosis has been studied in euploid plants belonging to populations which differ in fertility and frequency of aneuploids. In this connection the types of diakinese configurations, frequencies of bivalents and multivalent chromosome associations, co-orientation of chromosomes at first metaphase and chromosome distribution at first anaphase have been studied. The mechanism leading to differences in the frequency of aneuploids will be discussed.

13.17. Different Fertility of Mitotic and Meiotic produced 4x-Forms in Primula malacoides Franch. KURT SKIEBE (Quedlinburg/Harz., Germany).

The fertility of *Primula malacoides* was studied, both generated by colchicine induction and 2 x x hybridization. Plants within the types were crossed exclusively. Comparison is made from C_1 to C_5. The fertility of the meiotic produced 4x forms exceeds significantly the mitotic originated ones. Therefore in future 4x forms from unreduced gametes only should be used in polyploid breeding.

13.18. Study of Pollen and Pollination in Relation to Partial Sterility of Autotetraploid Rice. J. BOUHARMONT (Louvain, Belgium).

In autotetraploid rice, there is no correlation between the rate of meiotic irregularity and the level of sterility, but a number of factors take part in the reduction of seed setting. An important one is the relatively lower quantity of good pollen shed on the stigmata. We have to consider the quantity and the quality of the pollen produced in the anthers, the abundance of pollination and the pollen germination.

After chromosome doubling, the number of stainable pollen grains per spikelet is reduced by about 30 per cent: the number of PMC's is lowered (18 per cent) and some microspores fail during their maturation (12 per cent). The diploid pollen grains are larger than the haploid ones (20 per cent) and their size is more variable:

the coefficient of variation is 7.3 per cent for the latter and varies between 10.7 and 15.0 per cent for the former.

Though the quantity of viable pollen in tetraploid flowers is still sufficient, the number of grains deposited on the stigmata is generally lower than in diploid plants and a variable percentage of spikelets are not at all pollinated.

The pollen germinates on the stigma as soon and as well in tetraploid as in diploid plants. Nevertheless, many pollinated flowers fail to set seed; thus if the low pollination is one cause of the partial sterility of tetraploid rice, it is not the only one.

These data on pollination and the study of other causes of sterility will be published later, probably in *La Cellule*.

13.19. Pollen Morphology in Relation to Pollen Germination and Seed Set in Tetraploid Red Clover. MARIE BRAGDO, (Vollebekk, Norway).

Pollen morphology, judged by the aceto-carmine-glycerine-method, and seed set were studied in 1961 in 176 plants. The following characters were observed:

x_1 Per cent good pollen grains (well stained and not shrivelled).

x_2 Diameter of good pollen grains.

x_3 Variation in diameter of good pollen grains.

y Number of seeds per head after open pollination in the field.

As X_1 was closely correlated with X_2, the latter was discarded. No correlation was detected between X_1 and X_3. Y was positively correlated with X_1, and negatively correlated with X_3.

Some plants, selected for pollen quality according to morphology, were propagated by cuttings. In 1962, 15 plants of each clone were planted in a field and pollen morphology studied. There was a good agreement between the percentages of good pollen grains in 1961 and 1962. A test of pollen germination on sugar-agar showed this to be significantly correlated with percentage of morphologically good pollen. Thus, pollen morphology, judged by the aceto-carmine-glycerine-method gave a fairly good indication of the relative germinability. The average of germinated pollen grains was lower than the average of morphologically good pollen.

On account of the selfsterility in red clover, the seed set cannot be much influenced by the pollen quality of the same plant. The association must be due to some other factor, with which both are correlated, probably the quality of the female gametes.

13.20. The Location of a Gene for Male-sterility in Linkage Group I of Barley. J. MILOHNIĆ (Zagreb, Yugoslavia).

Crosses have been made between male-sterile (ms ms; SUNESON) lines of *H. vulgare* and normal (Ms Ms) *H. distichum*. The former species is six-rowed (v v) and has a low number of rachis internodes (rin rin). The latter species is V V Rin Rin. F_2 analysis shows that ms is linked to the two chromosome I loci. The order is as follows (rec. per cent by product-ratio): v - 30.7 - rin - 33.8 - ms. Rec. per cent between v and ms is 48.7. It was also found that ms ms reduces the number of rachis internodes in the Rin-rin genotypes.

13.21. Tetraploid Inheritance of Monogerm Character and Male-sterility in Beta vulgaris L. (Sugar-beet). V. F. SAVITSKY (Salinas, U.S.A.).

1. Monogerm character (gene *m*) is inherited in tetraploids on the basis of chromatid assortment at an intermediate frequency of double reduction (parameter α). Value of α is stipulated by an intermediate frequency of crossovers between the locus and kinetochore (parameter e), and by an intermediate number of quadrivalents formed (parameter a). Gene expression: nulliplex m_4 produces monogerm fruits; simplex M_1m_3 and the rest of genotypes produce multigerm fruits.

2. Gene *a*, responsible for mendelian male-sterility, and gene *m* are located in different chromosomes. Gene *a* is inherited on the basis of chromatid assortment, but the value of its parameter α is greater than the value of α for the gene *m*. Gene *a* is located further from its kinetochore than the gene *m*. Gene expression: nulliplex a_4 causes complete male-sterility; simplex A_1a_3 and all other genotypes are male-fertile.

3. Inheritance of cytoplasmic-genetic type of male sterility in tetraploids indicated the presence of only 1 basic gene which controls pollen fertility in cytoplasmic male-sterile races, not of 2 genes as often assumed in studies of diploid heredity. This gene is inherited on the basis of chromatid assortment.

13.22. (D.) Sterility in Broad Bean (Vicia faba L.). D. G. ROWLANDS (Bedford, Great Britain).

The demonstration shows that sterility in Broad Bean is not a simple system but operates at

different stages in the breeding system. Under field conditions only 10-20 per cent of the total flowers produced on a plant ultimately set pods, and under controlled self-pollination this pod set may be as low as 2-3 per cent.

In some genotypes, damage to the papillae of the stigma during pollination by insects or mechanical means is necessary for pollen germination and pollen tube development. In such genotypes, foreign pollen appears to have a developmental advantage over pollen from the same plant. Unsatisfactory pollen tube development causes the flower to absciss.

When pollen tube growth is normal, the young ovary develops until it reaches 20-22 mm in length, but if fertilization is not effective, the young ovary falls off at this stage.

After self-pollination of some genotypes, up to 80 per cent of the pods initially developed may reach a length of 40 mm. Pod development then stops and the ovules within these pods degenerate. Occasionally a single ovule develops normally, in which case, degeneration of the other ovules occurs at a much later stage.

Embryological studies show that this ovule degeneration in Broad Bean resembles the somatoplastic sterility in *Medicago sativa* as described by Brink and Cooper.

13.23. Effectiveness and Specificity of Ionizing Radiations and Some Chemical Substances in Inducing Mutations in Winter Wheat.
V.V. Khvostova, V. S. Mojaieva and N. S. Aigaes (Moscow, U.S.S.R.).

1. A comparison was made of the mutagenic effect of gamma-rays in dosages of 10, 15 and 20 kr and of fast neutrons with energy 1–2 MeV in dosages of 350 rad (210 rad for neutrons and 140 for gamma) and of 750 rad (425 rad for neutrons and 286 for gamma) and ethylene imine in concentrations of 0.01-0.04 per cent treating air-dry seeds (at room temperature) of winter wheat *Triticum-Agropyrum* hybrid-186.

2. Taking into account the largest number of progenies segregating mutants in M_2, the most effective mutagene proved to be ethylene imine in concentrations of 0.01 : 50.8 per cent of progenies segregated mutants; on the second place stood fast neutrons in dosages of 740 rad, producing 36.5 per cent of progenies with mutants, on the third place were gamma-rays in dosages of 10 kr: 6.2 per cent of progenies.

3. The spectrum of the mutations, appearing under the action of neutrons, gamma-rays and ethylene imine proved to be different. The narrowest spectrum was produced by the neutrons: a total number of 208 mutants showed 80 per cent of erectoids, squareheads and speltoids, and only 20 per cent other changes. Among 81 mutants, which appeared after gamma-ray treatment, the number of other types of mutants amounted to 33 per cent. After treatment with ethylene imine there were found 100 mutants, 34 per cent belonging to the above mentioned three types, 66 per cent various other forms.

Thus, ethylene imine appeared to be the most effective mutagene, producing at the same time the most broad mutation spectrum.

4. Under the action of these mutagenes, valuable agricultural forms are produced: resistant to lodging, to fungal diseases, and highly productive.

13.24. Mutation Research in Canary Grass.
D. E. Bremer-Reinders (Wageningen, The Netherlands).

In studying the influence of chemical mutagens and X-rays on the frequency and spectrum of mutations in *Phalaris canariensis* ($2n = 12$) comparable doses (seedling percentage surviving treatment) were chosen.

Irradiation with 15,000 r decreased the number of seeds per spike in the M_1-generation to about 50 per cent of the control. A corresponding dose of ethylene imine decreased the M_1-fertility to about 36 per cent of the control, while the corresponding E.M.S. treatment decreased the fertility of M_1 to about 15 per cent of the control.

Of 406 progenies tested after irradiation (15,000 r) on an average 20 per cent contained chlorophyll mutations. With ethylene imine this was nearly 27 per cent in 78 progenies, while with E.M.S. this amounted to nearly 30 per cent in 325 progenies. Related to the number of seedlings the percentage of chlorophyll mutants was nearly 3 per cent with 15,000 r and 2.6 per cent with ethylene imine, while it amounted to 7 per cent with E.M.S.

The spectrum of chlorophyll mutants, after chemical treatment, had more common chlorophyll aberrants, while in the case of irradiation with 15,000 r more of the rarer mutant chlorophyll types and other morphological seedling mutants occurred.

With 15,000 r and with E.M.S. there was a correlation between the occurrence of chlorophyll mutants and major morphological mutant types. In both cases (406 and 325 progenies respectively) the chance that morphological mutants will appear is more than twice as great in M_2-families with chlorophyll mutants than in

families without chlorophyll mutants. In the case of ethylene imine no correlation was found, perhaps on account of the low number of progenies tested (78).

13.25. A Contribution to the Effect of Acute Gamma-Radiation of Jonathan Apple Scions. P. D. Mišić (Čačak, Yugoslavia).

In order to improve Jonathan apple, besides the method of hybridization, acute gamma-radiation of dormant scions has been used. The applied doses of gamma-rays, ranging from 1500 rep (1 hr 7 min) to 10,000 rep (7 hr 20 min), showed the different effects on scion survival, length of growth and changes of leaves and shoots.

There was no visible suppressing effect of the lowest gamma dose (1500 rep) on scion survival. The doses increasing from 3000 rep to the highest one (10,000 rep), however, showed clear suppressing effects (3000 and 4500 rep) or full lethal ones (6000 and 10,000 rep).

Total new growth per scion showed no statistically significant difference between control and 1500 rep, but the reductions in growth were significant among 1500, 3000 and 4500 rep respectively.

The induced changes such as disturbance of ortostichy and appearance of rosette, pinnately-serrate leaves, adventive shoots and bifurcation of leaf veins became more frequent when the dose increased. On the other hand, the percentages of induced dichotomy per total number of shoots, leaf chlorosis and bifurcation of leaf petiols were less frequent when the dose increased. The doses of 1500 and 3000 rep, however, produced rather high and approximately the same rates of induced dichotomy shoot growth (19.5 and 19.4 per cent respectively) per cm of total growth.

To summarize: the lower acute gamma-radiation doses applied (1500 rep in 1 hr 7 min and 3000 rep in 1 hr 13 min) showed more numerous primary effects.

13.26. Formation Process in Avena sativa provoked by the Influence of Ionizing Radiation. G. M. Zakharova and I. E. Gloushchenko (Moscow, U.S.S.R.).

It is known that under the influence of different factors which affect negatively the development of plants (low temperatures, high doses of ionizing radiation, some chemical compounds) there occur plants in the oat, progeny (*Avena sativa*) with characters of wild oats (*Avena fatua*). The results obtained by us while studying the formation process in oats show the same phenomenon.

The experimental work has been carried out with two oat varieties: Pobeda (Victory) and Dippe. They had white grains and awnless spikes (or spikes with delicate awns). The dry seeds of these varieties have been irradiated by X-rays, the doses being 8000–13,000 r. Plants obtained out of non-irradiated seeds have been used as a control.

Cytological and field investigations have shown that the chosen doses of X-rays affect negatively the development of cultured oats. By the further studies of irradiated forms of progeny it has been established that the variety Dippe in X_3 had 4.3 per cent of plants with wild oat characters and 1.4 per cent of plants with the characters of both oats and wild oats; in x_4 5.5 per cent of plants with the characters of wild oats and 0.3 per cent of plants with oats and wild oats characters.

The Pobeda in X_3 had 9.4 per cent of plants with wild oat characters and 0.2 per cent of plants with the characters of oats and wild oats; in X_4 32.7 per cent of plants with wild oat characters and 0.6 per cent with oats and wild oats characters.

The newly-formed wild oat forms as a rule are dwarfish and completely or partially sterile. It should be noted that the plants with wild oat characters had white spikes (maternal type).

It is important that the modified forms have appeared only in X_3 and in the progeny of the plants which in X_1 and X_2 had coarse curved awns. No wild oat forms have been noticed in the control nor in the irradiated plant progeny which had spikes with awns, no awns or coarse awns.

At present it has been established that ionizing radiation disturbs physiological and biochemical processes and this no doubt changes different biological properties of the organism. To all appearance x-rays in the dose of 8000–13,000 r provoke such important changes in the metabolism that awnless oat plants or plants with delicate awns give forms with coarse curved awns. The latter have labile heredity, a fact demonstrated by the presence in the progeny of such plants of great diversity. As a result of the disturbance of physiological harmony there appear in the progeny of irradiated oat plants new specific forms with the characters of wild oats.

13.27. Variability of Anthocyan in Chimera Cabbage Plants. A. S. KRUZHILIN, I. E. GLOUSHCHENKO, Z. M. SHVEDSKAJA and L. K. SOKOLOVA (Moscow, U.S.S.R.).

1. The anthocyan colour of plants is a stable hereditary character which is inherited both by sexual and vegetative reproduction. Therefore we have studied possible variability of anthocyans in intraspecific grafting of cabbage forms of different colour—i.e. in cabbage chimeras (developed from the callus) and their seed progeny.

2. It has been shown that under the influence of non-coloured plants of the stock which do not contain anthocyan, the content of anthocyan of rubobrassicin type in the coloured scion plants is considerably reduced. The analogical phenomenon is seen in the chimera sprouts. In spite of their cutting off the coloured chimera sections, the content of anthocyan is very low. The same is also true for their further vegetative propagation (by cutting) and for their sexual reproduction. Therefore the hereditary character of the plants—their colour—is changed in chimeras obtained by grafting. This shows that callus tissues of the chimeras do not exist autonomously, but interact physiologically by means of metabolism. Their properties modify in the course of seed progeny development. Therefore we believe the intraspecific cabbage chimeras to be of the vegetative hybrid type.

3. As to the anthocyan content, the coloured plants of chimera seed progeny and of sexual hybrids of the same combinations are almost alike. This indicates that the variability of plants obtained by grafting and crossing is of the same type.

4. Anthocyan is synthesized and accumulated in leaves and stalks. It is not found in the roots, even of the plants with coloured surface organs.

13.28. The Study of Intraspecific Cabbage Chimeras and Their Seed Progeny. I. E. GLOUSHCHENKO (Moscow, U.S.S.R.).

The nature of intraspecific cabbage chimeras and their seed progeny has been studied by the author during eight recent years.

It has been determined that in the progress of callus tissues forming, there take part the products of scion and stock protoplasts destroyed by the cutting. The basic character of the callus is the small-celled tissue which is formed out of cambium as a result of the trauma.

When the callus occurs at the place where two varieties belonging to the same species are joined together (at the place of the cutting), the adventive buds of hybrid nature are formed. They have different amounts of genetical characters of grafting components. The highest percentage of hybrid plants in our experiments amounted to 30.7 per cent.

It has been shown by the research that the plants obtained out of the callus tissues are young as to their stage of development and are biologically identical with those obtained out of the seeds. The taking off of the developmental processes occurs when the places of multicellular callus tissue are formed. These places are characterized by the higher basophily of the protoplasm, which, as a rule, is an indication of cell youth.

The studying of seed progeny of intraspecific cabbage chimeras has confirmed the hybrid nature of the experimental plants. When crossing of the initial components (control) produces in the first generation only the plants of one type which have intermediate inheritance of the main characters (colour and form of stalks and leaves), the chimeras of the first seed generation possess a great variety of these characters (segregation).

There has been established the following regularity: when seeds are taken directly from the grafted plants, the diversity of the progeny is very great; when seeds are taken from the chimera cuttings the variation of the progeny is not so great. However, in the latter case the variation is different from that of the crossing.

The second generation of the chimera grafted group possesses greater diversity than the first one.

When a callus occurs as a result of different species grafting and then decapitation, there are also formed adventive buds and sprouts of the chimera type. The progeny of such sprouts however have, as a rule, the characters of one species.

Consequently the nature of intraspecific cabbage chimeras which are practically one of the forms of vegetative hybridization is different from that of interspecific chimeras.

13.29. Alterations of Hereditary Traits in Solanum melongena induced by Grafts with Solanum nigrum. C. C. MATHON, M. STROUN and J. STROUN (Poitiers, France).

The pupil plant is the "white round" eggplant, and the mentor plant a strain of black nightshade.

The pupil is the epibiota and the mentor the hypobiota. Controls are represented by homografts of the pupil variety. The technical con-

ditions are as follows: for the pupil plant: of a less advanced age than the mentor-plant and total removal of leaves all along evolution; for the mentor plant: maintenance of leaves and absence of flower-buds which are cut off as soon as they appear.

The influence of the black nightshade mentor on the "white round" eggplant pupil became apparent only in the offsprings of the third generation of grafts. Out of 7 symbionts carrying fruit at the third generation of grafts, 2 offer in their descendency an alteration that so far prevailed down to F_3: the colour of the stem is slightly purple. These anthocyanins are extremely thermolabile. Chromatographic analyses made so far show the R_f of anthocyanins to be different from those found in the black nightshade.

In these interspecific grafts we did not observe any mentor-oriented alterations. However, the new traits that have become apparent in the descendency of both modified plants are of the same type, therefore, the influence of the mentor may be assumed to work in both pupils in the same direction.

Article to be published in: *Comptes rendus des séances de la Société de Biologie de France*, 1963; *Archives des Sciences de Genève*, 1963.

13.30. Alteration of Traits obtained in Solanum melongena as a Consequence of Inter-variety Grafts. M. STROUN and J. STROUN (Poitiers, France).

The "Early Violet" variety was used as mentor-plant and the "White Round" variety as pupil-plant. The mentor-epibiont / pupil-hypobiont grafting procedure is applied as follows: the pupil-plant is totally rid of its leaves all along its development, whereas the mentor-plant retains its foliage but is rid of its flower buds which are cut off. The graft is repeated on each new generation until alterations appear. Sexual crossbreeds were also made "White Round" ♀ x "Early Violet" ♂.

The 19 homografted "White Round"/"White Round" controls did not show any alterations.

The influence of the mentor-plant "Early Violet" on the pupil variety "White Round" appeared only at the third generation of grafts. Out of 24 fruit-bearing symbionts, 9 presented various alterations in fruit and stem colour, shape of fruit, or in a trait of the stamen. These alterations resemble the traits of the mentor variety. They are found again in the sexual descendants of the modified symbionts, studied so far down to F_2. They often differ from the characteristics conveyed by sexual crossings between the two varieties used. There is a disjunction in F_1, only part of the alterations being transmitted. Futhermore, new traits appear on certain plants. To all appearances therefore, grafts, as practised by the authors, seem to influence the hereditary traits of a given variety.

C. R. Acad. Sci. **255**, 561-563, 1962; *Arch. Sci. Genève*, 1963, to be published.

13.31. To a Correlation of the Objectives in Tree Breeding. C. LAZARESCU and E. SĂNDULEAC (Bucharest, Rumania).

Since trees constitute the most constant vegetable covering of the earth, the approaching of the problem of tree breeding from the point of view of timber production—as that is applied usually in forest genetics—is only a single aspect of this question.

The multilateral task of trees in nature requires a simultaneous correlation of the objectives in their breeding: (a) fast growth and higher quality of the timber; (b) resistance to damages by biotical and abiotical factors and the achievement of a stable biological balance within the biocenoze; (c) increase of the task for protection of nature (soil, sources, rivers, mineral water sources, etc.); (d) increase of their healthy and aesthetical task in the crowded centres.

We refer here as an example to the breeding of black locust in Rumania. In the best conditions (light but rich sandy soils and more than 11°C annual mean temperature), the 25-30-year-old stands of this species realize yearly mean growths up to 18 m^3 per hectare. As a first objective in the breeding of black locust cultivated in this country, we have in view to propagate the descendants of the plus trees, which give maximum growth and better properties of the wood.

Regarding the second objective, we have identified a few hybridogenous populations obtained in the cultivation (*Robinia pseudacacia* × *R. viscosa* and *R. pseudacacia* × *R. neomexicana*), which realize a more large biocenoze, due to their more marked entomophyll character and their increased resistance against frost and disease. At the same time, these hybrids are faster in growth and give a higher nectariferous production (0.0700 g bloom instead of 0.0460 g, at the 62-63 per cent concentration).

On the other hand, the black locust is frequently used in erosion control and sand fixation. The selection of the forms with a

noted capability for land improvement can also be correlated, under the climatical conditions of our country, with the objectives of timber production in this case.

The existence of many black locust cultivares may be possibly an evident increase of the aesthetical task of this species in the crowded centres, due to the great variation of their habitus, to the colour of the flowers and the different time of the blossom. At the same time, it appears possible to choose and propagate as aesthetical forms in cultivation, only these cultivares which correspond with the other specified objectives. So we have: *Robinia Decaisneana* and the hybridogenous populations *R. pseudacacia* — *R. neomexicana* and *R. pseudacacia* — *R. viscosa*.

When we analyze the objectives of breeding for each species of trees, we can see that it is generally possible to correlate the majority of the four objectives above, in order to obtain more efficiency in the tree improvement work.

13.32. Some Important Characteristics of Autotetraploids of Tree Species induced by Colchicine Treatment. SIN-KYU HYUN and CHUNG-SUK KIM (Suwon, Korea).

With a view to produce new germ plasm to be used in tree breeding as well as to obtain triploid forms which would result in vigorous growth through hybridizing diploid with tetraploid, induction of polyploidy has been conducted by means of colchicine treatment for twenty-four tree species.

In total 9300 colchiploids were produced as the result of treating seed or apical growing point with colchicine and those are now 4-7 years old.

The optimum colchicine dosage as evaluated by the frequency of stable tetraploids was not related to the optimum colchicine dosage as evaluated by the frequency of abnormal seedlings. Apparently, the physiological condition related to the development of tetraploid tissue was of greater significance than the colchicine dosage.

Some important characteristics of induced autotetraploids so far observed are as follows:

1. In all induced tetraploids, the increased stomata-size as well as the decreased stomata-frequency which were observed at their seedling age are maintaining up to the date (age of 7).

2. The frequency of gigas pollen was much higher in $4n$ plants than in the corresponding $2n$ plants.

3. The frequency of deformed pollen as well as the pollen sterility were higher in $4n$ plants than in the corresponding $2n$ plants.

4. In both *Hisbiscus syriacus* and *Robinia pseudoacacia* the elongation of pollen-tube was slower in $4n$ plants than in the corresponding $2n$ plants as observed on artificial media (with *Robinia*) as well as in the styles (with *Hisbiscus*).

5. The corolla of $4n$ plant was apparently larger in *Robinia pseudoacacia* (Gigas type) than in the corresponding $2n$ plants, but it was not so with *Hibiscus syriacus*.

6. The induced tetraploids of *Robinia pseudoacacia* were classified into four types as follows according to the forms expressed by the grown plants.

(a) Gigas type
(b) Spinless and pendulous type
(c) Brush type
(d) Dwarf type

In the wild population of $2n$ *Robinia pseudoacacia* too, the similar four types of plant form are recognizable.

In order to elucidate the relations between the forms occurring in the wild population of *Robinia pseudoacacia* and the forms acquired by chromosome doubling by colchicine treatment, karyotypic analysis of those plants are now progressing.

13.33. Notes on Some Needle Characteristics of Soft Pine Species and Hybrids. HOWARD B. KRIEBEL (Wooster, U.S.A.).

Needle characteristics of 6 species and 8 hybrids of soft pines were studied. Many of the needles were replicated samples from trees analyzed independently by other workers. Other samples were taken from arboreta in Ohio and Illinois, and from hybrids of controlled pollinations made in Ohio.

Needle characters studied included length, relative cross-sectional area, margin, position and number of rows of stomata, position of resin canals, and number of resin canals.

Needle cross-sectional area was intermediate in hybrids *P. monticola* × *griffithii* and *P. flexilis griffithii*, but not in other hybrids. The *P. monticola* × *peuce* hybrid was intermediate between the two parent species in number of needle serrulations *on the inner edge*. Needles of *P. flexilis* James had from 2 to 4 rows of dorsal stomata (in previous studies *P. flexilis* was reported to have 2 rows).

The following species and hybrids were found

to have ventral resin ducts in some needles, in addition to those reported in a recent report by other workers: *P. flexilis* (0 to 2), *P. strobus* (0-1), *P. ayacahuite* (2 to 4), *P. strobus* × *flexilis* (0-1), *P. monticola* × *P. ayacahuite* (0-1). A ventral medial resin duct was found in a needle of a *P. monticola* × *griffithii* hybrid from Placerville, California.

Comparison of results with previous work indicates that more research is necessary on all species and hybrids of pines before definitive descriptions are prepared. Evidently more sampling is needed to avoid overlooking variation. Additional analyses are also needed because of variation caused by environment, intraspecific genetic variation, or a combination of both factors.

13.34. Some Biochemical Aspects of the Phenomenon of Heterosis in Maize. T. Crăciun, A. Ciofu, M. Ivănescu, M. Ferrando, M. Bîrsan, I. Bîrnaure and M. Ionescu (Bucharest, Rumania).

The present paper was written on the basis of research work carried out by the Chair of Genetics and Plant Breeding of the "N. Bălcescu" Agricultural College of Bucharest.

In the framework of the more complex problem of the development dynamics of some characters and special features in simple and double hybrids in F_1 in connection with the nature of the crossed organisms and of hybridization, detailed research work was carried out on the chemical composition in different phases both in the parents and in the maize hybrids.

After the chemical analyses, the following was found, *inter alia:*

In the first phases of growth the general tendency was observed that the inbred lines have a higher percentage of starch-protein than the simple hybrids. The relationship starch-protein is in favour of protein, namely higher in the inbred lines and lower in the simpler hybrids. The amount starch + protein is higher in the inbred lines and lower in the simpler hybrids, while the amount of cellulose and hemicellulose is higher in the simple hybrids than in the inbred lines.

At the end of July, in the milk-dough stage, and taking in view precocity, the starch contents of the simple hybrids have a tendency to equal or to exceed the inbred lines. The protein contents decrease and the relationship starch-protein is getting favourable to starch, generally in hybrids.

13.35. The Phenomenon of Heterosis and Vitamin Concentration in Maize Plant. W. N. Stoletov, Ye. N. Odintsova and M. Shinkovitz (Moscow, U.S.S.R.).

In our investigations of the biological foundations of heterosis the simple interlinear maize hybrid and its two parent forms were used.

We observed the growth and the development (in hothouses and under field conditions) as well as the distribution of the vitamins of the B group in root, stem, leaves and reproductive organs (panicle and corncobs).

Also the leaves development and the dynamics of vitamin accumulation in them were studied (up to the stage of the appearance of panicle).

Using Odintsova's microbiological micromethods the following six vitamins of the B group were determined: meso-inositol, biotin, pantothenic acid, thiamin, pyridoxin, nicotinic acid.

As was found, the vitamins of the B group are concentrated mainly in the leaves and in the reproductive organs—in panicle and corncobs. Their total amount in these organs is very high.

The above six vitamins are present in a very small amount in the roots and in the stems. Often they are absent entirely in these organs.

Therefore the vitamin content in the leaf and in the panicle can be used as the indicator of the concentration of the vitamins of the B group in the plant.

The phenomenon of heterosis correlates to the sharply increased concentration of the vitamins of the B group in reproductive organs—panicle and corncobs. In the leaves the more high concentration of the vitamins of the B group was observed for only two vitamins—biotin and meso-inositol.

The great ability to the accumulation of the vitamins of the B group by the heterosis plant in the process of its development, i.e. the ability to the higher concentration of biocatalitical substances is one of the important factors which provides the most high accomodation by this plant to the synthesis of plastic substances, to the most powerful development of the vegetative organs.

13.36. Observations on Heterosis in Zea mays L. Helena Bańkowska (Warsaw, Poland).

Twenty-one inbred lines of maize of American origin have been intercrossed. Their F_1 exhibited heterosis. Selection and inbreeding of some of the most vigorous plants in F_2 and in succeeding generations resulted in isolating stable inbred

lines, exceeding F_1 in vigour. To study the effect of heterosis several features are analyzed: height of plant, length and width of leaves, length and number of internodes, number of days from seeding to pollen shedding. From the experimental data total sum of leaf surfaces per plant was computed.

To establish the differences between the parents, F_1 and the stable vigorous line No 10 (cross WD × W9) an experiment in randomized blocks was performed. The differences in all features are significant and statistically proved. As concerns the heterosis for earliness, it was stated that in case of mutual differences in earliness of parents less than 7 days, their F_1 exhibited pronounced heterosis for earliness. If the difference surpassed 10 days, F_1 was asymmetrically located between the parents and closer to the earlier parent. A significant positive correlation has also been observed between the number of days from seeding to pollen shedding, expressed by mean value of both parents, and the plant height of their F_1 ($r = 0.905 \pm 0.034$).

Heterosis for earliness can be explained by presence of dominant genes reducing the number of days from seeding to pollen shedding. Besides, additive genes exhibiting blending inheritance can participate in determining the time of flowering. The vigorous growth is probably due to interaction of complementary genes, which assure more intensive growing rate of longer duration in vigorous lines than in parents.

13.37. Gene Action in the Inheritance of Quantitative Traits. H. F. Robinson and R. H. Moll (Raleigh, U.S.A.).

Mathematical approaches to the study of gene action in maize have involved development of procedures and experimentally estimating the additive genetic, dominance and epistatic variances for complex characters such as yield of grain. The research of the past fifteen years has provided a basis for concluding that (1) additive genetic variance exists in open-pollinating varieties in sufficient quantity to allow for progress in yield improvement from selection; (2) overdominance is not the predominant type of action of genes concerned with yield; (3) the existance of appreciable epistasis has not been demonstrated and (4) genotype × environmental interactions are very important in quantitative genetic studies and in research on breeding methodology. The results of the extensive research program on quantitative genetics will be summarized and implications of the results will be presented. These results provide a basis for evaluation and development of breeding procedures as will be illustrated in a discussion of reciprocal recurrent selection. The relevance of the results to the explanation of the issue of heterosis will be given.

13.38. Influence of Radiation on the Combining Ability of Inbred Lines of Maize. D. L. Palenzona and R. E. Scossiroli (Pavia, Italy).

A problem which may bring more understanding on heterosis in corn, and may have important implications in practical research, concerns the possibility to change under radiation the combining ability of inbred lines of corn.

We know the consequence of this phenomenon. Some inbreds possess a general high combining ability in crosses when final yield is considered, while others show high combining ability only with specific inbreds but low combining ability with others. Is it possible to change under radiation the combining ability from high to low or vice versa? Is it possible using radiation to improve further the combining ability of an already good high combiner?

A series of inbred lines derived from an autodiploid strain were used to establish a pilot experiment to test if radiation applied to the original stock could be effective in changing its combining ability when used as pollen parent in crosses with a good combiner, the well-known inbred WF 9.

The results obtained show that radiation may change combining ability in crosses when yield of single crosses is considered.

13.39. Response to Selection in Tangüis Cotton. Jose A. Giles (Lima, Perú).

A method based on phenotypic selection was used in Peru for seven years to improve Tangüis variety (*G. barbadense*). Individual plants were selected in the field and their fiber characteristics analyzed. Selected plants were sown in progeny rows. Progenies with best appearance were chosen and put into yield trials and also propagated in increased blocks. To start a new cycle of selection, individual plants were selected from progeny rows and increased blocks.

A selection differential of the average difference in phenotypic value between selected progenies and overall progenies was obtained for number of bolls per plant, lint percent, lint index, boll

weight, and fiber strength. These data were recorded for each season only at the end of the seven year program. The results showed that some improvement was obtained in number of bolls per plant with an average increase of 4 per cent per year. There was no improvement in the other characteristics. This may be due to lack of genetic variability for these factors and due to the fact that the progenies were selected only on the basis of their field appearance. The latter may explain why the only success was in increasing number of bolls. Variability was large for number of bolls; fair for lint percent; and small for lint index, boll weight, and fiber strength. After seven years the variability remained practically constant for each character. This method of breeding showed to be inefficient for making steady improvement once there is a genetic population which has reached a certain stage of progress. Procedures that can utilize new recombinations and that can measure accurately the genetic variability are suggested.

13.40. The Average Degree of Dominance of Genes determining Components of Lint Yield in the BP52 Upland Cotton Variety. J. T. WALKER (Namulonge, Uganda).

1. Estimates of the average degree of dominance of components of lint yield of Upland cotton were obtained from a field trial based on a design by Comstock and Robinson (1952.)([1]). The F_2 populations of hybrids between inbred lines of the BP52 variety were used in backcrossing and had previously been evaluated in diallel crossing trials.

2. Only additive genetic variance was found for the traits loculi per boll and lint per seed.

3. Genotypic interaction due to specific genotypes caused dominance for seeds per loculus, seeds per boll and earliness as measured by the number of days to first flower.

4. The characters lint percentage and seed weight showed generalized genotypic interaction and apparent overdominance with transgressive segregation, the interaction variance being distributed evenly throughout the material.

5. Findings are discussed in relation to previous inferences about the inheritance of yield in cotton made as a result of selection index and diallel crossing studies. It is suggested that interaction at the genetic level may be involved in the expression of lint yield heterosis.

6. It is concluded that non-additive effects must be exploited in order to realize the ful yield potential of Upland cotton in Uganda.

1. COMSTOCK, R. E. and ROBINSON, H. F. Chapter in *Heterosis*, Iowa State College Press, Ames, Iowa.

13.41. Genetics of Internode Length in a Wide Cross of Pisum sativum L. DONALD L. MAHONEY and JULES JANICK (Lafayette, U.S.A.).

The inheritance of internode length was analyzed in reciprocal F_1, F_2, F_3, and backcross progenies of crosses between the field pea (Black-eyed Susan) and the garden pea (Wisconsin Perfection). Associative characters (total number of nodes, total height, and number of nodes to the first flower node) were also analyzed and correlated with internode length.

The F_1 exhibits positive hybrid vigor in height and average internode length significantly greater than either parent (BES—height, 156 cm, average internode length, 6.3 cm; WP—90 cm, 4.9 cm; F_1—186 cm, 8.9 cm). The total number of nodes appears to be controlled by partially dominant factors; in most cases, the F_1 lies closer to the fewer noded parent (WP). The greater height of the F_1 is therefore attributable to heterosis in internode length. Analyses indicate that the heterotic expression of internode length in the F_1 through the F_3 is best explained by the dominance rather than the overdominance hypothesis. Lines were obtained in the F_3 whose average internode length was equivalent to the F_1.

Tetraploidy decreases the average internode length in both Black-eyed Susan and Wisconsin Perfection. Fertility in tetraploid peas is very low, and crosses involving tetraploids and diploids were unsuccessful.

13.42. The Somatic Variability of Plant Clones. G. HASKELL (Dundee, Great Britain).

Variability, the capacity for variation, is a property of living matter. It may be genetic, producing observable differences between individual organisms, and somatic, embodying differences in the same individual at different life stages (Raymond Pearl).

Biometrical variability of the plant clone, a group of individuals arising vegetatively from one

parent, has been neglected. Yet many horticultural crops are reproduced vegetatively, like apples which are artificially budded on rootstocks; these might in turn influence the free variability. Strawberries are more naturally reproduced from runners. These rot ultimately, so leaving self-supporting ramets at the rooted nodes forming an asexual population. Such clones provide opportunity for assessing the variability of a vegetative reproductive system not previously analysed intensely.

Plant clones can be of all ages, and of all vegetative stages removed from the original parent. A clone may be diploid or polyploid, homozygous or heterozygous, healthy or virus infected. Some clones may even transmit plasmagenes vegetatively through successive generations. The clone is subject to the rejection or accumulation of somatic mutations of oligogenic or polygenic origins; hence they may be homosomatic or hetero-somatic. Not surprisingly a clone may show vegetative segregation.

The variability in ramet size is analysed for octoploid virus-free cultivated strawberry clones and those carrying plasmagene infection. The internal variation is examined within grafted clones of very old and new apples for the biometrical character "stamen number". These data are then assessed in the light of contemporary views on genetic homeostasis, the "c" effect, and the individual in the plant kingdom.

13.43. Genetic Variability in Isogenic Lines of Barley. C. O. Qualset and C. W. Schaller (Davis, U.S.A.).

Backcross-derived isogenic lines of Atlas barley with two segments of chromosome marked by independent genes governing the length of the lemma awn are being used to study types of quantitative gene action and the effect of the awn on yield and various other characteristics. The theoretical length of chromosome associated with each of these loci retained from the nonrecurrent awnless parent through the backcrossing procedure has been calculated to be about 5 crossover units. Several quantitative characters are being studied to assess the amount of genetic activity in these short segments of chromosome. Many workers have found that photosynthetic activity in the awn contributes significantly to final kernel weight and hence total yield. In this material the four homozygous genotypes are phenotypically distinguishable by the length and distribution of the awn. They are designated full-awned (AABB), half-awned (AAbb), quarter-awned (aaBB), and awnless (aabb).

A study has been completed in which the four genotypes were grown at five plant densities (1/6 to 6 ft^2/plant) in two years. Over both years genetic variation was found for 5 of 8 characters measured. For the quantitative characters measured the genotypes were stable over the various densities but interacted with years in 5 out of 8 cases. Significant additive genetic variability was found at the A locus for kernel weight, plant height, and spike density. At the B locus only kernel weight and percent sterility indicated additive effects. All of the genetic variance for kernel number/spike was due to interaction of the A and B loci. This interaction resulted in a significant increase in total yield of half-awned over full-awned in the first year and equal yield in the second year in spite of a significant decrease in kernel weight due to the reduced length of awn in half-awned.

13.44. The Effect of Different Treatments on the Variability of Polygenic Characters in Wheat. S. Borojević (Novi Sad, Yugoslavia).

A study has been made of the effect of mineral nutrients, rate, mode and date of planting on the variability of ear and kernel characters as well as on the yield of wheat.

The absence of fertilizers, as well as the use of nitrogen in the absence of phosphorus produced the highest variability in all the characters studied. Phosphorus in the absence of nitrogen did not affect the variability of the length of ear and number of spikelets but increased considerably the variability of the number and weight of kernels per ear. Potassium does not seem to affect the variability. The lowest variability of the characters studied and the highest yield have been obtained when high doses of nitrogen, medium ones of phosphorus and low ones of potassium were applied.

Heavier stands lead to the increase in variability of the number and weight of kernels but not of other characters. The date of planting affected the variability in a similar manner. The mode of planting did not exert any great influence on the length of ear and number of spikelets. However, larger distances between rows resulted in lower variability and higher mean values.

These findings have interesting bearing on the exploration of the genetic capactity for yield and indicate that from the increase of heritability level in the number of kernels the most may be expected.

13.45. Percent Dry Matter as an Index for the Evaluation of Individual Plants and Strains of Sugar Beets (Beta vulgaris L.). P. BERGEN (Tober, Canada).

Data will be presented which indicate that percent dry matter of the petiole is under the control of a genetic mechanism which also influences the sucrose content of the root. Percent dry matter of the petiole is positively correlated with sucrose percentage in the root and negatively correlated with root weight. The progeny of plants selected for percent dry matter of the petiole showed that significant changes had been obtained for percent dry matter of the petiole, percent sucrose, percent dry matter of the root, root weight and sucrose yield. A further experiment demonstrated that percent dry matter of the leaves and petioles of young sugar beet plants may be used to predict the potential root yield and percent sucrose of sugar beet varieties and strains.

13.46. International Sources of Resistance of Wheat Tested in Respect to the Major Physiologic Races of Puccinia recondita Rob. ex Desm. in Yugoslavia. MOMČILO BOŠKOVIĆ (Novi Sad, Yugoslavia).

In order to select the best sources of resistance to the prevalent physiologic races of *Puccinia recondita* in Yugoslavia, i.e. 6, 20 and 77, as well as some races of minor importance, such as 17 and 105, a large number of varieties and hybrids of wheat was tested for resistance in the greenhouse at the seedling stage of plants to the various races, and in field tests at the adult stage of plants. These experiments were conducted in the years 1960, 1961 and 1962.

It has been observed that the races used segregate many of the wheat varieties and hybrids with a greater or lesser degree of resistance into resistant variants of plants on the one hand and susceptible ones on the other.

From international winter wheat rust nurseries six hybrids with the highest resistance were selected, in which segregation occurs only in respect to one race in the greenhouse. A still higher degree of resistance to all the races examined, and that without segregation, was found in Chinese X *Ae.umbellulata* P-54-44-16 and B.Y., selected from international spring wheat rust nurseries. Apart from the above varieties and hybrids, some other valuable material, selected on the basis of the present investigation, has been presented in this paper in the tables. The best hybrids selected are intended for use as sources of resistance in our breeding programmes.

It has been found that many sources of resistance used in various countries show either complete susceptibility or only partial resistance when used in Yugoslavia, while only a small number of these is fully resistant to our races of the parasite.

13.47. Seedling Reaction to Leaf Rust Races of Wichita Wheat Lines with Genes for Resistance Transferred from the Differential Varieties. E. G. HEYNE and C. O. JOHNSTON (Manhattan, U.S.A.).

A series of lines of Wichita hard red winter wheat has been developed for separating physiologic races of *Puccinia recondita* Rob. ex Desm. These lines have been obtained by transferring the genes for resistance from the eight standard differential varieties. Wichita was the recurrent parent and the backcross lines available for study have reached the 4th to 6th backcross. The resistance of Transfer, which has leaf rust resistance derived from *Aegilops umbellulata*, also has been transferred to Wichita. Tests with physiologic races from eight important race groups have shown that the backcross Wichita derived lines have a similar reaction to the standard differentials. The Wichita line with the Hussar reaction gives readings more like those originally described. Apparently the Wichita background results in less variation to leaf rust infection than when the factor for resistance is in Hussar. These Wichita backcross lines have an advantage in that they all have the same growth habit and virtually are the same except for reaction to various leaf rust races. In the present material apparently only one major gene from each donor parent has been transferred. For example, the Wichita^5 × Carina and Wichita^5 × Brevit do not have the resistance of Carina or Brevit to race 9 of leaf rust. Seed of these Wichita backcross lines are available for use by other investigators.

13.48. Inheritance of Rust Resistance in Stem Rust Differential Varieties of Wheat. [1] NORMAN D. WILLIAMS and F. J. GOUGH (Fargo, U.S.A.).

The inheritance of seedling resistance to physiologic race 111 of *Puccinia graminis* f. sp. *tritici* Eriks. & Henn. was studied in eight of the stem rust differential varieties of wheat. Physio-

logic race 111 (the most widely avirulent of the described physiologic races) was used because a culture avirulent on a wide spectrum of varieties would be expected to differentiate more resistance genes than a virulent one. The F_1, F_2, F_3, backcross-F_1, and backcross-F_2 generations from crosses of resistant tetraploid varieties Acme, Arnautka, Kubanka, Mindum, Vernal, and Khapli with moderately susceptible P.I. 192334, and from crosses of resistant hexaploid varieties Marquis and Kota with susceptible Little Club were tested with a single spore culture of physiologic race 111. Two resistance genes in Kubanka and three in each of the other resistant varieties were indicated. All of the resistance genes were dominant or partially dominant. Genes for high levels of resistance commonly appeared epistatic to genes for low levels of resistance. In some cases, notably in Vernal, the resistance genes were cumulative in effect.

1. Cooperative investigations at Fargo, North Dakota, of the Crops Research Division, ARS, USDA, and the North Dakota Agricultural Experiment Station.

13.49. A Monosomic Analysis of Leaf Rust Resistance in Five Varieties of Triticum aestivum L. R. C. McGinnis and W. J. R. Boyd (Winnipeg, Canada).

Five leaf rust resistant varieties of common wheat (Africa 43, Frontana, Klein Aniversario, Klein Titan, and Sinvalocho) were crossed to all 21 different monosomics of the susceptible varieties Red Bobs and Rescue, as well as in diallel in order to investigate the inheritance of rust reaction and associate genes for resistance with specific chromosomes. Chromosome counts made from root-tip squashes of germinated F_1 seed prior to planting were used to identify monosomic and disomic plants. The F_1's were inoculated with race 9 of *Puccinia recondita* Rob. ex Desm. at both seedling and adult stages and rust reactions recorded. In the seedling stage, resistance proved to be recessive in all cases; segregation for resistance occurred in only the critical chromosome lines. It was possible, therefore, to associate genes for resistance with specific chromosomes as follows: Africa 43—chromosomes 1B and 5D; Frontana —2A and 5A; Klein Aniversario and Sinvalocho 5A, 4B and 5D; and Klein Titan—4B, 4D and 5D. Resistance was found to be dominant at the adult plant stage.

F_2 populations from monosomic F_1 plants together with disomic controls were grown and also inoculated at seedling and adult stages. The data showed that the inheritance of adult plant reaction is complex and expression of reaction is influenced greatly by environment. No satisfactory genetic hypothesis could be proposed for the different varieties on the basis of the available data. Chromosome 5A of all varieties appeared to carry a gene for adult plant resistance and is particularly important in Frontana while chromosome 6B appeared to be important to all varieties except Frontana.

13.50. Genetic Studies on Geographical Distribution of Barley Varieties with Special Reference to Uzu or Semi-brachytic Form Native to Japan. Ryuhei Takahashi (Kurashiki, Japan).

The present writer has already shown that about 80 per cent of barley acreage in Japan is now occupied by a group of varieties which possess in common a recessive mutant gene called uzu (*uz*). They are characterized by short, thick culms and compact heads with short awn, which are especially preferred by the Japanese farmers. A further study was made to gather more evidence for the prevalence of this type in Japan. For this, effects of the genes, *Uz* and *uz*, on yield and its components were compared using 24 normal and uzu isogenic paired lines. The results indicated that *uz* was slightly less favorable than *Uz* in an average genetic background. However, productivity of uzu relative to normal line was found to vary considerably with genetic background. Thus, possibility of breeding higher yielding uzu variety by selecting good genetic background was suggested. Responses of several selected isogenics to three different levels of fertility disclosed that uzu barley was adaptive to heavy manured conditions, but was inferior to the normal one at the lower levels of fertility. It may be possibly concluded that progress in barley breeding and a marked trend of heavy manuring have fostered the spread of uzu barley, replacing the formerly existing normal type varieties.

13.51. Breeding Lodging Resistant Winter Barleys from "Uzu" Parentage. John M. Poehlman (Columbia, U.S.A.).

Lodging resistant winter barleys with short,

thick culms, dark green color, dense spikes and short awns, described as semibrachytic or "uzu", possess a recessive gene that has a pleiotropic effect on the plant, shortening stature, rachis internodes, and awns. One strain, C.I. 7439 from Korea, when compared with Mo. B-475 at different fertility levels was less productive, the higher grain yield of B-475 resulting from more tillers and larger seeds. C.I. 7439 set more seeds per spike and had a straw/grain ratio of 1.46 vs. 1.88 for Mo. B-475.

When C.I. 7439 was crossed with Mo. B-475, the F_1 was tall and had lax spikes and long awns like B-475. The F_2 segregated into three phenotypic classes—tall, lax-spike, long-awns; tall, dense-spike long-awns; short, dense-spike, short-awns—with minor variations in height and spike density within each class. Segregation was conditioned by one major gene affecting spike density in addition to the "uzu" gene. Effects of the spike density and "uzu" genes on several plant characters were measured by comparing sets of partially isogenic lines derived from different F_2 plants. Plants with the recessive spike density gene had reduced plant height and seed size in addition to the reduction in rachis internode length. Plants with recessive "uzu" genes had lower grain yields, reduced height, smaller seed size, increased sterility, and later heading date than "non-uzu" counterparts. In the "uzu" types primary selection for high tiller capacity and larger seed size will be needed to obtain maximum yield. Superior winter hardiness was observed in all three phenotypic classes.

13.52. A Genetical Study of "Cold Test" Reaction in Seed Corn. Shu Ting Chang (Hong Kong).

The maize material involved in this investigation included inbred lines, single and double hybrids, and open-pollinated progenies of single hybrids, grown on the Wisconsin University farm at Madison in 1957 and 1958. Representative ears of each of these classes were harvested at successive stages of maturity, the moisture content of the kernels ranging from 60 to 18 per cent. In order to simulate preharvest frosts as they might occur in the field conditions, the ears were exposed to freezing treatments of varying intensity and duration, under controlled conditions in a specially equipped freezing cabinet.

The following conclusions can be drawn from these studies:

1. Significant differences between inbred lines and between hybrids of which they are the parents are shown to exist in respect to tolerance to freezing injury as well as to pathogenic fungi attacking theerminating seed.
2. In general, hybrids are more tolerant to frost injury effects on germination and to attack by soil-borne seed-decaying pathogens than are their parental inbred lines.
3. The ability of seed corn to resist damage to germination by freezing treatments is not necessarily correlated with inherent resistance to attack by soil-borne seed-decaying organisms. The tolerance of hybrids to freezing injury was not as strongly affected by the maternal parents as was resistance to attack by soil-borne organisms under "cold test" conditions.
4. The effect of age on freezing seed viability under "cold test" conditions is significantly greater for inbred lines than for hybrids.

13.53. Resistance to Low Temperatures in Connection with the Stages of Organogenesis of the Ear in Some Genotypes of Wheat. Mišič Todor (Novi Sad, Yugoslavia).

The winter hardiness of six genotypes of wheat in dependence on the stage of organogenesis of the ear (21, 50, 58, 70, 91, 104 and 112 days after emergence) has been examined in a field test, conducted after the split plot method in conditions with and without a snow cover, as well as in a laboratory experiment. A regularity was found to exist in the gradual increase in the percentage of winter killing from the early stages of organogenesis of the ear to the later ones, which was particularly remarkable in genotypes with low winter hardiness. The winter killing resulted in a significant decrease in grain and straw yields, 1000 kernel-weight and hectoliter weight of grain. This decrease is in accord with the percentage of winter killing in dependence on the stage of organogenesis of the ear in a given genotype.

13.54. Inheritance of Frost Resistance by Wheat. A. Popović (Kragujevac, Yugoslavia).

At the Plant Breeding Institute in Kragujevac the frost resistance of 15 F_1, 7 F_2 and 4 F_3 hybrid combinations of wheat were studied. The highest level of frost resistance was by hybrids which originated from steppe × steppe-like varieties. The combination "Minhardi × Bankuty 1201" had 100 per cent of survived

plants under cold chamber temperature —12°C for 30 hr.

By crossing steppe-like varieties with mediteranean (mainly Italian) varieties we got the hybrids with 5.0-93.3 per cent of cold resistance. Among Italian varieties San Pastore showed the highest effect on frost hardiness. The hybrids from this variety had 53.3-93.3 per cent survived plants. The cultivars like Produttore, Leonardo and Mara have lower frost resistance. Especially the cultivar Mara demonstrated a high grade of susceptibility to frost.

Italian varieties intercrossed gave the F_1 hybrids with 1.6-5.0 per cent of alive plants.

F_2 hybrids were tested under —14, —16 and —18°C in the course of 16 hr and showed different reactions up to the severeness of test. By —14°C F_2 progenies were intermediate between their parents. The last two temperatures resulted in high winter killing.

From hardier parents came more frost-resistant plants in F_3 generation. Some F_3 progenies combined the spring character with good winter hardiness.

Breeding wheats with still greater hardiness is being continued and it is expected to produce high yielding material with satisfactory frost resistance.

13.55. The Transforming of Winter Italian Wheats With Low Cold-resistance into Spring Wheats in Moscow Region. R. D. GLAVINICH (Moscow, U.S.S.R.).

In the course of 1958-1962 an experiment was carried out on changing the heredity of soft Italian wheats with low cold-resistance (30 varieties) which are characterized by their non lodging and with high agrotechnics give 40-45c on 1 hec.(1)

The work has been carried out in two directions: (1) to obtain winter wheats with high cold-resistance for Moscow region and (2) to obtain spring forms out of the same initial material.

We have used the following method of changing Italian wheats with low cold-resistance into wheats with high cold-resistance. By twofold autumn sowing (18-20 October) under the influence of spring-summer conditions we have changed winter forms with low cold-resistance into spring forms. Then by optimal sowing (25-31 August) we have transformed them into winter forms with high cold-resistance. By this method we have changed 16 varieties out of 30, but only 6 of them have become real winter forms for Moscow region (80-100 per cent of their F_3 survived winter of 1961-62).

The vegetative period of new winter forms in F_3 did not differ from the standard winter varieties and in two forms was 4-7 days shorter. The absolute weight of 1000 grains of new winter wheats was more than 40 g, the weight of the standard being 38-39g. The yield of these forms averaged 44c on 1 hec, the yield of the standard being 36-37c on 1 hec. All six winter forms were morphologically alike. Three of them (Kvaderna, Forlani and Klavatino) are the perspective forms for the Moscow region.

After three years of spring sowing (1959-1961) 9 real spring forms have been chosen out of 30. 88-98 per cent of them ripened in 1961 (spring standard variety Moscowka). The vegetative period of new spring forms was 2-5 days shorter than that of the standard. The absolute weight of 1000 grains was 1-4 g bigger than that of the standard. Some of the new spring forms (Fortunato, 1373, Forlani) are of interest for selection.

1. 1 centner (c) = 50 kg. 1 hectare (hec) = 10.000 m².

13.56. Molecular Structure of DDT Analogues in Relation to Gene Action in Barley. G. A. WIEBE (Beltsville, U.S.A.).

The insecticide DDT kills certain barley varieties and not others following foliar applications of this chemical during the seedling stage. Inheritance studies show this reaction is controlled by a single gene and that resistance is a recessive character. Biochemical tests show that DDT enters the leaf tissue of resistant and susceptible plants in approximately equal amounts. Since the reaction appears to be highly specific, tests were undertaken to determine the reaction of a series of DDT analogs to this gene. On the basis of incomplete tests of a group of 50 analogs, 8 were found that reacted similarly to DDT. In comparing the chemical composition and structure of those analogs that gave a differential kill with those that did not, one finds that the structure of the molecule is of greater importance than its composition. This finding is in line with the growing concept in molecular genetics that the specificity of gene action may be governed in part by the secondary and tertiary structure of its primary product, an enzyme. For example, in the present case, where ordinary p,p′ DDT will kill one variety of barley and not the other, the o,p′ DDT form will kill neither variety. The specific action of DDT on barley has not been determined. It

can be stated, however, that in general, those analogs showing toxicity to insects also are toxic to barley, although there are exceptions to this rule.

13.57. Outlines of the Cacao Selection and Breeding Programme in San Thome. J. C. Ascenso (Lisbon, Portugal).

The cacao breeding programme in San Thome includes single plant selection in the local population, introduction of hybrid seed and selected parents and hybridization work utilizing clones selected locally and abroad.

13.58. (D.), Evolutionary Maize Breeding. Alexander Grobman (La Molina, Lima, Perú).

Recent information has become available on evolutionary rates of improvement of maize grain yield components, through studies of archaeological and living maize ears in Peru stratified over a period of nearly 2500 years. It has been possible to establish differences in evolutionary rates of change of quantitative ear characters between "tripsacoid" and "non-tripsacoid" maize races. The former have a steeper rate of improvement of ear length, which becomes evident after the introduction of "tripsacoid" maize germplasm in certain geographic areas.

Modern breeding evidence points also to the yield enhancing effect of the introduction of tripsacoid germplasm into intervarietal hybrids of maize studied in the highland region of Peru.

Phylogenetic relationships have been established on the other hand between more than 200 races of maize in Latin America, based on morphological, genetical, cytological, and other kinds of characters. Tentative degrees of teosinte and *Tripsacum* introgression into these races have been estimated.

On the basis of all this evidence, breeding schemes may be divided in different areas of Latin America to better utilize heterosis in intervarietal and interracial hybridization as a first step in the improvement of yield of grain of maize.

13.59. Parthenogenesis in Polyploid Populations. O. J. Eigsti (Chicago, U.S.A.).

Polyhaploid individuals appear in populations supposedly all polyploid. Parthenogenesis accounts for the origin of polyhaploids. These variants cause the entire population to produce more and more seed each generation. The discovery of a parthenogen was made from self pollination of a supposedly tetraploid *Citrullus vulgaris*. Subsequent observations confirmed continuing origin of polyhaploids during each generation and the data support the conclusion that a frequency of 1 seed per thousand tetraploid carry polyhaploid embryos. Such individuals are not easily recognized in field populations. Pollen from polyhaploids produces diploid hybrids instead of triploid hybrid seed in a field where such seed production is the goal. The practical problem created must be recognized and compensation made to eliminate the ultimate and unfavorable influence upon the production of seedless watermelon fruit. Similar origins of polyhaploids among populations of *Polygonatum commutatum*, a naturally occurring polyploid species, causes confusion taxonomically within the glabrous forms that make up the *P. biflorum* complex. Propagators of tetraploid cultures and studies dealing with evolution among polyploid species must recognize the role played by parthenogenesis. More and more cases are being discovered since this discovery was made.

13.60. Cytoplasmic Replacement through Androgenesis in Maize. Sherret S. Chase (Sycamore, U.S.A.).

In maize, as in many other species, cytoplasm is transmitted by the female gamete only.

Following a suggestion of the author, the Nebraska inbred line N6 has been converted (Goodsell, S. F. *Crop Sci.* **1**, 227-228, 1961) to the "Texas-sterile" cytoplasmic phase by utilizing the rare androgenetic individuals (paternal monoploids) resulting from crossing N6 as male to a diploid "Texas-sterile" cytoplasm stock.

The present report is concerned with the attempt to increase the effective rate of androgenesis by utilizing a specially synthesized "Texas-sterile" tetraploid, Emerson-brown, marker strain as the cytoplasmic donor. Initial results indicate that crosses of inbreds to this strain do yield higher frequencies of androgenetic individuals and, even more interesting, that an appreciable portion of the androgenetic individuals are diploid.

Cytoplasmic transfer through androgenesis has the advantage over the customary backcrossing technique of leaving the chromosomal system intact.

13.61. Inheritance and Utilization of Five Dwarfs in Pearl Millet (Pennisetum glaucum) Breeding. [1] GLENN W. BURTON and JAMES C. FORTSON (Tifton, U.S.A.).

Over 39,000 parent, F_1, F_2, and backcross spaced plants of hybrids involving five dwarf and four normal inbreds (Gahi-1 lines) of pearl millet were grown in replicated randomized blocks in 1962. The dwarfs ranged from 32 to 60 in. in height, one-half to three-fourths the height of normal inbreds. Most dwarf × normal F_1 hybrids were significantly taller (up to 38 per cent) than the normal parent. F_1 hybrids between four dwarfs were as tall as normal × dwarf hybrids proving that these dwarfs carried different recessive genes for dwarfness. One dwarf × dwarf hybrid was little taller than the taller dwarf parent, suggesting that both carried the same dwarf genes. Inheritance of dwarfness appeared to be conditioned largely by one or two recessive genes. Evidence that the normal inbred in some instances introduced modifying factors was observed.

Where pearl millet is grown as a cereal, converting existing tall varieties to dwarfs can reduce lodging and facilitate harvesting. Dwarfs can be used to reduce the cost of producing hybrid seed of tall forage millets. Experience indicates that a field planted to a mixture of equal numbers of seeds of four different dwarfs will by natural crossing produce seed, 75 per cent of which will be F_1 hybrids. These hybrids will be tall, will carry hybrid vigor (up to 50 per cent), will crowd out the inbred seedlings, and will give forage yields comparable to 100 per cent hybrid seed. Cytoplasmic male-sterile dwarfs recently developed can be used as females to facilitate single-cross seed production and will give tall hybrids if normal or different dwarf males are used.

1. Cooperative investigations at Tifton, Ga., of the Crops Research Division, ARS, USDA, and the University of Georgia, College of Agriculture Experiment Station, Coastal Plain Experiment Station.

13.62. The Nature of the Spelta Gene q. MIKIO MURAMATSU (Columbia, U.S.A.).

Deficiency of the *vulgare* gene *Q* on chromosome 5A (IX) in hexaploid common wheat, *Triticum aestivum* ssp. *vulgare*, results in a phenotype called speltoid because it is very similar to ssp. *spelta*. The assumption is sometimes made that ssp. *spelta* originated as a simple deficiency for *Q*.

A line with chromosome 5A of ssp. *spelta* substituted into ssp. *vulgare* var. Chinese Spring was used for testing the effect of increased dosage of the *spelta* allele *q*. Squareheadedness was chosen as the character that most clearly distinguishes the *vulgare* from *spelta* phenotype. Five doses of *q* resulted in squareheadedness, while six doses caused even further compaction of the spikes. Evidently *q* is not a deficiency nor an amorph but is an allele which has an effect similar to that of *Q* but of a lesser degree. Relative to *Q*, *q* is a hypomorph, and five doses of *q* corresponds to two doses of *Q*. The threshold level for squareheadedness lies between four and five doses of *q*.

Being *qq*, *spelta* should be closer to *vulgare* in phenotype than is speltoid, which is deficient for the *q* locus. That the reverse is true is attributed to the effect of modifying genes. Modifiers are also assumed to account for the ambiguities of dominance that are found in the F_1 between ssp. *vulgare* and ssp. *spelta*.

Evidence has been obtained for the existence of duplicates of *q* on the homoeologous chromosomes, 5B and 5D that are similar in effect, but probably not identical, to *q*.

13.63. The Nature of Supergenes in Polyploid Wheats. D. ZOHARY, M. FELDMAN and Z. BRICK (Jerusalem, Israel).

It is proposed that supergenes differentiating between the principal types of hexaploid wheats (i.e. spelta or Q factor, sphaerococcum or S factor, compactum or C factor) are alien chromosomal segments that have been incorporated into the polyploid wheats by means of distant introgression from several genera of the *Triticinae*. Such mode of origin explains why these factors are genetically compound and why they lack homology in the respective chromosomes of the primitive wheats (i.e. show null-allelic situation). These supergenes are confined to the polyploid level since polyploidy buffered the incorporation of alien segments.

13.64. Induced Mutations at the "Q" Locus in Relation to the Phylogeny of Hexaploid Triticum Species. M. S. SWAMINATHAN (New Delhi, India).

Induced mutations leading to the loss or duplication of the free-threshing gene Q located

in the long arm of chromosome 5A were studied in sub-species *vulgare*, *compactum* and *sphaerococcum* of *T. aestivum* ($2n = 42$). In *vulgare*, the loss of Q leads to the origin of speltoid characteristics and an overdose of Q results in subcompactoid and compactoid phenotypes. Various defects in floral organs and rachilla elongation as in *vavilovi* occur only in the absence of Q. Orderly flower morphogenesis is thus one among the many traits brought about by Q. In *compactum*, the loss of Q makes the plant grow taller and the ear less dense, in addition to making the glumes tight thereby rendering threshing difficult. A phenotypic resemblance to a speltoid form of *vulgare* is thus generated, though true speltoids are obtained only when both Q and C are deleted from *compactum*. An over-dose of Q in the presence of C gives rise to hyper-compactoid ears. Similar phenotypic consequences are observed in *sphaerococcum* when Q is absent or is present in a tetrasomic condition. Thus, the absence of Q alters radically the key characteristics of sub-species *vulgare*, *compactum* and *sphaerococcum* of *T. aestivum*. Q has not so far been found to occur either spontaneously or in irradiated populations in the non-free-threshing hexaploid sub-species *spelta*, *vavilovi*, *macha* and *zhukovskyi*. If Q is added to these sub-species by crossing them with Q containing sub-species they also lose their key characteristics. Q is thus a most interesting locus from the phylogenetic angle, besides being of immense importance from the agronomic and adaptive standpoints.

13.65. (D.). Genome Manifestation of Wheat in Aegilops Cytoplasm. HITOSHI KIHARA (Misima, Japan).

In order to investigate the effect of alien cytoplasm on genome manifestation, genome complements of 4x and 6x wheat have been introduced by successive backcrosses into the cytoplasm of *Aegilops caudata* or *Ae. ovata*. The backcrosses have been carried out 14 times for the most advanced materials and only twice in the least advanced ones.

Influence of the alien plasm has been noticed in morphological and physiological wheat characters. It was most remarkable in the floral organs. When their genomes were introduced into *caudata* cytoplasm, two varieties of 6x and four of 4x wheat showed complete or incomplete pistillody and all became male sterile. Fourteen other varieties or strains became also male sterile but no pistillody occurred. Those were seven varieties of 6x wheat, three of 4x wheat, three strains of synthesized 6x wheat and one Triticale. A 6x wheat, *Triticum compactum* var. No. 44, differed from the others in being normally male fertile. In general, genome manifestation of 4x wheat in this respect was more influenced by *caudata* cytoplasm than that of 6x wheat. This was critically shown in the difference between 4x wheat (pistilloid) and the hexaploids (male sterile but not pistilloid) synthesized from the former as one of the parents.

Genomes of four 4x wheats were introduced into *ovata* cytoplasm, and all became male sterile. Pistillody did not occur in any strains.

Effect of the alien cytoplasm was also noticed in the increased occurrence of haploid and twin seedlings. The extent of the increase varied greatly with the genotype.

13.66. Mutable Alleles at the R Locus in the Soybean LEONARD F. WILLIAMS (Columbia, U.S.A.).

In the soybean (*Glycine max*), four alleles are known at the *R* locus, *R* (black seed-coat pigment), r^m (brown base with concentric black stripes), r^x (black with brown hilum), and *r* (brown). *R* and *r* have been described. r^m and r^x are in use by soybean breeders. In the USDA World Collection of soybean lines, *R* and *r* are most common, r^m is uncommon, and r^x is rare. Varieties and genetic types with these genotypes breed true, and no mutation has been reported at this locus in established lines. Dominance is complete and in the order: *R*, r^m, r^x, *r*. In certain crosses of r^m with *r*, r^x sectors appear on a few F1 and F2 plants, and a few F2 plants occur that are completely r^x. Linkage associations indicate that the mutants are derived from r^m rather than *r*. Crosses between true-breeding r^x lines and mutant r^x lines gave no segregation in F2, indicating that the mutant is identical to, or very similar to r^x. In crosses with *R*, r^m and *r*, the mutant r^x and original r^x give similar segregations in F2. However, certain crosses of mutant r^x with *R* and *r* give a small proportion of r^x plants sectored with r^m and a very small proportion of r^m plants. One mutant r^x line has been maintained for 15 generations without change, but others tend to reverse mutate to r^m. No mutants to or from *R* or *r* have been observed in these crosses. These results indicate that the mutability of r^m and r^x is intrinsic, but the degree is influenced by other genetic factors.

13.67. Mutational Tendency of Different Genes with the Same Phenotypic Expression. L. B. MAZOTI (Llavallol, Argentina).

In a maize pedigree studied throughout 20

generations, 4 mutations have been produced: vp YW (I); vp YW (II); vp YW (III); vp YW (IV) (the three last ones were spontaneous), each one conditioning the viviparous character or premature germination of the embryo. The former mutations, with the exception of the last one, are not allelemorphs. The 4 mutations behave as simple recessive genes in the various genetic environments tested.

In this pedigree apparent cases of homozygous double crossing-over appeared, (theoretically probable) without interference, once in a million cases. Mosaics in aleurone color due to instability of the A_2 gene. Progeny with necrosed grooves.

To be able to interpret the conjunction of these phenomena in closely related individuals, we believe that there are interactions between certain loci with metabolic states or with molecules of variable localization of specific mutagenic action.

The mutant genes *vp* which determined a specific mutational tendency were saved, and crossed with lines from other stocks; they behaved as simple genes of monogenic mendelian segregation. The specific mutational tendency, even only in a limited number of generations, and in exceptional lines of a species, could clear up some of the phenomena of evolution. On the other hand, families with a certain advantageous mutational tendency could probably be used in breeding objectives.

13.68. On a Spontaneous Mutation of the Cauliflower-head Type in Medicago sativa L. A. Panella, F. Lorenzetti, A. Mariani and G. M. Briganti (Perugia, Italy).

On a wide program carried out at the Plant Breeding Department, University of Perugia, Italy, regarding methods and techniques applied in alfalfa breeding, in single plant selfed progenies, the appearance of a spontaneous mutation of the cauliflower-head type was observed.

A morphological and cytological research was made on the mutant material along with a study of the hereditary behaviour in three subsequent selfed generations.

Morphological and cytological description of the mutants and data gathered on the segregating material are discussed.

13.69. A New Inhibitor of Aleurone and Plant Colour in Maize. N. K. Notani and Chandra Mouli (Bombay, India).

A new inhibitor (proposed symbol I_2) of aleurone color has been discovered in maize. Its mode of action is somewhat different from the only other known inhibitor—C^1. Whereas $C^1\,C^1$ when reciprocally crossed with homozygous colored aleurone stocks (*AA CC RR*) gives only colorless aleurone, $I_2\,I_2$ does so only when used as the female parent. In the reciprocal cross, I_2I_2 only partially inhibits pigment formation. Occasionally a sector of lighter colored kernels has also been noted on such cobs.

The I_2I_2 stock also has the property of inhibiting plant color when crossed to intense—red (*AA BB PIPI*) plants. The extent of inhibition varies from partial to complete. Comparison between the results from reciprocal crosses has shown slight differences in inhibition.

13.70. The Heterogametic Sex in Dioecious Flowering Plants. J. N. Hartshorne (Manchester, Great Britain).

Dioecism in the angiosperms has probably arisen independently on many occasions. Of the 13 species or groups of species where the sex-determining mechanism is known, all except one have heterogametic males, although the animal kingdom shows that female heterogamety is an effective alternative. There are three possible explanations for this preponderance of male heterogamety. The first is that by coincidence an excess of species of the one type happen to have been investigated. The second is that dioecism based on male heterogamety evolves more readily than the alternative. The third is that although both systems are equally likely to arise, male heterogamety confers an advantage which increases its chance of persisting.

Evidence supporting the third possibility comes from Correns's demonstration that competition between two sorts of pollen leads to an excess of females in the progeny. Since only females produce seed, and a single male can fertilize several females, this arrangement could help to ensure survival of the species. Experiments to test for Correns's certation effect, using natural agencies of pollination, are being carried out with dioecious species of *Melandrium*, *Mercurialis*, *Rumex* and *Silene*. Results so far do not confirm Correns's observations, but show that:

(a) the quantity of pollen reaching the stigma has no effect on the sex-ratio of the progeny;
(b) sex-ratios either do not deviate significantly from 1 : 1 or in some species show a significant excess of *males*.

13.71. Sex Variability of Grape Vine Hybrid Offsprings. M. NEAGU (Bucharest, Rumania).

The development of characteristics and features with interspecific vine hybrids was studied in the experiment plot for genetics and selection of the Genetics Chair, Agricultural College, Bucharest.

Sexual offsprings were used, obtained by crossing the native with foreign varieties belonging to different geographical and ecological groups (*Proles pontica* and *Proles occidentalis*). Mother varieties such as Negru moale, Cabernet Sauvignon, Pinot gris, Italian Riesling, St. Emilion, Gordan and Aligoté have normal hermaphrodite flowers.

Sex differentiation of the offsprings studied so far proved to be a developmental phenomenon, subject to the heredity of genitors historically formed during ontogenesis and phylogenesis on the one hand, and to the environmental factors on the other hand. The percentage of individuals with hermaphrodite flowers and that of individuals with female flowers deviates from the classical ratio 1 : 1.

Each hybrid combination shows different segregation ratios between individuals with hermaphrodite flowers and individuals with functional female flowers. Thus, the percentage of individuals with hermaphrodite flowers is lower (38.9 per cent) with the hybrid combination Braghină × St. Emilion, while the same percentage is higher (63.6 per cent) with Braghină and Pinot gris.

Likewise, the mother variety influences these ratios in different ways. The hermaphrodite percentage ranges between 53.5-53.7 when Negru vîrtos is mother variety, while with the Braghină combinations the percentage is highly superior, ranging between 55.9 and 63.6.

The combination Braghină × Aligoté produced an individual with male flowers as well.

The optimum growth conditions enables the blossoming of a big number of plants during the third and even during the second year after seeding. That is very important both for the grape vine selection and for rational selection of genitors, in order to obtain a high percentage of plants with normal hermaphrodite flowers.

13.72. The Effect of Certain Chromosome II Genes on Fruit Size in the Tomato. L. Butler (Oakville, Canada).

Chromosome 2 of the tomato contains the loci of 26 known genes. It also has regions which control fruit size. Two F_2's which were segregating for tall/dwarf, smooth/peach, round/pear, simple/compound inflorescence, green/suflava, and green/diluta were grown. Individual fruit weights, measurements, and locule numbers were recorded for each plant. The mean fruit weight per plant varied from 18 g to 196 g. The order of the genes on the chromosome is *d,p suf dil o s*. Analysis of these data showed that peach and diluta had no effect on fruit size. Dwarf, pear and compound reduced fruit size by 7.0,18.5, and 7.7 g respectively. Suflava, in spite of its reduced chlorophyll, increased fruit size by 11.1 g. The decrease brought about by dwarf and compound is not the result of pleiotropy. The effect of the pear locus on fruit size mediated by two factors: locule number and fruit shape. All pear-shaped fruits have 2-4 locules as a result of either the dominant pleiotropic effect of this locus, or of a closely-linked dominant gene. In these crosses there is a linear relationship between locule number and weight; each locule adds 6.5 g to the weight of an individual fruit. Pear shape also reduces weight because the same number of cells in the locule wall, if arranged in a pear shape instead of a sphere, decrease weight from 100 g to 80 g. The effect of suflava is probably pleiotropic because diluta and suflava are both mutants of Condine Red.

13.73. Leaf-Shape Differences in the Tomato. J. A. JENKINS (Berkeley, U.S.A.).

Four recessive (dwarf, potato, entire, trifoliate) and three dominant (Lanceolate, Mouse ear, Curl) genes in the tomato modify leaf shape. None of these genes has a noticeable effect either on the initiation of leaf primordia or on the structure of epidermal or mesophyll layers. They do, however, markedly alter the length-width relationships of mature leaves. Moreover, in comparison with the normal, which has odd-pinnately compound leaves with large terminal segments and three or more pairs of laterals, each of the mutant phenotypes has fewer lateral leaflets and frequently a more entire margin. Most, if not all, of these genes have pleiotropic effects. Among the progeny of hybrids produced by crossing the mutant lines with each other, two hybrid combinations (potato-entire and potato-trifoliate) were similar to heterozygous lanceolate in having entire leaves with an almost complete absence of lateral leaflets. Thus, similar phenotypes may be produced by different gene combinations. Studies of morphological, anatomical and biochemical differences between lines that differ by single genes and by combinations of these genes are in progress.

13.74. A Novel Type of Gamete Elimination in Tomatoes. CHARLES M. RICK (Davis, U.S.A.).

Unusual segregations are encountered for genes on chromosome 4 in certain mating combinations, always involving var. Gondine Red (GoR) as one parent and Pearson (Psn) or related vars. as the other. Although normal recessive F_2 ratios are obtained in other combinations, the proportion of mutants is 51 per cent for *afl*, 70 per cent for w_1, and 77-81 per cent for $w4$ when entering the cross on the Psn chromosome and 12 per cent for *di*, 10 per cent for *e*, and 0.6 per cent for *ful* when on the GoR chromosome. All F_1's have normal phenotype and are cytologically normal in all respects. In a distorted $w4$ F_2, all of 96 + segregants proved to be heterozygous and 96 per cent of the F_3 families segregated in the same abnormal fashion. The cause of the disturbance can be transmitted equally well from either sex. The CoR factor is inherited in the fashion of a single gene as ascertained from 1 normal : 1 disturbed F_2 segregations from the cross CoR/+ X $w4$ (Psn). CoR/Psn hybrids show considerable abortion of pollen and ovules, although the extent to which abortion is associated with disturbed segregation is still not clear. The data discount dominance reversal, cytoplasmic influence, and zygotic elimination as causes of the disturbance, render unlikely paramutation or other mutational phenomena, and suggest gamete elimination affecting both sexes. Although no model is entirely compatible with all observations, the best fit is provided by a gamete killer of complementary determination and with nearly complete expression. Map distances from a killer with 100 per cent penetrance would be 9-12 for $w4$, 16 for $w1$, 29 for *afl*, 7 for *ful*, 32 for *e*, and 35 for *di*. These spatial relations are in the right order and roughly approximate map distances known for *ful*, *e* and *di*.

Research partly supported by grant GM 06209 of the U.S. Public Health Service.

13.75. Spontaneous Origin of a Balanced Lethal Condition in a Synthetic Oenothera Hybrid. H. T. STINSON (Ithaca, U.S.A.).

The F_1 hybrid between the complex heterozygous circle of 14 *angustissima* strain of *Oenothera parviflora* and the 7 paired *Douhat* 4b race of *O. argillicola* forms a circle of 14 chromosomes at meiosis. The hybrid has a half lethal system; the α *parviflora* (α*p*) complex is transmitted through the egg only, whereas the *argillicola* complex (*a*), being lethal-free, can be transmitted by pollen and egg. Consequently, the circle of 14 α*pa* hybrid should yield equal numbers of α*pa* and *aa* plants on selfing. This expected behavior has been observed in numerous F_2 families, although an excess of α*pa* segregates is sometimes seen. In one pedigree history in which α*pa* individuals were continually selfed, *aa* segregates failed to appear in the F_3 through F_2 generations. Also, no *aa* segregates appeared in progenies from the crosses F_3, F_4, F_5 α*pa* ♀♀ X *argillicola* ♂♂. The reciprocal crosses (*argillicola* ♀♀ X αpa♂♂), however, yielded only *aa* segregates. The *a* complex of the true breeding α*pa* hybrid is thus apparently no longer transmitted through the egg. Substitution crosses confirm that the aberrant behavior of the α*pa* hybrid resides in the *a* complex. In F_7, the last generation grown to date, seven *aa* segregates were present among 806 offspring. Reciprocal crosses revealed these *aa* segregates to be male fertile but female sterile. This unexpected result suggests that the failure of the *a* complex to be transmitted through the egg is due to an egg lethal (incompletely penetrant) rather than to megaspore competition.

13.76. Multiple Effect of Fertilization in Floral Plants. I. M. POLYAKOV (U.S.S.R.).

The widespread conception of fertilization considers this process only as syngamy (double fertilization in floral plants). Gamete combination is considered to be of random character (with some exceptions).

Physiological and biochemical aspect of fertilization is regarded as one that has no direct effect on formation and modification of hereditary disposition of the progeny. According to this conception fertilization is nothing but a "bridge" that helps the germinal plasma to pass from one progeny to another.

There is a considerable number of facts now showing that physiology of fertilization has a direct effect on heredity, variability and vitability of progeny. The main facts are connected with finding the *effect of polyfatherhood*. This effect is expressed both in general stimulating and sometimes specific modifying action on behalf of that part of the pollen, which physiologically "participates" in the process without participating in double fertilization (in some cases the pollen may belong to other species). This fact also manifests itself in changing the character and range of offspring variability when pollination changing conditions are changed (especially

under limited pollination). In connection with the aforesaid, well-known facts of metaxenis are becoming more important.

Our trials with 15 species of floral plants (especially with *Nicotiana* and *Zea mays* species) together with the investigations carried out by other authors showed the double role of the effect of polyfatherhood.

On one side physiological "combined action" of pollen tubes maintains typical hereditary disposition, on the other side the effect of polyfatherhood through changing conditions of pollination and fertilization (change of qualitative and quantitative composition of pollen, its physiological state and so on) may lead to the change of normal heredity.

It may be also considered that syngamy is not only a simple combination of both parents' heredity, it can lead to the development of new formations as a result of metabolic interactions of two gametes.

In trials with corn it was shown that the effect of polyfatherhood concerns not only the first progeny, in some trials this effect reaching even the fourth generation. Thus, the constant interrelation of fertilization processes and genetic phenomena was deeper than it had been supposed.

In our trials we tried also to find out physiological and biochemical foundation of the effect of polyfatherhood. In 1953-1962 we recommended and used the new method for studying fertilization processes with the help of the pollen labelled with radioactive isotopes S^{35} and P^{32}. For the first time these tests were *direct* physiological evidence that pollen without taking direct part in double fertilization is active in metabolic processes of growing seeds. We have partly studied these processes and tried to give their quantitative characteristics.

The further development of metabolic theory of fertilization and the study of two most important aspects of fertilization—polyfatherhood and selectiveness will be of great significance for genetics.

13.77. New Methods in Cotton Hybridization. D. V. Ter-Avanesyan (U.S.S.R.).

Achievements in biological science reached in the last decade are well known. It should be noticed, however, that as profound is our knowledge with regard to a living cell, so poor it is in the field of biology of pollination and fertilization. The experimental data remain unexplained so far, and they are strictly empirical. Here are some interesting results of a long period of our work at the Central Asia Experimental Station of the All-Union Research Institute of Plant Breeding. There was a task to work out the method of cotton hybridization using the pollen of heterochromosomic species and even of genera of Malvaceae family.

As it is known, in the case of hybridization of *G. hirsutum* L. cotton species with *G. herbaceum* L. and *G. arboreum* L. (African-Asiatic species) having twice less chromosomes in their cells, pollen tubes of the latter, as a rule, do not fertilize even if they reach embryo sac of diploid form. At the same time the pollen of the Malvaceae family (*Hibiscus esculentus*, *H. rosa sinensis*, *H. cannabinus* and *Malva neglecta*) does not germinate on the cotton stigma at all.

But in spite of the evident genetical incompatibility of chromosomic gametes we succeeded in using them when working out two breeding methods. The first method is as follows: at the growing period of maternal cotton there were 2-3 buds left on the plant, the rest being removed. This method stimulated the accumulation of nutrients in flowers. 10-20 pollen grains were placed on the stigma of emasculated flower followed by unlimited pollination with the pollen of a paternal form (in this case of an alien species) for 3 hours.

The second method with similar results was the following: in that very evening emasculated flowers were pollinated with unlimited quantity of paternal pollen. Next day unlimited quantity of parental pollen was again placed on the stigma of the same flower, and at noon (about 20 hours after emasculation) there was pollination with the mixture of paternal and maternal pollen in different amounts.

The first and second generations from seeds developed by the described method, gave some interesting plants. The progeny considerably differed from maternal plants. It got the characters of non-crossing cotton species and changed completely its qualitative and quantitative characteristics being affected by the pollen of Malvaceae plants. These characters became hereditarily fixed, and they were transmitted to progenies. The suggested methods can be used for cotton hybridization. They will improve our possibilities of using diversified *Gossypium* genus in cotton hybridization.

13.78. The Use of Heterosis, Polyploidy and Male Sterility in Sugar Beet Breeding and Seed Growing in the U.S.S.R. N. Negovsky (U.S.S.R.).

On the ground of extensive use of modern

breeding methods (hybridization, selection, training on high-fertile soil, etc.) many sugar beet varieties have been developed and are grown in the U.S.S.R.: Uladovskaya 752, Verkhnjachskaya 031, Verkhnjachskaya 038, Ivanovskaya 1745, Lgovskaya 059, Ramonskaya 06, Ramonskaya 023, Ramonskaya 065, Bijskaya 032, Kirghizskaya 056, Pervomaiskaya 028, Mezhotnenskaya 080, and others. Home varieties of monogerm sugar beet are sown on the vast territory. They are: Byelotserkovskaya Odnosemyannaya 1, Yaltushkovskaya Odnosemyannaya 2, Kirghizskaya Odnosemyannaya 8. A number of monogerm varieties are under State Variety Trials.

In conditions of intensive development of the U.S.S.R. sugar beet growing, the development and introduction of varieties and hybrids (including monogerm) and the use of heterosis in the first generation is of prime importance.

Together with developing of diploid varieties and hybrids, the development of polyloid varieties is of great interest, the latter including a large number of triploid hybrids.

A large number of polyploid varieties developed in the U.S.S.R. are under variety trials and in the near future they will be released.

According to the data of the State Variety Trials the best monogerm triploid hybrid obtained at Byelotserkovskaya Breeding Station in 1962 overcame the diploid monogerm variety of this Station for sugar yield by 3.7-7.9 centners per hectare in different localities.

To increase the productiveness of polyploid varieties we conduct a serious task on the development and use of male sterility in the process of breeding and seed growing.

In order to reproduce and stabilize sterility zero-pollinators are selected, inzucht (inbreeding) reproduction being accompanied with regular culling of half-sterile and fertile forms.

The best hybrids developed on the ground of sterility will be used in production in the nearest future.

13.79. Influence of Ionizing Radiation on Genetic Variability of Gladiolus Seedlings. I. V. Dryagina (Moscow, U.S.S.R.).

Hybrid seeds of fifteen varieties of the gladiolus varying not only in color and shape of flowers but in geographical origin were exposed to radiation.

Dry seeds were exposed to Co^{60} before sowing.

The doses: 0.5, 2, 5, and 10kr (1) were tested. Experiments showed that gladiolus seeds are more radiation-resistant than their bulbo-tubers.

The critical dose for gladiolus bulbo-tubers (under single irradiation) is a dose of 7-8kr and with such a dose only 9-25 per cent of irradiated plants survive. Under irradiation of seeds with a dose of 10kr the germination power was 80-90 per cent, while the germination power of the control seeds was 60-70 per cent.

Most seedlings having grown from irradiated seeds began to flower much earlier than the control seedlings. Thus in the control two years after sowing, 30-50 per cent of the seedlings flowered, while the percentage of flowering in the irradiated seedlings was 75-85 per cent.

It was observed also that genetic variability in irradiated seedlings was greater than in the control.

In colouring of flowers in the control it could be observed that the colour of flowers of the paternal and maternal type or of one of them inherited most often (for example, domination of deep-red colour). Irradiated seedlings (the dose 0.5-2kr) in the first generation (γ_1) showed great diversity in colour and shape of flowers (up to 5-6 groups). Amoug irradiated seedlings was observed the appearance of shortish and dwarfish forms.

The diversity of irradiated seedlings in hybrids obtained from geographically remote forms was especially great.

Among the seedlings having grown from irradiated seeds, I, together with G. E. Kazarinov, selected much more interesting seedlings from the point of their ornamental perspective than among the control hybrids.

In 1962 we selected for further research and studies, 140 seedlings valuable for their colour and corrugated, fringed and crocus-like flowers.

(1) kr = 1000 roentgen.

13.80. Induced Mutants and Their Suitability to Hybridization Breeding in Barley. Friedrich Scholz (Gatersleben, DDR).

Experimental induction of gene mutations is a promising tool in plant breeding, particularly for specific improvements, e.g. as to lodging resistance, early maturing, and the like. No lengthy backcross procedure is necessary as unavoidable if non-adapted material is used as source of a desired character. The induced mutants are especially suitable to hybridization breeding. By crosses between various mutants many new combinations of characters can be produced. Thus several breeding objectives may be realized simultaneously.

Model experiments were performed in con-

nexion with the Gatersleben mutation work with barley. As cross parents distinct mutant lines of high yielding capacity were used, belonging to the following groups: (a) stiff-strawed erectoides, (b) early maturing, (c) naked-grained, (d) smooth-awned. If two of the characters were to be combined, e.g. a × b, isolation of the desired double recessives followed in F_2 generation. These were used for a second crossing step, e.g. (a × b) × c or (a × b) × (c × d), if three or all the four characters were to be combined. The most promising progeny lines of numerous isolated F_2 plants were tested in yield trials for several years. The yielding capacities of these combination lines on an average are at about the level of the parent mutants and original varieties. These forms are, consequently, distinguished by two, three or even four desired specific characters and sometimes by increased yields too. Each of them seems to be more usable, in at least one respect, than the original varieties or any of the parent mutants.

13.81. Inbreeding and Hybrid Studies in Marrow-stem Kale Brassica oleracea L. var. Acephala D.C. T. D. JOHNSTON (Aberystwyth, Great Britain).

In this naturally outbreeding species it was found that inbreeding causes a marked depression in yield of leaf to an average at I_3 of 50 per cent of the original population. The depression was due to decrease in leaf size and not to reduction in number per plant.

On hybridization mean yield was restored and diallel cross analysis of the data for six inbreds and their hybrids showed additivity of gene effects on leaf number with no heterosis effects, whereas leaf size showed marked heterosis.

All possible double crosses were also produced from the F_1 hybrids in an attempt to determine a means of predicting the best combinations for commercial exploitation. As would be expected from this genetic situation the number of leaves per plant could be forecast with useful accuracy from the values for constituent inbreds or F_1 hybrids. Leaf size could not however be so predicted. Although the mean leaf size of the three possible double-cross combinations obtainable from a given set of four inbreds showed a significant correlation with the mean of those inbreds, the range of values among the combinations was very large and reference to F_1 data did not indicate which permutation would be best of the three.

Thus, as no correlation was found between number of leaves per plant and their size, it appears that to obtain the highest yielding double crosses severe selection can be carried out among potential inbred parents for leaf number, but for leaf size no useful preliminary selection can be performed.

13.82. The Genetic Control of Growth Rate in Tomato. W. J. WHITTINGTON and W. E. PEAT (Sutton Bonington, Great Britain).

A diallel cross experiment was carried out involving *Lycopersicon pimpinellifolium* and several varieties of *L. esculentum* in order to investigate the genetic control of relative growth rates. One investigation followed the relative growth rates for total dry weight and leaf area in F_1 and F_2 generations; another followed growth rate for leaf number in F_1, F_2, Backcross and F_3 generations. Growth rates were calculated as b_1' and b_2' from polynomial regression equations fitted to the log measurements of the data and as initial growth rate from the exponential period of growth. The results for dry weight and leaf area were similar in that b_1' was found to be inherited with complete dominance. Although there were significant differences between crosses in values of b_2', the large environmental component of variation masked the genetic effects. There was heterosis for initial relative growth rate in the crosses involving *L. pimpinellifolium* and negative heterosis in two of the crosses between varieties of *L. esculentum*. This was probably due to gene interaction. For leaf number b_1' and initial relative growth rate were found to be inherited additively with varying degrees of dominance.

The results showed that the inheritance of growth rate could be studied successfully by the methods of biometrical genetics and also that the successive measurements of growth with time allowed a greater understanding of the genetic and physiological data obtained. The experiment continues that described previously.[1]

1. KHEIRALLA, A. I. and WHITTINGTON, W. J. Genetic analysis of growth in tomato. I. The F_1 generation. *Ann. Bot.* **26,** 489-504.

13.83. Effect of Growing Conditions of F_1 Soft Spring Wheat on Variability in F_2. T. YA. ZARUBAILO (U.S.S.R.).

There were made crosses between soft spring wheat varieties with different duration of their growing periods.

The first progeny of hybrids (F_1) from these crosses was grown in various conditions: some F_1 plants from each combination were sown in spring as usual, the rest in late fall (early winter) (on the 5th of October). The second offspring (F_2) was sown in spring and was grown in the same conditions. It appeared that growing conditions of F_1 affected the characters of F_2 plants to a considerable extent, especially their segregation (variability) according to the duration of their growing periods, especially when parental forms (varieties) greatly differed in this respect. In this case the following phenomenon was observed: when F_1 was sown in spring F_2 plants entered their heading stage simultaneously with the early-maturing parental form or earlier, and finished it simultaneously with the late-maturing parental form. Most F_2 plants had passed through their heading stage by the time the earlier parent finished it.

If F_1 was sown in late fall (early winter), the behaviour of F_2 plants was quite different. The beginning of their heading also coincided with the beginning of that of early-maturing parental forms, but when F_1 plants had already passed this stage, the number of F_2 plants with developed heads was about 20 per cent.

Most hybrid plants formed their heads simultaneously with their late parent and the considerable number of plants (about 20 per cent) formed their heads later than their parental form. This latter part of hybrid plants formed their heads for a very long time and finished it a month later than the late matured parental form. In this case only 3 per cent of plants reached their tillering stage before winter dormancy, i.e. they became winter crops.

When parental forms did not considerably differ in the duration of their growing periods or if both of them were comparatively early-maturing, early winter sowing of F_1 also led to the obviously prolonged period of heading of F_2 plants, but this period was not so long as it was in the first case. There were no winter crops here.

Growing conditions of F_1 also affected the variability of other characteristics of F_2 plants, head productiveness in particular. When F_1 was sown in early winter F_2 plants of all combinations in our experiments were characterized by higher productivity index as compared with F_1 sown in spring.

In that way growing conditions of F_1 can affect the characteristic of F_2 to a considerable extent. This can be explained by the fact that the stability (conservatism) of the heredity of F_1 plants is shattered and therefore they are easily transformed being affected by changing growing conditions.

13.84. About Breeding of Sprouting Resistant Cereals. HERBERT W. MUELLER (Bernburg/Saale, Germany).

Beginning in 1953 we crossed the resistant "Wjatka-rye" with "Petka" and "Champagner". 293 ears were artificially pollinated and tested. In F_1, sprouting was dominant. Only 2.7 per cent of the kernels were as resistant as the "Wjatka-rye". This was partially caused by the low weight of the kernels. The average was 24 per cent lighter than the average of the parents. After crossing of barley the result in F_1 was, that there are reciprocal differences between the crosses of the sprouting resistant "Neuga" and the fast sprouting sorts "Mansholts" and "Nordland". The differences are caused by the matroclin parent, probably a result of the covering layers of the kernel. The F_1 in average was equal to the better sprouting parent.

The spring wheat combination "Koga" × "Bernburg 38626" was tested with 42 ears in F_1, and sprouting was as quickly as "Koga".

In following years the combinations were tested starting with the F_2. The descendants of the F_1- kernels were examined with the Eosin-test. The reaction of the populations was not uniform, but the effectiveness of an F_2-selection was very unsatisfactory. The correlation values of the selected resistant descendants in F_2 to reaction in following generations were only:

rye $r = 0.09$	wheat $r = 0.12$
oat $r = 0.10$	barley $r = 0.12$

The following conclusions are possible:

1. The heritability of sprouting is regulated by many genes.
2. The effectiveness of a selection in F_2 is so small, that for breeding purposes there is no applicability.

The intensive testing of 10 winter barley populations on the other hand showed the possibility of negative results of F_2-selection.

Comparing the populations selected in F_3, higher correlations between the selected parents and their descendants were possible. Averaging all combinations we get following values:

winter barley	$r = +0.46$
winter wheat	$r = +0.38$
spring wheat	$r = +0.48$
oat	$r = +0.39$

The values are satisfactory for cereal breeding.

The correlations of F_4-selections were somewhat higher but there are many technical difficulties to select a greater number of populations and to test their descendants. It would be more economic to test a greater number of populations in F_3.

13.85. Breeding Drought-resistant and High-yielding Spring Wheat. V. P. KUZMIN (U.S.S.R.).

Success of breeding depends on correct determination of plants' characters according to the character of environment that forms, preserves and intensifies these characters.

Central Kazakhstan has a peculiar arid climate: long cold periods in spring, abruptly followed by hot dry weather in the beginning of summer. Rains are typical for the middle of summer, while the second period of drought and early frosts are typical of autumn.

The main sources of moisture for plants are their deep subsoil reserves and rains in the middle of summer.

Thus, to overcome drought and to make full use of moisture, wheat breeding was aimed at developing various varieties with different development and growth rate, i.e. with proper growth activity during the cold spring period (early variety type) and with desirable growth dynamics during summer rains. As for morphological characters the greatest attention was paid to the development of the root system.

The general method of work consisted of hybrids training by environmental conditions and selection in usual field conditions.

Good results were obtained from Winter and Spring wheat breeding: higher resistance to spring frosts and drought. Hybrid training included different sowing dates: very early, middle and late.

Breeding stock selected according to its ability to produce a great number of good seminal and crown roots was sown on limited fertile, moderately warm and humid soil. This soil corresponded to usual field conditions. Plant varieties developed by this method had a good root system that did not degrade but became even stronger. As a result the plant productiveness increased.

Some Spring wheats were bred by this method; they show in commercial trials the highest yielding ability and annual stability of output on the territory with peculiar arid climate.

13.86. Controlled Modification of Heredity of Non-winterhardy Varieties of Durum Wheat, Pea and Two-row Barley into Winterhardy Crops. V. F. KHITRINSKY (U.S.S.R.).

T. D. Lysenko has worked out theoretical foundations of controlled modification of plant heredity by training. In the Genetic Division of the All-Union Institute of Plant Breeding and Genetics (Odessa) we conduct experimental work on controlled modification of heredity of durum wheat, pea and two-row barley into winterhardy varieties.

1. 19 strains of winter durum wheat (*Tr. durum* Desf.) developed by training were investigated at our Institute during the competitive variety trials in 1962. The trials showed that as a result of winter training non-winterhardy wheat changed hereditarily into winterhardy one. It changed some physiological characters, including the vernalization and light stages which became the same as they are in common winter wheats. The transformed durum wheat is safely wintering in the field and endures low temperatures—up to —20 °C. It is a good yielder (40-42 centners per ha) resistant to drought, diseases and lodging and its grain is of good quality. It was sown on 4 hectares in the autumn of 1962.

2. As a result of autumn training 22 non-winterhardy *Pisum arvense* varieties (originating from the U.S.S.R., Western Europe, Asia and Africa) are purposedly transformed into winterhardy varieties. They acquired a new property-winterhardiness. They have changed the duration of growing period, habitus, colour of seed and some varieties, even the form and size of seed. Winterhardy pea is blooming and fruiting 2-3 weeks earlier than the non-winterhardy pea and the yield of green mass is considerably higher in the first case.

Two varieties of non-winterhardy *Pisum sativum* plants were transformed into a winterhardy variety by the same method.

3. Experiments on controlled modification of heredity of spring and winter crops included two-row barleys: *Hordeum distichum* L. var. *nutanus* Schübl. and var. *medicum* Körn. There were commercial varieties among them—Odessky 9, Odessky 14 and others. As a result of training we have controlled hereditary modification of morphological, biological and valuable economic characters adequate to autumn conditions. Winter and alternate forms of two-row winterhardy barley were developed from spring non-winterhardy two-row barley plants. The Odessky 17 commercial variety produced the average yield of 34 centners per hectare and 97 strains of transformed two-row barley yielded 37.3-56 centners. 130 best strains of transformed two-row barley were sown on 1.5 ha in autumn of 1962.

In conclusion it is necessary to note that using plant training in particular conditions, it is possible to modify and control their heredity developing new varieties with desirable characters.

The method of controlled transformation of spring non-winterhardy plants into winterhardy ones has become a valuable method in the Soviet plant breeding.

13.87. Genetical Differences in the Sensitivity Against 2,4-D auxin-herbicide in Tissue Culture. BÉLA FALUDI (Budapest, Hungary).

The effect of auxin-herbicide 2,4-D consists of the induction of tumorous growth in the resting meristematic cells in the stem of dicotyledonous plants. In order to study the sensitivity against the herbicide it is conducive to exclude the environmental non-genetical differences and the morphological factors of uptake and transport as well. For this purpose the tissue culture method offers a good opportunity. The proof of varietal differences apart from its theoretical significance might form the basis of the selection decreasing the sensitivity of cultivated plants against the herbicide.

In tissue cultures of potato tubers (*Solanum tuberosum*) we tested 12 varieties, and found considerable differences in the extent and character of tumorous growth.

We subjected five varieties of characteristic behaviour to a detailed biometrical investigation to examine the extent of variance among the varieties, among the individual plants within the variety and to compare it with the variance of the method applied. The investigated varieties were the following in the order of their sensitivity: Margit, Aquila, Ella, Delta, Gül Baba. The extent of multiplication of the fresh weight of the cultures amounted in two weeks time: 0.8; 1.0; 1.5; 1.6; 3.2. The variances in the F-value were for varietal differences 1041, for intravarietal differences 70, and for differences between tissues of individual tubers 83 respectively. On the basis of these data we may conclude that differences among varieties are highly significant concerning the exclusively genetically determined resistance. The variance in the same variety was not significant. These data indicate that prospects for clone selection are not favourable. On the other hand the prospects of the varietal selection are promising to obtain resistant potato varieties against the 2,4- D herbicide.

13.88. Some Vine Clones Resistant to Plasmopora. P. COUTINHO (Lisboa, Portugal).

The extent of damage caused by *Plasmopora viticola* in most regions of Portugal is very important.

This paper refers to attempts made since 1945 with regard to this subject.

The adopted criterion of selection is described.

At present we are studying 110 clones of F_2 of interest on account of their resistance.

There are another 18 clones already in an advanced stage (experimental field scale), so far the most interesting being clone *C.27*, a cross of "Jaen × Azal branco".

Finally some of their productivity and quality characteristics are mentioned.

13.89. Mechanism of the Loss of Fusarium Wilt Resistance in Hybrid Bananas. D. L. RICHARDSON and D. J. HUTCHISON (La Lima, Honduras).

In the classical method of banana breeding a *Fusarium* wilt susceptible triploid clone "Gros Michel" is used as the female parent. The majority of functional eggs produced by this clone are triploid, resulting from a meiotic restitution of the chromosome complement. Genes conferring wilt resistance are introduced through the use of wilt-resistant diploid male parents and the resulting tetraploid hybrids are screened for commercial usefulness. Many cases are now recorded where resistant segregates have been grown for years before giving rise to susceptible sub-clones, but no satisfactory explanation of this loss of resistance has been proposed. That resistance genes are found in only a single dose in these autotetraploids, presents a situation where the somatic loss of chromosomal material containing resistance genes could result in the appearance of wilt susceptible sub-clones, Cytological studies indicate that this explanation is probably correct.

13.90. Breeding of Tobacco for Resistance to Ordinary Tobacco Mosaic (Marmur tabaci Holmes). SERAPION J. BAYUBAY (Los Baños, Philippines.).

Crosses between mosaic resistant varieties Ambalema and Ky 52 (White Burley) and the susceptible but otherwise desirable varieties Marogui, Simmaba C, Vizcava and Romero were made in the Central Expt. Station, Manila, in March 1950. Including reciprocals, 16 cross combinations were obtained.

The F_2 seeds and seeds of the parental plants were planted in rows with the corresponding parents planted beside each hybrid and were inoculated with the mosaic disease by rubbing mosaic infected leaves over the surface of each of the three young top foliage of the plants. Bagged F_2 seeds from each line that had no mosaic were again planted in progeny rows the following season and F_3 plants were evaluated for their agronomic characters and degree of

resistance to the mosaic disease. F_3 seeds of the selected plants within a line were bulked.

Evaluation of resistance in the late segregating generations was made by counting the number of mosaic infected plants one month after transplanting and thereafter counting of mosaic infected plants was made every week until harvesting of leaves.

For a single entry, selection for mosaic resistance and other desirable characters from generation to generation was performed following the bulk method, assuming that continuous selfing leads to homozygosis.

Experimental Results. The F_1 hybrids of the reciprocal crosses were identical and were intermediate in most characteristics between the parents, especially with respect to height, position and shape of leaves.

In all cases the majority of the F_1 plants were very susceptible to the mosaic disease under artificial inoculation indicating that recessive factors control mosaic resistance. Selected mosaic resistant F_2 plants, assuming that one pair of factors controls resistance should have produced all resistant plants in the succeeding generations, but the fact that they did not, like the resistant parent, suggests that resistance is controlled by multiple recessive factors.

The segregating hybrids showed significant difference in their degree of resistance in all four years of testing. The hybrid lines Amsim (Ambalema × Simmaba) and Simam (Simmaba × Ambalema) were the most resistant followed by Maram type B (Marogui × Ambalema); Amar type A (Ambalema × Marogui) and Vizam type A and B (Viscava × Ambalema).

Generally, hybrid lines were significantly more resistant than the susceptible parents, and as resistant as the introduced parents.

13.91. Method of Crossing Geographically Remote Forms in Winter Wheat Breeding. P. P. Lukyanenko (U.S.S.R.).

The lasting use of the method of crossing geographically remote forms which had been first suggested by I.V. Michurin proved highly efficient in winter wheat breeding at the Krasnodar Agricultural Institute. In combination with controlled individual selection this method permitted to create regularly new varieties with the complex of necessary economic and biological characters and to raise the upper limit of the yield of the developed varieties. As a result the yielding ability of varieties doubled and during a short period rose from 21.1 up to 50.6 centners per ha.

Interspecific hybridization of selected geographically remote forms of soft wheat supplemented with repeated crosses and controlled individual selection led to the development of the new, for the North Caucasus highly productive winter wheat varietal type: Bezostaya 1 variety (Lutescens). It is one of the first Soviet intensive varieties that is distinguished among all winter wheats for its short stem, lodging-resistance, productive heads, weak susceptability to sriple and brown rust, high yielding ability and good milling and baking quality of grain.

Wheats of rather remote geographical and ecological origin which belong to varieties cultivated in various countries and continents Argentine, Italy, Japan and others) had become components of the genealogy of Bezostaya 1 variety.

Many years of the State Variety Trials showed Bezostaya 1 to be one of the most productive varieties among winter wheats in main winter wheat growing areas of the U.S.S.R. (except regions of severe climate). This variety yields over 50-60 centners per hectare. Having unprecedented yielding ability this wheat variety is characterined by high grain quality and features of "strong" wheats. Bezostaya 1 is the first winter wheat in the Soviet breeding which is widely cultivated not only in the Soviet Union but in some foreign countries (Hungary, Rumania, Bulgaria, etc.).

The report describes in brief the adopted method of breeding; the initial breeding stock; correlation coefficient that determines the main trends of breeding; the method of individual selections in hybrid populations and particularly selection for brown rust resistance. The report touches on a problem of the further rise in wheat productiveness in humid areas by means of developing varieties with still lower stems and highly productive heads. These varieties have a complex of biological characters on which their adaptability to local conditions depends. The report presents a brief survey of obtained material and perspectives of winter wheat breeding for high productiveness.

13.92. A Homozygous Heterozygote. Benjamin H. Beard (Brawley, U.S.A.).

After all recombinations have been achieved, varietal improvement by plant breeding methods would appear impossible. Generally speaking additional chromatin indicates an evolutionary advance, i.e. as the chromosome number increases the organism becomes more complicated. Drastic changes such as the addition of one or more chromosomes probably require

many generations before complete compatability is achieved. Another addition of chromatin is a duplication. Stern showed that duplication of certain genes in *Drosphila* leads to the production of a larger quantity of the substance normally produced by that gene. Wallace and Vetukhiv showed an advantage for heterozygous *Drosphila* if there is a mechanism for maintaining heterozygosity. Two alleles in a disease conditioning allelomorphic series such as found by H. H. Flor in flax and J. G. Moseman in barley may give resistance to two or more races of a parasitic organism. *A priori* reasoning leads one to believe that a duplication of a specific locus offers many possible means for improving a species. In a self-pollinating organism, a true breeding heterozygote might be possible.

Detecting an irradiation-produced duplication of a specific locus may be possible by genetic techniques. The proposed method involves irradiating crossed barley seeds and following the pollen sterility of F_1X_1 and phenotypes of F_2X_2 plants. The desired aberration, an unequal reciprocal translocation between comparable arms of homologous chromosomes should give 50 per cent pollen sterility. The progeny from such plants can be studied for segregation. Alleles showing partial dominance may express the hybrid phenotype in the homozygous duplicated condition.

13.93. On Natural Periodical Change of Inbreeding and Crossbreeding in Plants. J. P. Mirjuta (Novosibirsk, U.S.S.R.).

Many papers on selective fertilization have appeared, in a number of which it is stated that when applying a mixture of pollen from the variety itself + other varieties of the same species, the strain-population is mainly fertilized by its own pollen, whilst deep inbred lines are to a greater extent fertilized by pollen of the other varieties.

In our own experiments on corn and spinach, we compared two pollen mixtures, *viz.* (1) variety itself + other variety, and (2) plant itself [1] + other variety. The strain-population plants selected related pollen to a higher degree from mixture (2) than from mixture (1). With deep inbred lines the reverse is true. In addition it was found for deep inbred lines that selection for own line pollen is higher than selection for own plant pollen but lower than selection for initial variety pollen.

This means that in not-inbred plants, pollen selection is directed towards inbreeding (adaptation to natural inbreeding), whilst during the course of inbreeding a shift towards the opposite takes place, because deep inbred plants select unrelated pollen, that is they are directed towards crossbreeding (adaptation to natural crossbreeding).

It should be noted that natural inbreeding is widely spread even among such crosspollinators as corn, spinach and hemp. Consequently, deep inbred plants should be present in strain-populations as the result of natural inbreeding. We found that the corn variety contains 15 per cent of plants at a level of inbreeding where other variety pollen is favoured.

---

1. Or brothers

13.94. Some Biochemical and Physiological Properties of Plant Reciprocal Hybrids. S. I. Issaev and V. V. Vartapetyan (Moscow, U.S.S.R.).

Numerous investigations on morphological, physiological and biochemical properties of reciprocal hybrids of woody plants (apple-tree) as well as other plants (tomato) were carried on since 1935 in I. V. Michurin Horticulture Institute and since 1955 in Moscow State University, Chair of Genetics.

The choice of the maternal plant often determines the difference in properties of the hybrid generation derived from the same pair of initial forms.

Initial varieties influence more actively the expression of properties of the hybrid generation, when they are used in the initial pair as maternal plant.

Hence it was demonstrated, that crossing of frostresistent (northern varieties) and frost-sensitive (southern varieties) of apple-trees produced more frost-resistant hybrid F_1, in the case where the northern variety was used as maternal plant.

Content of sugar and vitamin C in fruits of reciprocal tomato hybrids was higher in the case, where the variety with high level of these substances was taken as maternal plant. The choice of the maternal plant influenced also the degree of heterosis. Heterosis of a certain property increased, when the maternal plant of the pair was characterized by the same property.

Special experiments were carried out with grafts and the reciprocal crosses on these. These experiments showed that additional influence of the maternal plant on the properties of the progeny could be explained mainly by metabolic action of plant plastic substances on the embryo, which developed on this plant.

The obtained data could be valuable in a scientific as well as in a practical sense, namely for selection and seed-growing.

13.95. Effect of Genetic Albinism on the Photosynthetic Utilization of Light. ISTVÁN GYURJÁN (Budapest, Hungary).

The efficiency of photosynthesis in plants, and also the distribution of $^{14}CO_2$ over the different compounds are greatly influenced by the intensity and the wavelength of light. Photosynthetically, light affects in the first line the amount of pigments which, in its turn, influences the extent to which carbon dioxide is incorporated, and influences moreover the further part of carbon and oxygen.

The assimilation of $^{14}CO_2$ by albino maize seedlings, particularly sensitive to light, has been studied at different intensities of light. The activity of the alcohol-soluble fractions (amino acids, organic acids, sugars) revealed a linear connection with the concentration of pigment at the employed intensities of light (5, 100, 1000 and 10,000 luxes). The rate at which $^{14}CO_2$ was incorporated by alcohol-soluble compounds in the range of optimum light intensity for albino (from 100 to 1000 luxes) proved to be much higher in albino than in normal plants. The rate of incorporation became lower in strong light (10,000 luxes) on account of the destruction of pigment in the albino leaves.

Qualitative and quantitative analysis of autoradiograms made in respect of the alcohol-soluble fractions revealed the fact that activity of the organic acids became higher in albino leaves, while the activity of amino acids characteristic of photosynthesis (alanine, glycine, serine, aspartic acid) as well as that of sugars appeared to be higher in normal leaves. This difference was wider at higher intensities of light. The qualitative differences make it probable that deviations in the light tolerance of normal and albino leaves affect the mechanism through which $^{14}CO_2$ is incorporated. This manifests itself in the fact that—apart from affecting the amount of pigments—light does not uniformly influence the different ways of carbon-dioxide incorporation.

13.96. Different Types of Carotenoid Abnormalities in Albino Leaves. ÁGNES FALUDI-DÁNIEL (Budapest, Hungary).

A considerable part of chloroplast mutants of higher plants possess the ability to synthesize chlorophyll. These mutants have the genetic block elsewhere in the metabolic pattern. Investigating the carotenoid content and synthesis of albino leaves in maize and barley we found abnormalities characteristic of individual chloroplast mutants.

We have shown that leucine is an effective early precursor of carotenoid synthesis of the leaves during chloroplast formation. Consequently albinos with abnormal leucine content might have disturbances in the carotenoid synthesis. Another type of albinos contains carotenoids with partially saturated double bonds, lacking the ionon ring too (phytoen, phytofluen, Z-carotene). A further type of chloroplast mutants produces carotenoids with normally conjugated double bonds but the process of ionon cyclization is blocked (lycopene).

The qualitative and quantitative deviations in the carotenoid content are related to an increased lability of the protein-pigment complex of the chloroplasts. It was found that the lack of the ionon ring decreases the amount of stable carotenoid-protein complexes by about 30 per cent. If the lack of ionon ring is associated with the unsaturation of double bonds the amount of stable protein pigment complexes decreases by 45 per cent below the normal level.

On the basis of our data we are able to set up a hypothetical scheme concerning the localization of genetic blocks in the albino mutants with deviating carotenoid content.

13.97. Variability and Inheritance of the Protein Level in Plants. S. BARBACKI (Poznań, Poland).

Research of several years' duration into the variability and inheritance of the protein level in barley and lupin has proved that this character changes its value in dependence on the following factors: climatic conditions (in main rainfall), soil conditions, term of sowing, spacing, manuring (in main with nitrogenous fertilizers) and time of harvest. The genetic background is also important as it determines the metabolic type.

The protein level cannot be studied independently from the environmental factors and other qualities of the plant. The type of development, earlier or later ripening, the accumulation of other compounds—in the first place of carbohydrates—are all of importance in this case. In considering these elements it is possible to distinguish genotypes greatly differing by their reaction to the environmental conditions, carrying a tendency to accumulate lesser or greater amounts of protein. Crossing of various

genotypes brings about different kinds of segregations in the offspring which result among others in the arising of transgressive forms.

The elements of "crude protein" are also peculiar to the genotypes. Various forms of nitrogen are differently represented in them. Owing to the particular importance of the level of certain forms of nitrogen, in main of exogenous amino acids, in the nutrition of man and animals, it may be of practical value to study the tendency of their accumulation.

13.98. Biochemical Interspecific Differences within the Genus Lathyrus. J. PRZYBYLSKA (Poznań, Poland).

Within the genus *Lathyrus* there were found clear interspecific differences in the composition of free amino acids in seeds. Individual species are characteristic of an accumulation of different peculiar new free amino acids (α, γ-diaminobutyric acid, homoarginine, β-(-2-aminopirymidine-4-yl) alanine and other substances with amino acid properties unidentified as yet) which suggests the existence of different pathways of nitrogen metabolism. It can be assumed that the amino acids accumulated in seeds and peculiar to the individual species are not metabolic by-products; they are rather forms of storage, or storage and transport of nitrogen. These compounds are sure to have originated as a result of genetically conditioned changes in the activity of enzymes involved in the amino acid transformations. At the present moment comparative studies are being carried out on the role of individual peculiar non-protein amino acids in the nitrogen metabolism of several species of *Lathyrus*.

13.99. Genetical and Morphological Analysis of the Regularity of Right- and Left-handedness in Wheats. HINAKO SUEMOTO (Kyoto, Japan).

Right-handed and left-handed leaves (direction of folding) and spikelets (direction of first floret) alternate regularly (are "concordant") along stem and spike. "Discordant" leaves and spikelets are often found below the 3rd or 4th foliage leaf and above the 10th spikelet.

The degree of regularity or the intensity of polarity *at a given position* is measured by the "Concordance proportion" (C). Concordant and discordant leaves or spikes are scored 1 and 0 respectively. The mean per position for a number of shoots is C, with binomial standard error. Averaging over positions one obtains $\bar{c}$, the mean concordance proportion, which *a.o.* serves as a measure of species differences in R/L-handedness.

Each species of *Triticum* and *Aegilops* has its own specific value of $\bar{c}$ and the specific curve of C. A certain relation between $\bar{c}$-value and the genome constitution is found. The results of the polygenic analysis on two Einkorn species (*T. monococcum* var. *flavescens* and *T. aegilopoides* var. *boeoticum*) support the view that a polygenic system takes part in the species difference of $\bar{c}$-value.

The R/L-polarity is induced at about the time of germination, and is maintained throughout the developmental stages and disappears, according to the genotype of the plant. But the type of c-curve and the direction of the polarity is modified by the various artificial treatments. The deficient nutrition and X-ray irradiation causes a reduction of $\bar{c}$-values of developing organs. The gravity affects the induction of the R/L-polarity at the time of germination. This sensitivity is specific of the genotype. Furthermore, growth-hormone treatment modifies the R/L-polarity induced by the gravity.

These results will be reported in this paper.

13.100. Pathogenicity of Radiation-induced Mutants in Two Important Phytopathogenes of Rice. YOSHITO YAMASAKI, TAKASHI SUWA and NOBUO MURATA (Tokyo, Japan).

Nutritional deficiency mutations were induced by u v X-, and γ-irradiation in *Xanthomonas oryzae* and *Piricularia oryzae*, pathogens causing bacterial leaf blight and blast respectively in rice, and changes in pathogenicity of mutants were investigated with the view to make clear the significance of mutations for nutritional characteristics in specialization in pathogenicity in each of these organisms.

In *Xanthomonas*, mutants requiring histidine (4), nicotinic acid (2), and tryptophan (2 out of 6) were as pathogenic as wild strains whereas those requiring arginine (2), leucine (2), isoleucine and valine (4), threonine (1), and tryptophan (4 out of 6) showed a marked decrease in pathogenicity.

In *Piricularia*, mutants with requirement of NH_4-nitrogen (6), glutamic acid, etc. (1), glycine (1), leucine (2), methionine (1), cystine or methionine (1), cystine, methionine or thiosulfate (1 out of 2), histidine (1) tryptophan or nicotinic acid (1), nicotinic acid (1), inositol (1), and choline (1) remained pathogenic and those with requirement of phenylalanine (2), adenine

or hypoxanthine (2), and cystine methionine or thiosulfate (1 out of 2) proved to be of decreased pathogenicity.

In the latter organism, restoration of pathogenicity by supplementing infected tissue with the nutrient required was observed in one of adenineless strains and a close correlation between the nutritional characters and the loss of pathogenicity was confirmed, though trials with the other adenineless and the two phenylalanineless mutants have been unsuccessful, suggesting other mutations for decreased pathogenicity operating in these strains.

Experiments are under way to induce revertants with respect to the nutritional deficiency and observe whether they restore the pathogenicity.

13.101. Initial and Adaptive Tolerance of Ustilago scitaminea and U. maydis on Various Concentrations of Sodium Arsenate. ELISA HIRSCHHORN (Llavallol, Argentina).

102 monosporidial cultures of *U. scitaminea* and 70 of *U. maydis*, from different origins, were tested in various concentrations of sodium arsenate, in order to determine the limits of initial tolerance and the limits of adaptability to increased concentrations.

U. scintaminea. The initial tolerance ranged from 1.1 to 3.5 mg/ml. The following results are obtained for initial tolerance and adaptive tolerance (number of strains between brackets). 1.1→1.1 (15, no adaptation); up to 2.2→3.0-4.8 (62); up to 1.75→4.8 (16); up to 3.5→7.0-8.0 (9). Adaptation was transitory, as found after 4-7 retransfers on arsenate-free medium. Tetrad analysis showed for initial tolerance (tolerance: no tolerance) 0 : 4 and 2 : 2. The acquired tolerance, however, was not transmitted sexually.

U. maydis. Initial tolerance ranged from 4.0 to 10.0 mg/ml. All 70 strains first adopted to 20.0 and then 59 strains to 50.0 and 11 strains to 65.0. Transitory adaptation was found in 60 strains, though only after 20-25 retransfers on arsenate-free medium. However, 6 strains which adapted to 65.0, did not loose adaptation during 4 years.

Tetrad analysis showed for initial tolerance (tolerance: no tolerance) 4 : 0, 2 : 2, 3 : 1 and 1 : 3. Sexual transmission of acquired tolerance was found in 2 strains (adapted to 20.0 mg/ml), with tetrads (tolerance: no tolerance) 0 : 4, 2 : 2, 3 : 1 and 1 : 3. The nature of adaptability is considered.

13.102. (D.) Changing of Tomato Heredity under the Influence of Light and Low Temperatures. L. RASTUNKOVA and R. GLAVINICH (Moscow, U.S.S.R.).

Under the simultaneous influence of light and low temperatures we have succeeded in changing the heredity of the late Moscow region tomato variety No. 7, created by R. Glavinich by means of vegetative hybridization, as well as of the middle variety The Best. The two varieties have round red tasty fruit, but in Moscow conditions often give many green fruit. The temperatures of the experiment have been + 2 and —5 and the day length 10 hours. The germinated seeds have been kept in low temperature conditions for the time from 6 to 10 days.

The influence of light and low temperatures on the tomato plants of these varieties decreased the vegetative period of the experimental plants as compared to the control plants as well as to the plants of the standard variety Gruntovoj Gribovskij. Beside that, the experimental plants had different fruit size to compare with the control ones. The experimental fruits have become smaller but more numerous. Some of the experimental plants had changed colour of the fruit, as well as their form. They were elongated and of crimson colour.

All these acquired characters have been inherited by the second and the third generations and some of the plants of No. 7 and The Best varieties can be used as good seed material.

13.103. Ionizing Radiation in the Evaluation of the Genetical Constitution of Some Characteristics in Plants. A. TAVČAR (Zagreb, Yugoslavia).

1. From genus hybrid (*Triticum aestivum* × *Secale cereale*) × *Triticum aestivum* a homozygous *Tr. aestivum* ($2n = 42$ chromosomes with hairless upper internodium of the stem was selected. In R_2 gen. from seed irradiated with 5000 to 7500 r of gamma-rays, some speltoids with hairy upper internodium, similar to the male parent *Secale cereale*, have developed. During 8 generations of nonirradiated seed only *Tr. aestivum* of hairless upper stem were observed.

2. In (*Vicia Faba maior* × *V. F. minor*) homozygous *V. F. maior* and *V. F. minor* plants were selected. Some of their seeds were irradiated with gamma-rays. From their *V. F. maior* seed also some *V. F. minor* and from their *V. F. minor* some *V. F. maior* mutants were selected. From seeds of the parents used for hybridization after irradiation no changes from *V. F. maior* to *V. F. minor* or vice versa were observed.

3. In F_2 of *Vigna sinensis* hybrids: black × white seed coat, segregated plants with (a) black, (b) white, (c) black with small brown dots, (d) brown with large white sector seed coat. Through selection homozygous plants with the mentioned seed coat colours were obtained. After irradiation of black seed of parental genotype in R_2, somatic segregation of the same seed colours as in F_2 hybrids, plus some reddish seeds ocurred. From the irradiated white seed of the parental genotype only plants with white seed coat have developed. The parental genotype of white seed is recessive for the mentioned characteristics.

4. In year 1927 a natural mutation of decussated position of leaves and branches on tassels was first observed in one maize inbred. Now, between many dozens of genetically very different inbreds of *Zea mays* which have developed from seed irradiated with gamma-rays only in one inbred, a plant with decussated position of leaves and branches on tassel has developed. The mentioned inbred line has probably genetical ability for the development of the mentioned characteristics.

5. From seed of inbreds irradiated with 2000 and 3000 r the pachytene chromosomes in R_2 were spread through the whole cell and this facilitates the study of their structure in comparison with the nonirradiated genotypes where the pachytene chromosomes are clustered round the nucleolus.

Conclusion: From the mentioned examples it is possible to conclude that ionizing radiation can be very helpful in evaluation of the genetical constitution of some characteristics in plants.

13.104. Induced Biochemical Mutants in Corn. B. ANDOR (Gödöllö, Hungary).

In 1958 an open pollinated variety (Fk) and different lines have been irradiated at dosage rates of 10 and 15 kr according to the usual methods.

Sensitivity to radiation proved to be different: most resistant was the previous heat-treated (aneuploid) material. Most sensitive were several strains with high combining ability. Analysing the variability of protein content in the progeny of controlled ears, it ranged from 8.2-13.2 per cent. The mean values of the X_2 plots of lines showed a variation range from 8.3 to 16.1 per cent. In the X_3 generation we succeeded in obtain forms with hereditarily higher protein contents from 6 lines (2.2-3.9 per cent). Germination tests performed on the strains in the laboratory under exposure to low temperature (8°C) proved that the stock is suitable for the breeding of cold-resistant corn varieties.

13.105. Inheritance of Alkaloids in Interspecific Crosses in Lupinus. EDMUND NOWACKI (Poznań, Poland).

Among the fully successful interspecific crosses of lupines, e.g. *L. arboreus* × *L. polyphyllus*, *L. arboreus* × *L. argenteus*, *L. hartwegi* × *L. elegans*, *L. mutabilis* × *L. ornatus* and other species, the inheritance of alkaloids was studied. In the mentioned species there occur the following main alkaloids: sparteine (I), lupanine (II), and hydroxylupanine (III). The aim of this work was to investigate the genetical and metabolical relation shipof alkaloids. There was also breeding of alkaloidless fodder plants and on the other hand high sparteine-containing winter hardy plants for pharmaceutical purposes.

The observed segregation in progeny of hybrids between I and II containing plants was of a type that could be explained only when assuming the conversion of I to II. Those results were confirmed by feeding of labeled alkaloids.

13.106. The Culture of the Eggs of Plants. G. W. R. WALKER and H. F. DIETRICH (Edmonton, Canada).

The microslide culture of unfertilized eggs dissected from the ovaries at floral induration into hanging drops suspended in liquid paraffin has been used with *Hordeum sativum*, *Tradescantia paludosa* and *Hilium tigrinum*. Readily identifiable spherical eggs are obtained from *H. sativum* but no success has been achieved with the other species. The effects of chemical additives to the culture medium, including kinetin and ATP, have been studied by continuous observation under phase-contrast. Progressive changes following kinetin administration are interpreted by the authors, in the light of data from anther-culture as probable manifestations of kinetin-induced cell division. The administration of ATP results in a rapid and coarse vacuolization of the egg.

13.107. Breeding Aspects of Embryo Size Variations in Wheat. A. FASOULAS (Thessaloniki, Greece).

The significance of embryo size variation in wheat was studied within varieties, among

varieties and to a lesser extent among species.

In order to evaluate the importance of embryo size variations within varieties, it was necessary to eliminate the influence of endosperm using two different approaches. The first was to isolate large and small embryos, place them on a suitable culture medium and look for differences among the derived plants. The second was to compare plants derived from seeds of the same weight which however had embryos of different sizes. This last approach became possible after a careful study of the means by which embryo varies in relation to the endosperm at different places in the spike.

It was found that kernels of the same weight have not necessarily the same size of embryo. This depends mainly on the position of the kernel in the spike and on the variety.

The differences among large and small embryos consist primarily of differences in the number or degree of development of basic primordia, namely, those of the seminal roots, of the three first leaves and of the tiller at the base of the coleoptile.

Large embryos in comparison to small embryos gave plants with greater (a) number and length of seminal roots, (b) number and length of the first leaves, (c) number of tillers, (d) weight of green matter, and (e) number of spikelets per spike.

Varieties show differences in the size of embryo. Varieties with comparatively large embryos were found to have a greater competative ability in mixtures, a greater number of seminal roots, and a greater tillering capacity.

The tendency was for varieties with relatively large embryos to fit better on soils of lower fertility and lower water-holding capacity. The opposite was true for varieties with small embryos. This tendency is also supported by the observation that *Triticum durum* wheats which were found to have on the average embryos of greater size than *Triticum vulgare* wheats show better adaptability in drier regions.

In this work are discussed in detail the bearings of the results on the breeding of wheat, as well as the importance of endosperm in relation to the biological significance of double fertilization.

13.108. (D.) Pictorial Technique in the Genetical Analysis of Horticultural Crops in Scotland. A. B. Wills (Dundee, Great Britain).

Habit is a fundamental characteristic of plants which is partially dependent on the internode pattern of the vegetative shoot and inflorescence. The ideogram method of Anderson and Schregardus allows a graphical comparison of variation in internode patterns and so provides a partial analysis of habit. The method is adaptable and can be used for the comparison of genotypes within species or genera. Diagrams and preserved plant material will demonstrate the method used at Mylnefield in the analysis of tomatoes and *Ribes* species, with particular emphasis on adaptation in these crops.

Evidence will be presented that tomatoes of determinate growth, with either shorter internodes or fewer internodes, probably have evolved from determinate types. Further evolution has given rise to dwarf varieties with fewer, shorter internodes and dwarf bushy types in which apical dominance is suppressed.

The selection of suitable material and construction of the ideograms of woody perennials present special problems. The methods devised to solve these problems in the genus *Ribes* will be illustrated. It is believed that differences between ideograms of black currant varieties reflect adaptation to different environments, whereas the heavy pruning practised on red currants leads to similar ideograms in many varieties.

13.109. Inheritance of Awnedness and Glume Colour in Some New Vulgare Wheat Varieties. A. A. Omar, A. K. A. Selim and S. H. Hassanein (Cairo, Egypt).

1. The inheritance of awnedness was studied on the F_2 and F_3 generations of eighteen crosses including nine varieties. This character was found to be affected by three pairs of genes. The gene B_1 for apically awnleted, B_2 for short awned, the combinations B_1, B_2 for awnless and the recessive genes b_1, b_2 for fully awned type. The gene A was suggested as a promoter for tips and awns and its recessive allele (a) has no effect. The interaction of these genes gives rise to new types as B_1-B_2-A- for awnless beak, B_1-b_2b_2A- for long apically awnleted approaching awnleted and $b_1b_1B_2$-A- for short awned approaching fully awned.

2. The parental varieties were arranged in three classes: awnless, apically awnleted and fully awned. The awnless class includes the variety Ramona with the genes B_1B_2A. The awnleted class includes the varieties N.A.101 and hatcher with genes B_1b_2a. The fully awned classes involve the varieties Giza 144, Giza 145, Giza 147, Giza 139, Lee and Mida with the genes b_1b_2a.

3. The inheritance of glume colour was studied on the F_2 and F_3 or eight crosses including six

varieties. This character appeared to be governed by two pairs of genes. The gene Gc_1 for brown colour, the gene Gc_2 for yellow colour, and the combination Gc_1Gc_2 for light brown colour, while the recessive genes gc_1gc_2 give white colour.

4. The parental varieties were arranged in four classes, brown, light brown, yellow and white. The brown class includes the variety Ramona with the genes Gc_1 gc_2, the light brown class contains the variety N.A. 101 with the genes Gc_1Gc_2 and the yellow class involves the variety Giza 139 with the genes gc_1Gc_2. The white class includes the varieties Giza 144, Giza 145 and Giza 147 with the recessive genes gc_1gc_2.

SECTION 14

ANIMAL GENETICS AND BREEDING

14.1. A Possible Case of the Genetic Assimilation of Behaviour. NEVILLE MORAY, KEVIN CONNALLY and PAUL ARNOLD (London and Sheffield, Great Britain).

In 1961 Clutterbuck and Beardmore reported that *Drosophila melanogaster* reared on media containing peppermint oil, to which they were aversive, became less aversive to the adulterant over several generations.

In a replication of this experiment with further controls a stock of wild-type *Drosophila melanogaster* were artificially selected for aversion or non-aversion to peppermint. These two groups and two unselected groups were divided into two and reared either on normal food or on food adulterated with peppermint oil. The offspring from each group were tested at each generation for their aversion to peppermint. In the artificially selected groups on normal food the degree of aversion showed only a very slight upward trend, the unselected control remaining almost constant. The line "Unselected on peppermint" also showed little change.

In the lines which had been subjected both to artificial and natural selection results were striking. Non-aversive flies reared on peppermint (artificial and natural selection in co-operation) showed a marked decrease in aversion, resulting in the almost complete extinction of the line. Aversive flies reared on peppermint (artificial and natural selection in opposition) also became *less* aversive over six generations.

When this line was released at F_6 onto normal food, the degree of aversion remained the same as those flies still left on peppermint. We suggest that this effect bears similarities to the genetic assimilation of a character which does not have a threshold, as discussed by Waddington.

14.2. Genetic Transmission of Alcohol Preference in Mice. JOHN L. FULLER (Bar Harbor, U.S.A.).

Four inbred strains of mice and 6 interstrain F_1 hybrids were given access to 6 different concentrations of alcohol over a 6-day period. A measure of preference was obtained by adding 1 (for elimination of negative values) to the logarithm of the alcohol concentration corresponding to the midpoint of an animal's total accumulated fluid intake from all solutions. Homogeneity of variance was demonstrated in the group scores. No significant sex differences were found. The mean scores of the pure strains were: C57BL/6J, 1.74; C3HeB/FeJ, 1.61; A/J, 1.39; and DBA/2J, 1.08. The C57BL/6J and DBA/2J strains had significant general combining effects in their hybrids. However, specific combining effects were greater indicating the importance of non-allelic interactions in the determination of the phenotype of the hybrids. The variation between the hybrids in genetic control of alcohol preference (or aversion) suggests a similar variability in its physiological basis.

14.3. Factors Affecting Mating Competition in Mice. L. LEVINE (New York, U.S.A.).

Albino males of strain, ST/Jax, were paired with pigmented males of strain, CBA/Jax, and placed in cages with single albino females, St/Jax. The albino males fathered 83 per cent of the offspring. Cage size does not appear to affect the results. When pigmented females, CBA/Jax, were used, the albino males fathered 70 per cent of the offspring.

Single albino males were taken at birth and reared with pigmented litters. At weaning, each albino male was paired with one of its foster brothers, and both were placed in a cage with a single albino female. The albino males fathered 93 per cent of the offspring. When single pigmented males were fostered to albino litters and treated as above, the albino males fathered 50 per cent of the offspring.

Albino and pigmented males were fought in round-robin fashion. In two separate series, eleven matched males from each strain were used. In the first series, the fights were concluded at the end of the first submission or after thirty minutes if no submission occurred. In the second series, the fights were observed for thirty minutes regardless of number of submissions. In the first series, the initial fights included 5 albino victories, 3 pigmented victories, and 3 no-victories. In the second series, the initial fights included 6 albino victories, 3 pigmented victories, and 2 no-victories. Thereafter in both series, the pig-

mented males won no less than 9 fights per session and eventually won all 11 fights each time.

Supported by NSF Grant GB-312.

14.4. Behavioral Effects of Single Genes in Mice: an Investigation with the Use of Ethological Methods. J. H. F. v. ABEELEN (Nijmegen, The Netherlands).

Until now screenings of known genes and their influences on behaviour have been carried out only to a very limited extent. The same holds for the appliance of ethological methods in behaviour genetics, i.e. the drawing up of ethograms. In the present study a combination of these two approaches was attempted. For this purpose the behaviour of the mice was analyzed in a few tens of behavioral elements that can be seen performed by a solitary male in an observation cage, several special elements by two males placed together, and a number of elements by one male and one female placed together. The screened alleles were: yellow, pink-eyed dilution and jerker. Environmental factors were equalized for all subjects as far as possible. Frequencies of the defined elements performed by mutants were compared with those from non-mutants from the same strains. Also qualitative comparisons were made.

In spite of the endocrinological effects attributed to the yellow allele no evidence was found for a behavioral effect of this allele.

Homozygotes for pink-eyed dilution showed significantly less staring at observer and lifting one paw (intention-reconnoitering) and perhaps less reconnoitering also; more grooming and shaking the fur are indicated. Thus these animals show an autistic type of behaviour, probably because of a more or less impaired vision. Other explanations are possible but must remain open at this time.

Qualitatively the behaviour of the jerkers differed in many respects from normal behaviour. Quantitatively: feeding, fighting and sexual behaviour were disturbed the least, exploratory and comfort behaviour very clearly.

By the use of ethograms, strain differences may be discovered quickly. Full details will be published in *Genetica*.

14.5. The Ways of Reorganization of the Reproductive Function in Seasonly Reproducing Mammals. D. K. BELAJEV and L. N. TRUT (Novosibirsk, U.S.S.R.).

This work is devoted to the investigation of the evolutionary mechanisms of reorganization of the reproductive function in mammals from monoestrousness to polyestrousness.

It has been shown that the selection of the silver foxes (*Vulpes fulvus* Desm) kept under farm conditions for mating at the beginning of the breeding season has no effect and cannot change the time and the number of reproductive cycles during the year. The heritability of the mating time in these animals during the breeding season is very low. Nevertheless, some reorganization of the reproductive function has taken place in the evolution of domestic animals; the majority of modern forms has lost its monoestrousness and the strictness of seasonal reproduction which is characteristic of their ancestors; they became polyestrous. The causes which called forth such a hereditary reorganization of a stabilized function have not been revealed.

In our investigation we have shown that in regulation of the reproduction time of foxes the cerebral cortex is implicated. Its properties, in particular, are manifested in the type of the defensive behaviour of animals; the foxes with calm type of behaviour mate earlier in the breeding season than the aggressive ones and their litter sizes are significantly higher. This correlation is not only phenotypical but genotypical as well. The differences in the type of behaviour of animals are based on the genotype and it gives the possibility to select animals according to this character. Ten year's selection carried on by the authors for receiving foxes of calm type of behaviour called forth in some females the reorganization of the reproductive cycle: there were recorded obvious signs of estrus in 4 females out of the breeding season—in September and October. During the breeding season (February) these females were mated and gave litters, but their estrus was prolonged up to 18-20 days instead of 5-6 days as it must be usually. In females selected for calm type of behaviour some changes of the fur structure were noted as well.

The change of a character with law heritability became possible by selecting a correlated character which regulates the main selected character. The reorganization of the reproductive function realized in the evolution of domestic animals was probably a result of unintentional selection of animals "for domestication".

14.6. Parity and Mendelian Segregation. L. C. STRONG and F. N. JOHNSON (Springville, U.S.A.).

Luxoid has been reported in several species.

In 1956, Strong and Hardy reported on a new luxoid condition in mice. It arose in a mouse of the F_5 after crossing a polydactylous mutant of the Brpb subline, of the NHO/St strain, to C57/St. (This condition has been designated first.) The condition, according to Strong and Hardy, was similar, but differed in some respects, from previously reported luxoids. In 1962, Forsthoefel reported that there were, in fact, differences between Strong's luxoid and those reported by Carter and by Green.

The present communication will introduce a concept of parity and its effects on the segregation of genetic traits. Mice were bred throughout their reproductive life with some having as many as thirteen litters. Points were plotted for each parity for these traits: percent having normal feet, percent having either poly on one hind foot (Poly 1) or on both hind feet (Poly 2), percent having some form of luxoid, and percent of total defective mice. It was learned that the percentage of animals having some defect did not change with increased parity. However, if the percent of mice with some form of luxoid and percent of mice with Poly 1 or 2, were compared with parity, trends were apparent. To get a straight line regression was applied giving a positive slope to the luxoid curve and a negative slope to the polydactylous curve. To determine the significance of the curve, a test was applied to the regression coefficient. A positive coefficient of the luxoid slope ($r = 0.895$; d.f.-11) was significant at the <0.01 level and the negative coefficient of the polydactylous slope ($r = -0.792$; d.f. -11) was also significant at the <0.01 level. Thus, it is concluded that increased parity does not effect the total number of defects but does increase the severity of the morphological defects.

14.7. Two Unusual Mutations Affecting Pigmentation in the Mouse. H. Glenn-Wolfe (Bar Harbor, U.S.A.).

A mutation to pink-eyed dilute (*p'*) that occurred in the Production colony of C57BL/6J has been maintained continuously on the same inbred background by cross-intercross to C57BL/6J. Breeding tests established its allelism, but not its identity with existing pink-eyed dilute (*p*). An unusual feature is the sporadic occurrence of mosaic coat-color patterns in mice of genotype *p'p'*. These vary from a few dark hairs at only one place on the body to heavily mottled animals. Eye color in these same mottled animals ranges from pink eye to full color, and often shows bilateral asymmetry. Mosaics have produced wild type progeny in addition to mosaic and normal pink-eyed dilute progeny when test-crossed to non-mottled *pp* or *p'p'* animals. In outcrosses of C57BL/6-*p'p'* × 129/Re-*pp*, deviants varied from light mottled to wild type (8 observed in approximately 3000 classified). Besides effects on pigmentation, *p'* has been shown to affect the superovulation rate.

Five mutant animals, borne in two different litters, appeared in the cross C57BL/6J ♀ × C57BL/6J-Mi^{wh} + ♂. Mutants are pale yellow with white spots. Breeding tests showed the mutant gene to be located at the microphthalmia locus and yellow-spotted animals to be Mi^{wh} mi^{sp}. An unusual feature of mi^{sp} in heterozygous (mi^{sp}+) or homozygous ($mi^{sp}mi^{sp}$) form is the absence of any visible expression distinguishing it from wild type. Allelic interaction between mi^{sp} and Mi^{wh} or *mi* is being investigated, particularly effects on pigment granule size and distribution in selected hair types.

14.8. Inheritance of Leucocyte Counts in Rats. W. G. Downs (Cockeville, U.S.A.).

In an effort to obtain a strain of rats having a consistent leucocyte count, studies have been conducted on the method of inheritance of these counts. Animals were from our own strain of Wistar × Sprague-Dawley, out of a Wistar male crossed to four Sprague-Dawley females, then very closely inbred for fifteen or more generations.

Originally, total leucocyte counts varied between 6800/mm^3 and 17,800/mm^3. Initially, sixteen crosses were made between animals with a very low count and those with a very high count. Of the 207 of these offspring, 192 had counts between 7900/mm^3 and 15,700/mm^3. Of these latter animals, 16 crosses were made, and 197 offspring resulted. Of these 178 varied between 9000/mm^3 and 14,200/mm^3.

In three successive generations 16 crosses were made, in each instance discarding approximately the 10 per cent of animals at higher and lower extremes. The last, or fifth generation, resulted in 190 offspring whose leucocyte count varied between extremes of 9600/mm^3 and 10,900/mm^3, being fairly evenly distributed between these figures, though with a tendency to concentrate around a mean of 10,400/mm^3. Considering the inherent error in blood-cell counting, these figures are believed to have attained the initial objective. As the limits of the total count narrowed, so did the differential, as between lymphocytes and granulocytes, the fifth generation having a ratio of 67 per cent

lymphocytes to 30 per cent granulocytes 5.

A polygenic type of inheritance is hypothesized with not less than four, and possibly more, pairs of factors.

14.9. Adrenocortical Variation in Mus musculus. F. M. Badr and S. G. Spickett (Cambridge, Great Britain).

Study of the adrenal cortex of the house mouse reveals wide variation in the ratio of adrenal weight to body weight both between long standing inbred strains and between laboratory bred wild strains. This variation is, in part, the expression of variation in adreno-cortical zonation. Different strains show variation in the levels of circulating corticosteroids and in the levels of excreted steroid metabolites. This is due to variation in adrenocortical synthetic activity as determined by *in vitro* incubation, and in the $\Delta 4$ hydrogenase activity of the liver, and adrenal cortex.

Studies of hybrids indicates that this variation is, in large measure, of genetic origin.

14.10. Genetic Studies on the Resistance of Polyoma induced Mouse Tumour Cells to Polyoma Virus Infection. David Gershon and Leo Sachs (Rehovoth, Israel).

Resistance to challenge infection with polyoma virus (PV) was studied in 88 tissue culture clones from PV induced mouse tumors, to determine whether there exists in these mammalian cells any similarity to the immunity to superinfection found in lysogenic bacteria. There was no spontaneous virus production by any of these clones. Cloning of two resistant uncloned mass populations yielded about 50 per cent susceptible clones, while cloning of two susceptible clones yielded 7 and 37 per cent resistant clones. A segregation of resistant from susceptible clones was observed through two cycles of recloning. Although the resistant uncloned populations segregated susceptible clones in the first cloning cycle, second cycle cloning of resistant clones yielded only resistant clones. In contrast to lysogenic bacteria, susceptible cells thus segregated back to resistance in the absence of new virus infection. Treatment of two resistant clones with acridine orange did not render them susceptible. Both resistant and susceptible clones produced tumors in mice, and possessed the PV induced cell antigen. There was no apparent correlation between differences in the chromosome number of the tumor cells and their response to challenge infection. The results will be discussed in relation to the possible mechanisms that determine resistance and susceptibility of PV induced tumor cells to PV challenge infection.

14.11. Selection and a Maternal Effect. Nigel Bateman (Edinburgh, Great Britain).

Individual, family and within-family selections are often regarded as alternative methods, merely differing in efficiency, for selecting the same genes for improving the genotypes of individuals. The possibility that family selection especially, and to a lesser extent individual selection, could give qualitatively different results from within-family selection by procuring heritable maternal effects semes to have been mostly overlooked. The importance of such effects for 5-week weight in the mouse is described.

Both sexes were selected on individual 5-week weight. After ten generations heavy mice weighed 34 g and light mice weighed 17 g. Their difference amounted to 32 per cent of the accumulated selection differential. Differences between offspring of the worst individuals and the selected lines averaged 39 per cent of selection. Presumably on account of maternal effects, reciprocal hybrids differed by 43 per cent of the weight difference between parental lines. The responses of the selected lines and of offspring of the worst individuals could be accounted for if one-quarter of the maternal effect stemmed from maternal 5-week weight and maternal genotype (selected through family differences which constituted two-thirds of the superiority of selected individuals) determined the remainder.

At the beginning of the experiment a simple 1: 2: 3 relationship obtained between weights at 3, 5 and 9 weeks of age. Unexpectedly, the selection on 5-week weight alone left this relationship unchanged in both selected lines though the hybrids did not conform. In fact, the inherent growth curves for the selected lines had changed, but were masked by changes in the maternal effects.

14.12. The Spermatozoa of Mouse-Strains selected for Body Weight. R. A. Beatty (Edinburgh, Great Britain).

When n strains of mice are crossed in all possible ways, the n^2 types of progeny fall into

a table, with (say) the rows, M, representing the *n* kinds of male parent, and the columns, F, the female parents. Analysis of variance gives mean squares for the M, F and MF interaction effects. Alternatively, after adding the M and MF interaction effects. Alternatively, after adding the M and F marginal totals (strain by strain) a mean square *a* for "additive variation" (parental effects averaged over sex) is obtainable; after a corresponding subtraction of the totals, the differences can yield another mean square *c* for "maternal effects" (differences between strains when used as male parent and as female parent [1]. The MF interaction is further divisible into "mean dominance," etc. In a diallele cross (material of D.S. Falconer and R. C. Roberts) of four large strains of mice selected for high body weight, spermatozoan dimensions studied were: head area in optical projection; maximum head breadth; midpiece length. There was much significant additive variation, with a tendency towards a greater *male parent* effect than female parent effect. The (log) *within-male variance* in spermatozoan dimensions showed significant *female parent* effects and non-significant male parent effects. Difference between parental effects were sometimes reflected as significant "maternal effects". No significance was attached to "mean dominance" or any other items within the MF interaction. In a diallele cross of three small strains of mice there were no genetic differences in spermatozoan dimensions. There was no correlated response to selection; spermatozoan dimensions of small strains in general were similar to those of large strains. A detailed paper will be submitted to *Genetical Research* (Camb.)

1. See B. I. HAYMAN, *Biometrics* **10**, 235-244, 1954.

14.13. (D) Genetics of Reproductive Mechanisms and of Color Variations in the Freshwater Planarian Dugesia lugubris. M. BENAZZI and LENTATI G. BENAZZI (Pisa, Italy).

Dugesia lugubris presents a diploid amphimictic biotype and some polyploid pseudogamous ones, namely: a triploid biotype with ameiotic oöcytes, and a triploid biotype with hexaploid meiotic oöcytes; the female germ line of the last biotype becomes hexaploid through a chromosome doubling. The male germ line is always diploid with normal meiosis in all biotypes.

The genetic background of chromosome cycle of the above mentioned biotypes was experimentally analyzed by crossing amphimictic diploid specimens, used as females, with pseudogamous polyploid ones. The results obtained have shown that asynapsis, chromosome doubling in female line and pseudogamy may be transmitted through the sperm, and therefore must be considered as controlled by chromosomal genes. However, the inheritance of such characters is very peculiar, since the oöcytes may be both synaptic and asynaptic, diploid and polyploid, amphimictic and pseudogamous in the same hybrid: this variable expression of the characters is not easily framed in formal genetics.

Starting from diploid, asynaptic and amphimictic F_1 it was possible, through repeated crosses or backcrosses, to obtain the 2nd, 3rd and 4th generations, which are triploid, tetraploid and pentaploid, respectively.

Moreover such experimentally produced polyploids allow the study of the genetics of the pigmentation by crossing differently colored specimens (white and dark respectively). A gradual increase or decrease of the color intensity occurs at each successive generation, related to the increase or decrease of the chromosome sets derived either from the white or the dark parent. This result postulates a cumulative action of the color factors, which can be quantitatively analyzed.

14.14. Linkage Map of Sex-chromosomes in the Fish, Oryzias latipes. TOKI-O YAMAMOTO (Nagoya, Japan).

Artificial control of sex differentiation as advanced in *Oryzias*, where normal sex-determining mechanism is XX=♀ and XY=♂, renders it possible to obtain estrogen-induced XY ♀♀ *ad libitum*. Of 57 sons of induced X^rY^R♀ × X^rY^R ♂, where *R* stands for orange-red colour and *r* for its recessive allele, only two proved to be Y^RY^R. Y^RY^r males, however, are as viable as normal males. All evidence points to the supposition that there is an inert section (–) in the regular Y^R and a "viable" section (—) in the ordinary X^r as well as Y^r.

The linear order in the normal male is (×) 0.2 *r*— (+) in the X^r or (y) 0.2 ^{R}— (—) in the regular Y and that in the induced X^rY^R female is (×) 1.0 *r*— (+) or (y) 1.0 ^{R}— (—), where (x) and (y) stand for non-homologous sex-differentials and the numerals represent observed recombination values. To estimate recombination value of the interval *r*— (+) or *R*— (—), the sum of all fractions relevant to viable Y^RY^R of induced X^rY^R♀ × X^rY^R♂ is taken as the numerator and the sum of all fractions of

viable orange-red (*R*) sons as the denominator and the ratio is equalled to 2/57. The recombination fraction of the interval *r*— (+) in the induced X^rY^R♀ is assumed to be five times as high as that in the normal X^rY^R♂ as that in the interval (×) —*r*. The established linkage map of Y^R in the normal male is (y) 0.2 *R* 1.2 (—) while that in the induced estrogen-induced X^rY^R♀ is (y) 1.0 *R* 6.0 (—). A full account will be published in *Genetics* (U.S.A.) in the near future.

A strain has been fixed for extra-abdominal legs in some 30 per cent of the flies, but the character does not segregate after outcrossing.

The coiling direction of the hypopigium and the orientation of the asymmetrical sclerites completing the male terminalia seem to be pleiotropic effects of the same gene and independent developmental processes.

Close inbreeding leads quickly to sterility.

14.15. (D.) Formal Genetics of the Housefly.
M. G. Rubini-Franco (Pavia, Italy).

The complement of the housefly (*Musca domestica* L.) is $n=6$; somatic pairing is generally very good in metaphasic plates from supra-aesophageal ganglia; colcemide can easily be supplied with good results both to larvae and to adults by ingestion or to larvae by injection.

Variability of paleo- and neo-arctic populations shows great similarities in abdominal pigmentation and load of minor wing venation abnormalities, all having incomplete penetrance and variable expressivity.

Complex mutant phenotypes with lethal effects are common; they are of little value for formal genetics, requiring cumbersome breeding methods; good mutants have allowed identification of the five autosomal linkage groups. The complement includes big X Chr.; however no diaginic mutant has been found so far. The loci of the II linkage group show occasionally holandric inheritance (flies from Australia and Florida); cytological evidence of two distinct Y-chromosomes has been obtained.

Dominant mutants are rare and doubtful, apart from some genes for insecticide resistance, giving intermediate hybrids.

Three not allelic recessive genes causing similar yellow eyes are known mutant larvae developing with normal ones and can result in various pinkeyed flies.

Genetically determined eyelessness or deformed eyes are fairly common, but can hardly be fixed in true-breeding strains; narrow Bar-like eyes are recurrent in some strains but their inheritance is still obscure.

Among mutants affecting appendages differentiation special mention is deserved by (1) *aristapedia* which turns the arista into tarsal segments and generally reduces bristles; (2) *antennapedia*, which is irregularly dominant and of erratic expressivity and (3) *tarsi-fusi* (fused tarsi).

14.16. (D.) The Inheritance of Insecticide-Resistance.
M. T. Lanna (Pavia, Italy).

The response of houseflies to DDT can be measured either by the time required for the onset of well-defined symptoms of intoxication (knockdown) or by the probability of survival to dosages directly applied to the fly (topical application).

The first approach is adopted when samples are confined into containers having the walls coated or impregnated by the toxicant and the knock down times are recorded; the second approach is followed when dosage/ response regression lines are needed; either methods has its shortcomings and its points in favour for genetical research.

Tolerance levels for DDT generally show great variability within populations, and response to selection is prompt and speedy.

Three different genes are known, all of the II linkage group, which independently cause DDT-resistance; all provide clear F_2-segregations and in the following generations behave according to expectation of monofactoriality.

Genetical research based on the study of knockdown times or on that of dosage/mortality correlations have provided parallel results, but the evaluation of dominance differs somehow.

Dieldrin resistance is usually measured by topical application and has been found widely distributed among laboratory strains of various origin, some of which can positively be assumed as never having had any experience of dieldrin.

Hybrids from susceptible and resistant flies are intermediate; the F_2s give clear indications of simple inheritance, with free recombination with linkage groups II and V on which genes for DDT- and for OP- resistance respectively are located.

Free recombination of Dieldrin resistance occurs also with IV Chr. markers.

14.17. The Genetics of Insect Resistance to Insecticides. R. MILANI (Pavia, Italy).

About seventy independent strains of resistant insects belonging to twenty species have already been studied genetically.

Toxicological and genetical studies agree indicating specificity of resistance mechanisms for groups of chemically related toxicants and, to some extent, within groups.

Most of the information on inheritance comes from resistant strains developed under pest-control condition rather than under controlled selective pressure on laboratory populations. For many species the studies on the inheritance of resistance are the only property genetically analyzed.

Resistance and susceptibility generally are discrete properties, inherited as simple genetic differences; resistance is almost always dominant or partly dominant on susceptibility, as would be expected for a property selected through survival to sudden extreme adverse factors. The very few instances of recessive resistance known to the present author through the literature refer to forms of resistance developed to toxicants different from those which have been exerting pressure and genetically independent from the resistance primarily caused by selection.

Selective pressure with Organophosphorous (OP) insecticides on the housefly and on mosquitoes appears to cause resistance also to DDT; it has been shown for a strain of flies and one of *Culex tarsalis* that independent genes are involved. Other instances of multiple resistance have been genetically resolved in their components in various species. Linkage relations of various genes for resistance and genetic markers are on records for *Musca domestica*, *Aëdes aegypty*, *Anopheles albimanus*, various *Drosophila* species, *Blattella germanica*.

High dechlorinating activity is always joined by DDT-resistance, both properties being jointly inherited as a simple mendelian character.

Low-aliesterase activity is often coupled with OP-resistance in the housefly; they are then inherited as a unit.

Cholinesterase activity is inherited jointly with DDT-resistance in *Culex tarsalis*, but, like aliesterase activity, independently from malathion resistance; in the same species carboxyesterase activity and malathion resistance have been inherited jointly.

The full text has been accepted for publication in a special issue of the Bulletin of the World Health Organization.

14.18. Artificial Allotetraploids (2n Bombyx mori L. + 2n B. mandarina Moore) in Silkworm and Their Bisexual Reproduction during Three Successive Generations. B. L. ASTAUROV and V. N. VEREISKAYA (U.S.S.R.).

As reported earlier (*Proc. X Inter. Congr. Genet.*, 1958), by crossing mixoploid parthenogenetic females ($3n + 6n$) with normal $2n$-males autotetraploids could be easily obtained in the silkworm *B. mori* L. Females $4n$ display normal fertility, whereas autotetraploid males are sterile. Repeated attempts to obtain a bisexual autotetraploid race failed. The usual presence of several tetra- or polyvalents in $4n$-males meiosis suggests the aneuploidy of spermatozoa to be a cause of sterility. Consequently amphidiploids produced by crossing domestic *B. mori* L. ($n = 28$) with wild *B. mandarina* Moore (Ussury and Shanghai races, $n = 28$) could be expected to be fertile. 220 crosses of partheno-females $4n$-*mori* × ♂ $2n$ *mandarina* gave many offspring $3n$ ($2n$ *mori* + $1n$ *mandarina*) of both sexes. Analysis of 595 males proved their and their sisters triploidy. By heat activation of unfertilized ova extracted from 532 $3n$-females many parthenogenetic $3n$-daughters were produced in 52.9 per cent of batches. Percentage of hatching made 6.4 per batch, reaching 63.0 per cent. A portion of parthenofemales was expected to be mixoploids $3n+6n$. After reduction of $6n$-oocytes ($4n$ *mori* + $2n$ *mandarina*) in case of preferential homologous pairing, $3n$-pronuclei ($2n$ *mori* + $1n$ *mandarina*) must be formed. When fertilized with *mandarina* haplosperm, amphidiploid ($2n$ *mori* + $2n$ *mandarina*) progeny of both sexes is expected. 1049 crosses of hybrid partheno-females $3n$ and $\frac{^{3}n}{_{6}n}$ × ♂ $2n$ *mandarina* were performed and 1439 larvae obtained. Percentage of batches with hatched larvae (17.9) and total percentage of hatching (0.36) are close to those observed in matings of partheno-females $3n$ *mori* × ♂ $2n$ *mori*, yielding autotetraploids. Sex ratio in 235 V-instar caterpillars was 1.00♀ : 1.05♂. Cytogenetican alysis proved their tetraploidy and presumably—amphidiploidy. Phenotypically they resemble $2n$ *mori* × *mandarina* F_1-hybrids. 103 males were repeatedly mated and 51 of them (49.5 per cent) proved to be partially fertile. Hatching percentage fluctuates from 0 to 35.8 per batch, total one being 1.1 per cent. Poor fertility indicates the probable segmental allotetraploidy. Up to 1963 three successive generations of allotetraploids were reared: 1°—primary "amphidiploids" (A_1); 2°—"amphidiploids" A_2 and R_1—hybrids from back crosses ♀$4n$ *mori* × ♂ $4n$ "*amphi*"; 3°—progeny of

♀ 4*n* R_1 × ♂ 4*n* R_1 and of ♀ 4*n mori* × ♂ 4*n* R_1 crosses. Presently we have 274 batches of the 4th allotetraploid generation, a part of which is expected to yield larvae in spring. In all, from 1353 matings 2263 allotetraploid larvae were produced; 452 were reared up to Imago stage. In 314 crosses 4*n* × 4*n* percentage of hatching fluctuates from 0 to 39.5 per batch; total hatching making 1.0 per cent 54.3 of males proved to be partially fertile. In all generations sex ratio remains close to the normal one.

To our knowledge, it is the first case of bisexual reproduction of artificial allotetraploids in animals.

14.19. The Present Status of the Formal Genetics of Tribolium castaneum, T. confusum, Latheticus oryzae and Gnathocerus cornutus (Tenebrionidae). ALEXANDER SOKOLOFF (Berkeley, U.S.A.).

Linkages established for *castaneum*: Chromosome I (=X) from left to right in the order given: spotted elytra (*sp*); lethals 2 and 4 (^{12}, ^{14}); divergent elytra (*dve*); lethal-3 (^{13}); pygmy (*py*); miniature-appendaged (*ma*); red eye (*r*); paddle antennae (*pd*); truncated elytra (*te*); red-modifier (M^r); lethal-1 (^{11}). II: pearl eye (*p*); pink eye (p^{pk}); pegleg (*pg*). III: black (*b*); light ocular diaphragm (*lod*). IV: Bar eye (*Be*); sooty (*s*); deformed legs (*dfl*). V: microcephalic (*mc*); jet (*j*); split (*spl*). VI: Microphthalmic (*Mo*). VII: blistered elytra (*ble*); chestnut eye (*c*); curved appendages (*ca*); fused tarsi and antennae (*Fta*); short antenna (*Sa*). VIII-X: Vacant.

Unassigned: cut prothorax (*cp*) and juvenile urogomphi (*ju*) (which are linked to each other); fused antennal segments 1-3 (*fas*-1, *fas*-2, *fas*-3); abbreviated appendages (*aa*); squint (*sq*); extra urogomphi (*eu*); prothoraxless (*ptl*); engraved metasternum (*em*); incomplete mesosternum (*ms*); warped elytra (*we*); pointed elytra (*pe*); short elytra (*sh*); dent (*dt*).

For *confusum:* Chromosome I (X): Striped (*St*); eyespot (*es*); labiopedia (*lp*); reduced antennae and elytra (*rae*); lethal-1. II: pearl (*p*); ebony-2 (*e*-2); pegleg (*pg*). III: black. V: ebony. VI;I blistered. IV, VI, VII, IX to be filled with following: short elytra (*sh*); light ocular diaphragm (*lod*); ruby spot (*rus*); ruby (*Ru*); dirty pearl eye (*dpe*); chestnut (*c*); stilted legs (*stl*); engraved metasternum (*em*); dent (*dt*); warped elytra (*we*); split (*sp*); melanotic stink glands (*mgs*); deformed legs (*dl*); fused antennal segments (*fas*).

For *L. oryzae:* I (X): red (*r*); truncated elytra (*te*). II: pearl. Unassigned: brown body color (*bwb*).

For *G. cornutus*: II: pearl; III: light ocular diaphragm (*lod*).

14.20. Genetic Correlation and Asymmetry of the Correlated Response from Selection for Increased Body Weight of Tribolium in Two Environments. A. EARL BELL and H.W. MCNARY (Lafayette, U.S.A.).

Genetic parameters needed for predicting direct and correlated responses from selection for increased body weight (pupa stage) of *Tribolium castaneum* in each of two environments (70 per cent versus 40 per cent relative humidity) were estimated in a random mating Base Population. Heritabilities of body weight in the two environments were not significantly different (0.58 in Wet and 0.55 in Dry), but the phenotypic variation in Dry was approximately twice that in the Wet Environment. The genetic correlation between the two traits was estimated as +0.98. The predicted direct and correlated responses in both environments were checked in a replicated selection experiment spanning nine generations.

Good agreement between predicted and observed direct response was obtained for each of the four selected populations. Also, the average observed correlated response including both environments was accurately predicted (Predicted/observed = 147.5/141.5 µg and 139.5/138.5 µg per generation for Replication 1 and 2, respectively). Yet the observed correlated response in each replication for the population selected in Dry was approximately twice that predicted, while those observed in the population selected in Wet were only half of the predicted values.

When the *realized genetic correlation*, $\bar{r}g$, for each population was calculated from the observed heritabilities, direct and correlated responses and phenotypic variances [1], it was concluded that the observed asymmetry of correlate response was due to the effective genetic correlations being different for the two environments ($\bar{r}g$ = +0.64 and +0.67 for the two replications of selection in Wet and +0.93 and +1.12 for selection in Dry).

Supported by Grant G-15824, National Science Foundation.

1. Falconer's Formula 2, *J. Heredity* **45,** 42-4.

14.21. Selection for 13-day Larval Growth in Tribolium under Two Nutritional Levels. YAMADA YUKIO and A. E. BELL (Lafayette, U.S.A.).

Selection for large and small 13th-day larval weight in *Tribolium castaneum* has been investigated for 16 generations to evaluate the effec-

tiveness of various selection methods and the importance of genotype by environment interactions under two levels of nutrition. The principal difference between the levels consisted of 10 per cent dried brewers yeast in the Good ration while the Poor ration contained no yeast. All populations were raised under the conditions of 33°C and 70 per cent R.H. In addition to an unselected control, there were eight experimental populations as follows: GL = selected large on performance under the good level each generation, PL = selected large on performance under poor level each generation, gpl = selected large on average performance under both levels, gpl = selected large under good or poor levels in alternating generations, and similar four populations for small direction. Each population was represented each generation by 40 single pair matings. Sets of full sibs from each mating were reared on both good and poor rations. In addition, each mating was placed in standard wheat medium for reproduction. Selection was made on the basis of full sibs reared either under good, poor or average of both levels, depending on the selection methods. After choosing the best eight families in each population, five males and five females for each selected family were taken from the sib groups raised in the standard medium. They were mated at random with the restriction that full-sib mating was avoided.

As selection proceeded, the behavior of populations under two environments showed striking interactions. The gains per generation in GL populations under good and poor levels were 65 µg (direct response) and 60 µg (correlated response), respectively, and corresponding values in PL were 55 µg (correlated response) and 98 µg (direct response). GPL and gpl were intermediate between GL and PL or a little more similar to those of PL. In other words, regressions of population means on generation under two environments are nearly parallel for GL but not in PL, where the absolute difference observed in early generations lessened or disappeared in later generations. On the other hand, selection for small gave a completely reverse picture. Regressions (gain per generation) for PS under the two environments were alike (87 µg vs. 73 µg) but were greatly different in GS (106 µg vs. 53 µg). An interesting response developed in one of the GS populations. Its average weight under the poor environment was consistently heavier than under good for the last 4 generations. Asymmetrical selection responses observed were entirely dependent on the environment, i.e. larger response toward small under good but reverse under poor level. Realized heritability seems to be higher for small direction than for large, although differences between environments were not appreciable.

Supported by Grant G-15824, National Science Foundation.

14.22. Transfer to Descendency of Alterations Induced in the White Leghorn by Repeated Injections of Heterologous Blood. J. STROUN, L. STROUN-GUTTIÈRES, J. ROSSI and M. STROUN (Geneva, Switzerland).

The authors have repeatedly injected blood by the intra-peritoneal route from the grey guinea fowl to successive generations of cocks and hens descending from a white Leghorn strain with stable characteristics. Concurrently with this *test Leghorn group*, they raised a *check Leghorn group* treated under identical conditions with blood from white Leghorn, and also a *control Leghorn group* left untreated. In each new generation obtained through artificial insemination, fowls remaining up to the standards of the white Leghorn are selected from both treated groups and submitted to the blood injections. Moreover, from among the F_4 birds in the control group, the authors set up *three new Leghorn groups* treated under identical conditions with blood from Austhralorp, Rhode Island Red and white Leghorn respectively.

Results from F_0 to F_6 (February 1963): No alterations are noticed in either check or control Leghorn groups. In the test group, however, which was submitted to injections of blood from grey guinea fowl, there are in each generation from F_2 onward a few animals with alterations in the colour and quality of their feathers and in the pigmentation of their feet. Such alterations do not appear in the blood treated Leghorn fowls, but in birds of the following generation, independently of any injection whatsoever. They are maintained in the descendency which is no longer treated and are transmitted both by the mother and the father birds. In the groups treated with blood from Austhralorp and Rhode Island Red, alterations begin to appear as from F_2.

C.R. Acad. Sciences **255,** 781-783, 1030-1032, 1962.

Biol. Méd. 1963, to be published.

Arch. Sc., Genève, 1963, to be published.

14.23. (D). Technic of Repeated Heterologous Blood Injections to Induce in the White Leghorn Alterations Transferred to Descendency. L. STROUN-GUTTIÈRES, J. STROUN and M. STROUN (Geneva, Switzerland).

To successive generations of cocks and hens descending from a white Leghorn strain with stable characteristics, the authors have repeatedly injected heterologous blood by the intraperitoneal route. The blood is injected every 3 to 5 days as soon as the subjects are 10 to 30 days old and for a period of 6 to 7 months. In each new generation, fowls remaining up to the standards of the white Leghorn are again submitted to the blood injections. In some birds whose ascendants have been treated, from F_2 onward the authors have noticed alterations in the colour and quality of the feathers and in the pigmentation of the feet, which are maintained in the descendency independently of any injection whatsoever. No alteration is noticed in a check Leghorn group treated under identical conditions whith blood from white Leghorn. The authors demonstrate their technic and their results with diagrams, pictures and preserved specimens of altered and normal birds.

14.24. Egg White as a Modifying Factor in Heredity. LUBEN G. ANGELOFF (Sofia, Bulgaria).

The present experiments reported were concerned with influence of foreign egg-white (from 5 to 11 ml) of unfertilized eggs of other birds, namely, condor (*Sarcorhamphus gryphus*, Linn.), wild goose (*Anser anser*, L.), Chinese goose (*Cygnopsis cygnoides*, L.) and Indian turkey hen (*Meleagris galloporo*, L.) on the process of development of chick embryos. They were designed to determine whether the transmission of hereditary effects could be brought about by transforming foreign egg-white from one strain to another and if the yolk of the unfertilized egg is a bearer of inherited characters with a view to obtaining a new race of domestic hens possessing more valuable characteristics as regards meat, eggs and resistance to infections.

By injecting eggwhite into eggs of representatives of different orders of birds the following conclusions were drawn: transfer of traits by injection of egg white between representatives of different orders of birds is possible when the sexual crossing is impossible. The egg white of the unfertilized egg was a bearer of hereditary traits. The experimental birds grew rapidly, were bigger, and weighed more than the controls. The hereditary traits appearing in the first generation were transmitted to the fourth generation.

14.25. Experimental Change in the Hereditary Character of Gametes in Birds. B. G. NOVIKOV (Kiev, U.S.S.R.).

This note generalizes the results of the author's investigations on the change in the hereditary characters of gametes in birds by cross transplantation of the testicle and injection of heterogenous desoxyribonucleic acid.

Proceeding from the principle of the formation of the hereditary characters of gametes in the process of the entire ontogeny, it may be presumed that during development in the organism of another breed of animal these characters should alter in the gametes. The answer to this question was found in experiments on cross transplantation of the testicle in fowls and ducks. The essence of these experiments is that soon after hatching several testicles from Rhode Island chicks were transplanted into the body cavity of castrated Leghorn chicks, while Leghorn gonads were transplanted to Rhode Island chicks. In the experiments on ducks interchange transplantation of testicles was carried out on fledglings of mallard and peking ducks. After 1-2 years sperm was taken from the transplanted testicle during the breeding season and used to fertilize artificially females of the breed to which the transplant belonged.

The investigations showed that in posterity obtained as the result of such insemination varied hereditary changes occur which cannot be obtained by ordinary crosses of the initial forms. The rate of variability was intensified in subsequent generations. By selection and closely related crossing of altered forms it was possible to stabilize several breed groups of fowls differing in plumage colour, comb structure, constitution and final body dimensions. A similar result was obtained in experiments on the cross transplantation of testicles in ducks.

In later investigations, on analysis of the mechanisms of the phenomenon under consideration, an attempt was made to connect the hereditary changes of the gametes that had developed in the organism of another breed of ducks with the changes in their desoxyribonucleic acid. The study of this problem was begun with experiments on the injection of heterogenous DNA in ducks. The essence of these experiments consisted in systematic injections of DNA from the erythrocytes of a wild duck into peking ducks during several years, beginning at the age of eight days; in the converse experiments

DNA of peking ducks was injected into a wild duck. Under the influence of heterogenous desoxyribonucleic acid varied hereditary changes appeared in subsequent generations in a small percentage of cases. On this basis two dwarf forms of snow-white ducks with lighthorn and yellow beaks were obtained from a wild duck treated with peking duck DNA. The variability in ducks due to the effect of DNA injections was only partially similar to the changes obtained in cross transplantation of the testicle.

14.26. Abnormal Segregations for the Alleles "single Comb" and "Rose Comb" in the Domestic Fowl. PH. MÉRAT (Jouy-en-Josas, France).

A study of the segregations of the alleles R ("rose comb") and r ("single comb") in a poultry population reveals the main following facts:

(a) In the cross Rr × rr (with about 51.000 pedigree progeny) the male chicks show a highly significant excess of single combs over the rose combs; the proportion is normal by the female progeny.

(b) In the cross Rr × Rr (about 8000 progeny), the female chicks show a highly significant excess of single combs over the expected proportion of 1/4; there is a deviation in the opposite sense among the male chicks, so that, grouping the two sexes, the ratio of the comb types is normal, near 3/1.

For these two crosses, the observed deviation comes only from the progeny of some males.

(c) For the cross rr × Rr (about 15.000 progeny), on the whole, the ratio of the two comb types is normal in both sexes.

The hatching percentages, the sex ratio for each comb type, and the different results according to the type of cross, do not support the hypothesis of a differential embryonic mortality or of a differential fertility among the ova.

A progeny-test of single comb males from "abnormal" families, the compared results in the 3 crosses studied, and also in numerous RR × rr crosses, do not lend support to the hypothesis of modifying genes.

The hypothesis of a selective fertilization will be discussed.

14.27. Effects of Selection on Reproductive Fitness in the Fowl. A. W. NORDSKOG and MARGRITH WEHRLI (Ames, U.S.A.).

Populations subjected to intensive artificial selection for specific metric traits are expected to decline in reproductive fitness. The latter is defined here as a product of the components: rate of egg production, fertility, hatchability, and adult viability. Five lines starting from a common Leghorn base population have each been selected for a single trait over six generations. The breeding population of Line A, selected for high egg production, consists of 16 sires each mated to 9-16 dams. Lines B and C are selected for high and low body weight respectively, and Lines D and E for high and low egg weight, respectively. These lines are maintained by 8 single-male pens of 9-16 dams each. Line A has not improved consistently in egg production or reproductive fitness. Body weight has increased 54 per cent in Line B and fitness has declined steadily by 47 per cent. Line C has decreased 26 per cent in body weight; fitness has declined 16 per cent. Egg size has increased 15 percent in Line D, while fitness decreased 39 per cent. In Line E egg size has decreased 14 per cent and fitness declined 16 per cent, but the latter is not statistically significant. A similar selection experiment in the Fayoumi breed shows good agreement. The results fit the hypothesis that intermediate values of body size genes are optimum to fitness. However, egg size in the original stocks appears to be above the optimum for fitness such that downward selection has either improved or maintained reproductive fitness.

14.28. Genetic Variation in Chick Bio-assays for Gonadotropins. I. Testes Weight and Response.[1] P. B. SIEGEL and H. S. SIEGEL, (Blacksburg, U.S.A.).

Genetic influences on testes weights at hatching and testes weight response to anterior pituitary homogenates and purified gonadotropins were studied in White Rocks. Heritabilities were estimated from paternal half-sib correlations. Preparations were subcutaneously injected with a saline carrier and responses measured as increase in testes weight/100 g of body weight.

Significant differences among sire families were obtained from injections of anterior pituitary homogenates. Responses among sire families to 10 and 20 μg. of FSH (Armour) were also significantly different. The rank correlation of family responses for the two dosages was 0.74 and the mean heritability of response was 0.92. No significant differences among sire families were noted for injections of either 100 or 200 μg

of LH (Armour), however the mean heritability was 0.24.

Overall response to a combination injection of LH and FSH (Armour) was significantly greater than that obtained when either was administered singly. An additive effect was noted within some families while in others the response was similar to that obtained when either was administered alone.

When NIH-FSH and NIH-LH were injected, differences among sire families were not significant for the FSH but were for the LH.
Mean heritabilities were 0.03 for FSH and 0.80 for LH.

Significant differences were observed among sire families for two sources of PMS. The rank correlation of the familial response to each source was 0.80.

This experiment demonstrated genetic influences on the response of target organs to gonadotropins. Genetic control of assay animals facilitated excellent precision in bioassays of gonadotropins.

---

1. To be published in full in *The Virginia Journal of Science* **15.**

14.29. Genetic Variation in Chick Bioassays for Gonadotropins. II. Histological and Histochemical Responses.[1] H. S. Siegel and P. B. Siegel (Blacksburg, U.S.A.).

Histological and histochemical observations were made on testes of newly hatched progeny of White Rock sires after subcutaneous administration of purified gonadotropins. Hematoxylin-eosin for structural details, methyl green-pyronin for nucleic acid differentiation and sudan black for lipids were employed.

Non-significant differences were observed in the response of seminiferous tubules among progeny of 5 sires in a dosage range of 5 to 40 μg/chick of FSH (Armour). However, significant differences were found when the range was extended to 625 μg. Similarly, significant sire effects were observed with NIH-FSH levels ranging from 5 to 125μg. Differences in tubule response among families were not significant when NIH-LH was used at doses of 1 to 25 μg. Rankings of families for tubule diameter responses were not necessarily the same as those ranked for testes weight response.

Significant linear increases in tubule diameters were found in a range of 5 to 125 μg per chick of Armour FSH. When sire differences were considered, precision (λ) for these assays were 0.35 and 0.11, respectively. A significant quadratic response between the 125 and 625 μg levels indicated that this was above effective assay limits for this method. Precisions with NIH-FSH and LH were 0.23 and 0.25, respectively.

No noticeable differences were noted in lipid depletion among families. At 25 μg/chick of LH, lipoid depletion and increased nuclear activity were observed. This appeared as an all or nothing response and could not be effectively quantitated. Although FSH significantly increased tubule diameters, neither increases in mitotic figures nor nuclear activity were observed.

---

1. To be published in full in *The Virginia Journal of Science* **15.**

14.30. The Effect of Microdose Irradiation of Hen's Eggs upon Hatchability and Other Characters of Chickens. H.F. Kushner, I. G. Kostin, L. A. Zubareva, L. I. Shershunova, N. I. Kuznetzov, and M. G. Salganick (USSR).

1. The eggs of Russian White and New-Hampshire hens were used to study the effect of microdoses of γ-rays on economic and reproductive characters of hens.

2. The eggs over the incubation period were irradiated continuously or intermittently in different trials with uranium and thorium salts.

3. If the eggs during the incubation period were irradiated intermittently with total doses of 0.003-2.9 r the embryonic mortality was decreased and owing to it the average hatchability was 3.5 per cent higher than in control.

4. The results of irradiation depended upon the initial quality of the eggs: the lower the hatching properties of the control eggs, the higher was the degree of hatching improvement of irradiated eggs.

The effect of irradiation on New-Hampshire's eggs was superior to that on Russian White's eggs.

A high negative correlation between hatchability of control eggs and the difference in hatchability between the experimental eggs and controls was established ($r = -0.585 \pm 0.0695$).

5. The effect of intermittent irradiation of 0.003 — 0.021 mr doses was equally good.

6. Morphological and physiological studies on embryos under the intermittent irradiation showed that the hen's embryos were more sensitive to irradiation at their earlier stages of development. Treated embryos developed more rapidly than controls.

7. Respiratory metabolism in irradiated eggs during 12-18th days of the incubation period was more intensive than in the control eggs. The difference in O_2 consumption and CO_2 output in favour of treated eggs varied in different trials from 5 to 40 per cent depending on initial quality of the eggs and consequently depending on the effect of irradiation.

8. The postnatal growth, development and viability of the chickens hatched from treated eggs were the same as in the control group.

9. The egg productivity of over 4000 pullets hatched from treated eggs and maintained at the range or in the cages exceeded the control by 10 per cent.

10. The quantity, quality and fertilizing capacity of the sperm of the experimental cockerels (developed from treated eggs) were the same as the sperm of the control cockerels.

11. The preliminary observations show that the progeny of the 1st and 2nd generations from hens hatched from the irradiated eggs didn't reveal any abnormalities.

14.31. Selection on X-ray Induced Variation in Chickens. Hans Abplanalp, Dorothy C. Lowry and Everett R. Dempster (Davis, U.S.A.).

Populations of SCWL chickens were given 8000 r X-ray irradiation administered to the semen of males during seven generations. The populations used for this study derived from a flock which had been under selection for high egg number over 18 years. While irradiated populations were prepared without artificial selection, a control of equal size was reproduced under similarly relaxed selection, and the original line continued under artificial selection for egg number. Following the irradiation phase, all lines were subjected in replicate to four generations of artificial selection for egg number. Response to selection for egg number and correlated response in egg size and viability are discussed.

14.32. Quantitative Developmental Genetics of Body Shape and Size in Domestic Fowl. A. G. Cock (Edinburgh, Great Britain).

From a population of mixed breed origin, two lines, H (high) and L (low), have been selected in opposite directions for an index of *relative* shank length, $i_{10} = y_{10} - 0.4x_{10}$, where $y_a = \log_{10}$ shank length, and $x_a = \log_{10}$ body weight, at a weeks of age. The coefficient 0.4 is approximately the mean value of k, the growth rate of y relative to x (coefficient of heterauxesis) between 2 and 10 weeks. Two further lines, B (big) and S (small), have been selected up and down for x_{10}. After 4 generations of selection H and L differ by 0.067 in i_{10} (= 17 per cent difference in shank length at the same body weight), the realized heritability being 0.57. After 3 generations B and S differ by 0.143 (= 39 per cent) in x_{10}; r.h. = 0.47. Two estimates of the genetic correlation between i_{10} and x_{10} (− 0.32 from the correlated response in H-L; + 0.26 from B-S) differ significantly.

Genetic variation in i_{10} arises by (at least) two distinct developmental pathways: by changes in post-natal k, and by embryonic changes without change in post-natal k. The former pathway accounts for only 20 per cent of the H-L difference. Measurements of adult skeletons show far-reaching and anatomically complex differences in shape between H and L. There are anatomical and developmental similarities between H-L and ectomorphy-mesomorphy in humans. Possible interrelations between different anatomical and developmental kinds of variation will be discussed.

Full publication in *Genet. Res.***4**, No. 2, 1963.

14.33. Response to Selection for Body Size at Two Ages in the Fowl. E. S. Merritt and S. B. Slen (Ottawa and Lethbridge, Canada).

In broiler or meat-type chickens rapid early growth is highly important for efficiency of production. Large adult size, on the other hand, results in increased maintenance and feed requirements of breeding stock. A study was undertaken to determine whether a selection response for increased broiler weight could be achieved simultaneously with a decrease in adult weight.

Three lines were drawn from a random-bred control strain (Ottawa Meat Control). One was selected for increased weight at 63 days of age, one for increased weight at 147 days of age, and the other for an increase in 63-day weight but a decrease in 147-day weight. A sample of the random-bred control population, bred independently of these lines, was reared with each generation of the selected lines.

Heritability estimates for 63- and 147-day weights calculated on the control or base population were approximately 0.55 with a genetic correlation estimate of 0.75 between them. After

four generations of selection, the response in lines 1 and 2 was in reasonable agreement with expectations, being greatest in each case for the trait under direct selection. In the third line the males showed both an increase in 63-day weight and a decrease in 147-day weight. Females, on the other hand, showed a slightly greater increase than males in 63-day weight, but showed no change from the controls in 147-day weight. These results would indicate that the growth pattern can be changed in the desired manner by selection.

14.34. Selection for Growth Rate and Correlated Responses in Chickens. R. GEORGE JAAP (Columbus, U.S.A.).

The paired mating system suggested for minimum inbreeding in control populations by Gowe, Robertson and Latter[1] has been used for two random-bred control lines and five lines with random-breeding of growth-selected parents. All lines arose from crossbred ancestry, the two control and two of the selected lines arising from the same base populations.

In the growth-selected lines, 20 to 25 per cent of each generation has been used for reproduction in 40 paired matings per line. The inbreeding coefficient (F) in the selected lines increased approximately 1.0 per cent per generation. In the random-bred control lines, F has increased slightly less than 0.5 per cent per generation. Little change from inbreeding depression or random gene drift was expected. The present number of generations of selection is 8, 5, 2, 4 and 3 for lines G, A, AG, RG and GRL, respectively.

Realized heritability of weight at 8 weeks of age from the first four generations of selection in line G was about 0.4. Recently, those selected lines having most rapid growth rate (A, AG and the last 4 generations of G) have failed to respond as rapidly as RG, GRL and the first four generations of G. This slackening of the progress in line G is the first evidence of a plateau in the rate of progress from selection for growth rate in chickens.

When week body weight was increased by about one-half a pound, the following correlated responses were observed: Egg production decreased 5 per cent, egg weight increased 2.8 g, eggs became relatively broader in shape and their albumen height increased 0.3 mm.

1. *Poultry Sci.* **38,** 462-471, 1959.

14.35. Influence of Feed on the Heritability of Some Chicken Traits. J. P. BOYER, X. DE LAAGE and C. CALET (Jouy-en-Josas, France).

In May-June 1962 3527 chicks were hatched from 20 sires and 164 dams during 8 weeks—the hatches 1, 3, 5, and 7 received an all mash ration with high energy and a coccidiostat; the hatches 2, 4, 6, and 8 received an usual mash ration.

At 8 weeks of age, weight, breast angle, keel length, diameter of shank and weight / shank length ratio were appreciated. All data were transformed on a probit scale to avoid the hatch influence.

Heritabilities of the five characters were estimated by hierarchical analysis of variance.

For males, no great difference was observed between the two regimes: the percent heritability was, for "special" and "usual" ration respectively: weight 47.1-48.6, breast angle 38.1-21.3, keel length 59.2-59.2, diameter of shank 68.4-56.6; weight / shank length ratio 46.7-39.6.

For females, the differences were spectacular ones: weight 67.3-41.5, breast angle 42.3-17.2, keel length 62.1-49.0, diameter of shank 71.4-59.3, weight / shank length ratio 65.5-42.4. It is interesting to note that in all cases, the sire component of variance was greatly increased.

It is concluded that an appropriate feeding plan permits the manifestation of a genetic sex-linked variability for meat characters. Such an environment is able to facilitate selection.

It is suggested that the absence of response of males was perhaps caused by a limitation of available energy in ration.

14.36. Heritability in the Progeny of Hens with Contrasting and Similar Constitution and Productiveness. E. E. PENIONZHKEVICH (U.S.S.R.).

Investigations that are carried out at the poultry breeding laboratory under the auspices of the author on heretability of productive, exterior and interior characters in farm poultry show hereditary variability of the same characters depending on methods of reproduction (interbreeding, inbreeding, somatic hybridization), environmental conditions (mainly temperature), various physiological states of the organism, age of parents and selection of parental forms among contrast or similar breeds.

Progeny is affected by parental forms including at least two main factors: (a) nutrition during

embryonal development connected with the maternal organism; (b) hereditary peculiarities of paternal and maternal organisms.

When crossing birds with contrasting productiveness and constitution (egg-producing and double-purpose breeds) the progeny mostly inherits egg productiveness and physiological earliness from the paternal organism and live-weight, hatchability and fattening characters from maternal organism.

While selecting fathers and mothers with similar productiveness and constitution (double-purpose poultry breeds) we observe that in-breed mating and interbreeding do not result in the regular paternal or maternal influence on live-weight.

Only daughters are inclined to inherit live-weight from mothers and even less so from fathers.

High meat qualities are inherited by the progeny particularly from the paternal organisms. The above-mentioned regularities in heretability of characteristics when selecting poultry of contrast or similar breeds are efficiently used by poultry breeders in their work. To produce highly productive hybrids of double-purpose breeds, parental forms are selected from egg-producing breeds or strains, and maternal—only from double-purpose ones. For the development of hybrids, growing for meat, paternal and maternal forms are selected from special double-purpose breeds and strains. In this case the parental form should have high meat qualities which positively correlate with the live-weight, and the maternal form should have high egg production and vigorous constitution, which is characterized by high vitability, hatchability, intensive metabolism and by some interieur features (development of thumus).

14.37. Diallel Crosses of Inbred Lines of Egg-type Poultry Repeated over Locations and Years. I. The Relative Importance of Different Kinds of Genetic Effects. L. H. Baker and R. B. Arvidson (Des Moines, U.S.A.).

The now classical concepts of general and specific combining ability have been extensively applied in evaluating inbred lines of corn. However, all published evidence concerning the relative importance of these genetic effects in the fowl may be of limited value, since it has all been based upon results from individual performance tests.

Because genotype and environment have interacting effects on the phenotypic expression of quantitative characteristics, the data from a specific experiment pertain only to the genetic variance of the population with reference to the environment (s) actually sampled by the experiment. Therefore, points of major interest in the design and analysis of a hybrid breeding program are:

(1) The consistency of estimates of general and specific combining ability variances from experiments repeated over a series of locations and years,

(2) Estimates of variance in genetic and reciprocal effects defined as averages for the totality of environment pertinent to the destiny of the genetic populations studied, and

(3) The relative importance of additive and specific effects in lines selected over years.

The study reported here represents an attempt to evaluate these points of interest and compare the results obtained with estimates of genetic and reciprocal effects available from other studies in the fowl.

14.38. A Case of Hairlessness in Cattle. V. Derlogea (Bucarest, Rumania).

Two calves of the Pinzgau breed (♀ and ♂) hairless were born in 1962 in the Năsăud county —Cluj region. Their birth weight was normal and except the above-mentioned characteristic, they did not present any other phenotypical peculiarity. Beginning with their second week of life, they suffered from repeated indigestions, lost weight and died, one 52 days old, the other but 16 days old.

As compared to the previously described cases of semilethal allopecy (Surrarer, Hut and Saunders), these calves presented the particular characteristic that even their eyelids and tail-end were bare of hair.

The histologic cross-sections through their skin showed very rarely distributed hair follicles, the sheaths of which were much enlarged and distorted resembling small lodges in which the hair roots were coiling around several times. There was a total disturbancy as regards the orientation of the follicles and an utter impossibility for the hair to push towards the exterior (Fig. 2).

Breeding history. The mothers of these calves were daughters of an imported Pinzgau bull used in the A. I. Centre of the Năsăud county. These cows were at their first calving. Their mating had taken place in the conditions of mountainous pasture land where they had been put together with three bulls of the same herd, two of which were closely related with the cows, one even being a half-brother.

It is supposed that the imported bull, the grandfather of the calves, is the carrier of the semilethal hairlessness factor, that has been transmitted to his descendants. Through inbreeding, this hereditary recessive defect has finally become obvious.

Observations upon the descendants of this bull are being continued.

14.39. Gene-Hormone Interactions on Hair Pigmentation in Cattle. L. O. GILMORE and N. S. FECHHEIMER (Wooster, U.S.A.).

Blackish Pattern is a sex-influenced trait with the responsible gene being found in Ohio Ayrshire and Jersey cattle in the respective frequencies of 0.44 and 0.54. It is assumed to modify *Bs* although an insufficient number of test matings has been made to eliminate the alternate hypothesis of allelism. All eighteen bulls in a castration experiment lost blackishness in hair on the body proper but fewer did so in the area of the crest and head and none on the legs, ears or switch. The difficulty involved precludes establishing that the bulls were heterozygotes. This opens to consideration the possibility of hormone action overriding that of both genes present in homozygotes. Testosterone replacement by administration has been found to restore the pattern although not with complete duplication of the original phenotype.

To further test the validity of the premises underlying the theoretical considerations an effort was made to superimpose the administration of testosterone on the heterozygous cow with intact gonads. Heterozygosity was established by inference from observed phenotypes of dam and/or daughters. Several such cases point to the validation of the original concept. To date no attempt has been made to test the resistance of the homozygous genotype in the female.

It has now been shown that this is a likely system for studying a simple phenotype resulting from the combined action of gene and hormone.

14.40. Investigations into the Hereditary Origin of the Double Cervix Condition in the Meuse-Rhine-IJssel (MRY) Breed. A. VAN LOEN (Geleen, The Netherlands).

In a long-term project of research on bovine fertility within the Meuse-Rhine-IJssel breed (Netherlands) it was examined whether the double cervix condition (DC) is inheritable and, if so, how it is inherited under normal conditions of farm-management.

A total of 607 DC-cases recorded as cervix duplex was encountered among 16,375 cows, i.e. 3.7 per cent.

DC is regarded as a gross genital abnormality and is characterized on vaginal examination by two ora uteri externa. The occurrence of this malformation has to be considered a result of arrested development of the Müllerian ducts.

The population under study is a random-mating population, the influence of inbreeding and assortative mating being negligible. No selection has taken place according to DC. SC (single cervix) and DC cows do not differ in reproductive efficiency.

Heredity is the most obvious cause of the manifestation of DC in the female offspring of a relatively large number of sires. The sires definitely influence the incidence of DC in their daughters ($P<0.0001$). Affected dams produce relatively more affected daughters than normal ones ($P<0.01$).

Regarding the mode of inheritance there are strong indications that the pre-disposition to DC is due to an autosomal dominant gene (or gene-complex) ($p = 0.17$) with incomplete, and possibly strongly environmental, penetrance ($z = 0.13$). For the period in which cattle are moved from pasture to cowhouse and the period in which they are inside notably affect the critical stage of development of the Müllerian ducts following which normal development is arrested.

14.41. Genetical Study of "Tête de mouton" Cattle. J. J. LAUVERGNE and B. VISSAC (Jouy-en-Josas, France).

The abnormality called "Tête de mouton" was observed for the first time in France in the progeny of a Limousin bull (Charmant) used by the A.I. Center at SOUAL (Tarn) from November 1957 to January 1960.

The abnormals have a typical appearance: fronto-nasal trench, convex profiled nose, brachygnathia of the lower jaw, exorbited eyes and marked macroglossia. Ossification defects of skull and abnormalities of the heart (persistent Botal hole) and of the omasum are equally established.

Charmant was apparently normal. Both sexes are equally frequently affected by the abnormality, which had a total incidence of 5.4 per cent among 811 of his progeny, born or aborted. There was no relationship, either between the mothers of the abnormals or between the mothers and Charmant, who was used for beef production

crosses. In addition, matings between abnormal half-sibs produced normal and abnormal calves. Thus, the factor appears to be monofactorial, autosomal, dominant with incomplete penetrance.

The viability of the abnormal heterozygotes is reduced up to adult age.

(1) Out of 9 "Tête de mouton" calves kept under special nutritional conditions only 4 lived more than $2\frac{1}{2}$ years.
(2) The perinatal mortality of Charmant's calves was 32 per cent ± 12.2 among the "Tête de mouton" calves and only 4 per cent ± 1.4 among the normals.
(3) The embryonic mortality—estimated indirectly by the proportion of returns for service among inseminated cows, between 30 and 120 days after insemination—was higher in the case of Charmant (8.62 per cent ± 0.44) than in the total of all other Limousin bulls used in the A.I. Center during the same period (6.02 ± 0.14).

Considering all degrees of expressivity (early embryonic mortality, perinatal mortality of the abnormals and viable "Tête de mouton"), penetrance of the factor can be estimated at 21 per cent for heterozygous fertilized eggs.

Further information on this study will be published in *Annales de Zootechni.*

14.42. Sex Chromosome Mosaicism in Unlike Sexed Cattle Twins. N. S. Fechheimer, M. S. Herschler and L. O. Gilmore (Columbus, U.S.A.).

Female calves born co-twin to males exhibit maldevelopment of the reproductive apparatus when there has been vascular anastomosis of the twins' circulatory systems *in utero*. Such twin pairs also exhibit an erythrocyte mosaicism and a tolerance to reciprocal skin grafts. This fact has led previous investigators to postulate that cells other than erythrocyte precursors may be exchanged.

This hypothesis has been examined using the leucocyte culture method In spite of the large diploid chromosome number in cattle (60), the X and Y chromosomes are morphologically unique. All autosomes are acrocentric. The X is large and submediocentric, while the Y is mediocentric and much smaller than the X. Only the expected XX or XY sex chromosome constitution was observed in intact cells from seven single born females and six single born males respectively.

Among 13 animals from 11 sets of unlike sexed multiple births 7 females (presumed freemartins) and 4 males possessed cells of both male and female origin. Sufficient numbers of cells from the remaining two calves were not examined. Mosaicism of erythrocytes for antigenic properties was manifested by the cells of 9 of 10 animals for which a test was made.

These findings raise the possibility that the intergrade sexuality of females co-twin to males is caused not by a humoral agent produced by the male but may be a function of the sex chromosome mosaicism.

14.43. Heterosis from Crosses among British Breeds of Beef Cattle. R. C. Carter, W. H. McClure, J. A. Gaines and D. W. Vogt (Blacksburg, U.S.A.).

This experiment was designed to estimate heterosis in beef calves resulting from various levels of breed crossing, not confounded with heterosis in maternal traits of their dams. Purebred or high grade Aberdeen-Angus, Hereford and Shorthorn cows, here designated as "straightbreds", were mated to purebred bulls of these three breeds to produce straightbred and reciprocal two-breed cross calves. Similar straightbred cows were bred to crossbred bulls (F_1) to produce three-breed and back-cross calves. This design permitted comparisons among straightbred, two-breed, three-breed, and back-cross calves, born in the same year, and all from straightbred dams. Five calf crops were produced, 1957 through 1961, with a total of 504 calves born alive and 480 weaned.

The most important differences were in fertility and viability. Cows in crossbred matings had 8 per cent more calves born and 11 per cent more calves weaned than those bred to a bull of the same breed. There was evidence for heterosis in birth weights, growth from birth to weaning, and postweaning growth. Average birth weights, adjusted for sex difference, were, in pounds: straightbreds, 68.4; two-breed crosses, 71.4; three-breed crosses, 70.4; and back-crosses, 69.3. Average weaning weights for the four groups were respectively: 402, 426, 433 and 413 lb. There were significant deviations from mid-parental means for birth weight in two breed and back crosses; and in growth from birth to weaning and weaning weights, in two-breed and three-breed crosses. Differences in post-weaning growth rates were generally smaller than in growth to weaning, but crossbreds were heavier at all periods. Differences in feeder

grade at weaning, live slaughter and carcass grades were small.

Calves sired by crossbred bulls averaged 13 days earlier in date of birth than those sired by purebred bulls.

14.44. The Relationship between the Performance of Mothers of Dairy Bulls and the Progeny Tests of their Sons. L. K. O' CONNER (Thames Witton, Great Britain).

The purpose of this study was to obtain information on the relationship between the performance of mothers of bulls and the progeny tests of their sons, which would be useful in developing a practical method of pedigree evaluation for young dairy bulls.

The data consisted of the progeny tests of 736 Friesian bulls with contemporary comparisons based on a weighting of 10 or more, and the 305-day lactation records of their 640 dams. Where possible, the first five lactation records of the dam were included. The differences between the dams' lactation records and the appropriate herd average, and the contemporary comparisons of their sons, were used in the milk yield analyses. In the case of fat percentage, the dams' own records and the first lactation daughter averages of their sons were used.

For milk yield and for fat percentage, regressions of sons' progeny test on dams' rating were calculated for each lactation of the dams separately, and for averages of increasing numbers of dams' records up to five. Estimates of the repeatability of the dams' milk yield and fat percentage were also obtained. It was found that whilst the regressions for fat percentage were in good agreement with those expected in theory, the regressions for milk yield for second and subsequent lactations of the dam were less than expected.

A full report of this work will be submitted for publication in *Animal Production.*

14.45. A Genetic Study of Feed Efficiency in Holstein-Friesian Cattle. R. D. PLOWMAN, N. W. HOOVEN JR. and W. R. HARVEY (Beltsville, U.S.A.).

This study was conducted to determine if genetic differences existed in gross efficiency of feed conversion among progeny groups and if feed efficiency could be estimated from a portion of the complete lactation. Efficiency in this paper is defined as:

$$\frac{\text{Fat Corrected Milk Yield}}{\text{Therms of Estimated Net Energy Consumed}}$$

The data consisted of 584 lactation records completed on 332 cows. Eighteen sire groups were represented and 254 daughter-dam pairs. The variables considered were: FCM yield, feed efficiency, body weight, body weight change and therms of estimated net energy consumed. Results from the first lactation analyses indicated significant differences among the sire groups in feed efficiency and body weight. Significant positive correlations were found between feed efficiency and FCM yield. Body weight and body weight change were negatively associated with feed efficiency. Heritability and repeatability estimates of feed efficiency are similar to those of milk production.

Correlation between the third 60-day period and the total lactation feed efficiency ranged from 0.88 to 0.94 for different seasons of the year. Corresponding correlations for the second one hundred days as compared to the total were 0.92-0.96. It seems possible to predict feed efficiency from part lactation data.

14.46. Effects of Crossbreeding and Crisscrossing on the Birth Weights and Gestation Periods of Dairy Cattle. R. W. TOUCHBERRY (Urbana, U.S.A.).

The data for this study consists of the birth weights and gestation periods of 1205 calves arising from various crosses and crisscrosses of the Holstein and Guernsey breeds. The calves were born over a period of twelve years and are categorized into five generations. The first generation consists of purebred Holsteins and Guernseys and reciprocal crossbreds and was produced by mating unrelated Holstein and Guernsey heifers to Holstein and Guernsey sires. The second generation consists of Holsteins, $\frac{3}{4}$-Holsteins, $\frac{3}{4}$-Guernseys and Guernseys and was produced by systematically mating the first generation females to purebred Holstein and Guernsey sires. The third, fourth and fifth generations were produced in a similar manner by systematically mating the females of the second, third and fourth generations to Holstein and Guernsey sires. For the first generation data, crossbreds were 2.8 $\pm$ 1.6 lb heavier than purebreds, calves out of Holstein dams were 18.7 $\pm$ 1.6 lb heavier than those out of Guernsey dams, while those by Holstein sires were only 6.9 $\pm$ 1.6 lb heavier than those by Guernsey sires. Crossbred calves were carried 0.54 $\pm$ 0.78 days less than purebreds, those out of Guernsey dams were carried only 3.2 $\pm$ 0.76 days more than

those out of Holstein dams, while those by Guernsey sires were carried 4.8 ± 0.76 days longer than those by Holstein sires. The results of analyses of the data for other generations will be presented. This paper will be submitted to the *Journal of Animal Science* for publication.

14.47. Some Observations on Reproduction, Weight Changes under Lactation Stress and the Mothering Ability of British and Crossbred Zebu Cattle in the Tropics. G. H. LAMPKIN (Kikuyu, Kenya) and J. F. KENNEDY (Rockhampton, Australia).

Data collected at Belmont on the performance of British, British cross Africander and British cross Brahman females was examined. Details of 2871 matings were available. Of the cows mated only 56 per cent of the homebred British cows produced live calves compared with 77.3 and 73.8 per cent from Africander and Brahman crossbred cows respectively. Evidence was found that the low calving percentage for British females was connected with lactation stress, the British cows being frequently unable to maintain weight if two pregnancies occurred in two successive years. Under similar stress crossbred zebu cows could thrive and crossbred Brahmans in particular would normally gain weight.

Although F_2 calves out of crossbred zebu dams were significantly smaller at birth than contemporary crossbred calves obtained from British dams, this ability of crossbred zebu mothers to thrive whilst lactating was not at the expense of their ability to rear their calves. Calves out of crossbred Brahman cows, in fact, grew significantly faster than crossbred Brahman calves obtained from British dams. Between birth and weaning this difference for males and females was 0.23 and 0.26 lb/day respectively.

14.48. Serum Transferrin Type and Milk and Butterfat Production in Dairy Cows. G. C. ASHTON, G. R. FALLON, and D. N. SUTHERLAND (Rockhampton and Brisbane, Australia).

The serum transferrin types of 225 Jersey cows in 13 herds, and 433 Australian Illawarra Shorthorn cows in 24 herds, in two regions of Queensland were determined. The effect of transferrin type on lactation length, milk yield, butterfat yield and butterfat percentage was assessed. The results for the two breeds did not differ significantly. Combining the data from both breeds, it was found that on average Tf D/D cows had lactations 13.9 days longer than Tf A/A cows ($P < 0.01$), and yielded 450 lb (204 kg) more milk ($P < 0.01$). The results for Tf A/D cows were intermediate. No effect on butterfat percentage was found.

The proportion of the genetic variance in milk yield due to the transferrin locus was 10.4 per cent in the Jerseys and 6.0 per cent in the Australian Illawarra Shorthorns. This implies that the manipulation of this gene could raise milk yield by about 5.1 per cent in two generations in these herds by quite simple selection.

The nature of the effect is now known but is not due solely to increased length of lactation.

14.49. Blood Groups in Czech Brindled Cattle and Czech Large White Pigs. K. HÁLA, J. HOJNY, J. MATOUŠEK and J. SCHRÖFFEL (Liběchoý, Czechoslovakia).

Besides the formation of antibodies against a great number of new blood factors following isoimmunizations of the Czech Brindled cattle a large number of phenogroups in the B and S systems is characteristic for this breed.

In 656 bulls and cows of this breed, both phenogroups having been determined by family analysis, 144 phenogroups of the B system and 24 of the S system have been proved. In the Czech Brindled breed all types of transferrins have been found. From the above results and in comparison with homogene breeds it may be assumed that the Czech Brindled breed is heterogene.

In the Czech Large White pig 28 blood factors have been found to this day, the majority of which are classed in 11 known genetic systems. Five new blood factors are being studied as to their relationship to genetic systems.

By electrophoresis on a starch gel between the start and the alpha-fractions a protein fraction has been found, appearing on sexual maturing and lasting till old age. By means of antisera gained by isoimmunization of sows by boar sera a close relationship of this serum protein to the protein parts of the fluid contained in the seminal vesicles of boars has been observed.

14.50. The Variability of the Number of Vertebrae in Domestic Pigs and Their Relation to Economically Important Criteria. J. K. HINRICHSEN (Giessen, Germany).

The numbers of vertebrae can be of interest for the producers and the consumer of pork, if

they are in correlation with the content of lean meat and the content of high prized cuts in the carcass. The importance of this criterion has been discussed in numerous publications. The fact, that longer and leaner pigs are prefered nearly all over the world, can not be without influence on the distribution of animals with different numbers of vertebrae.

The material to be discussed comprised 822 pigs of German improved Landrace (veredeltes Landschwein) in the years 1954 and 1955, all of them animals of the old type of German pigs.

The amelioration with boars and sows of Dutch or, if we look further back, Danish origin, began later. Therefore the material can be seen as sufficiently uniform. The distribution of thoracal and lumbar vertebrae is presented. A comparison of body length and number of vertebrae shows that length is in dense correlation with vertebrae numbers.

The figures show that the average length of vertebrae is nearly the same in all classes of vertebrae numbers. It is to be stated that pigs with a higher number of vertebrae are longer in the average. A selection with the aim of increasing the number of vertebrae must be successful in increasing the length of the animals and if length is connected with lean characteristics, also in improvement of quality. Practical breeding is following this trend.

14.51. Selection for High and Low Fatness in Duroc and Yorkshire Swine. H. O. HETZER, W. R. HARVEY and W. H. PETERS (Beltsville, U.S.A.).

Individual selection based on backfat thickness at a live weight of 175 lb has been carried in both upward and downward directions through 7 generations in Duroc swine and through 5 generations in Yorkshire swine. By generation 7, b.f.t. in Durocs averaged 2.01 in the high-fat line, 1.22 in the low-fat line and 1.50 in a randomly selected control line. The corresponding values for the 5th generation Yorkshire lines were 1.43, 1.15 and 1.28 inches, respectively. Realized heritabilities computed as the regression of generation means on cumulative selection differentials were 0.64 $\pm$ 0.06 and 0.42 $\pm$0.07 for high- and low-fat Durocs and 0.36 $\pm$0.12 and 0.35 $\pm$0.05 for high- and low-fat Yorkshires. The significantly slower change in the low-fat than in the high-fat Duroc line ($P < 0.05$) indicates that the response in the two lines was asymmetrical. Heritability of b.f.t. in the Duroc and Yorkshire control lines was estimated at 0.53 $\pm$ 0.07 and 0.32 $\pm$0.09 from the intra-group regression of offspring on mean of parents. These values agree very well with the corresponding averages of the realized values and indicate that Yorkshires are less variable genetically for fatness than Durocs. Litter size and litter weight differed little among the Yorkshire lines but declined in both the high- and low-fat Duroc lines. Post weaning daily gain tended to be higher for low-fat than for high-fat Duroc pigs (1.44 vs. 1.38 lb), while the reverse was true for high- and low-fat Yorkshires (1.38 vs. 1.29 lb). Feed requirements per 100 lb gain were substantially lower for low-fat than for high-fat lines in both breeds with control lines intermediate. Low-fat Durocs produced substantially higher yields of lean meat and less fat than high-fat Durocs. Low- and high-fat Yorkshires showed similar though somewhat smaller differences.

14.52. Genetic Change of Backfat Thickness in the Danish Landrace Breed. CHARLES SMITH (Edinburgh, Great Britain).

Data from the Danish progeny test reports were used in an attempt to measure the genetic change in backfat thickness in the Danish Landrace breed from 1952 to 1960. Over this period the average backfat thickness of tested pigs fell from 34.2 mm to 28.5 mm, a change of two standard deviation units.

The method used to measure genetic change depends on the difference in performance in two or more years of progeny from particular sires of dams. Environmental differences between the years are avoided by measuring performance relative to the year mean. However, allowance has to be made for selection among parents on the basis of their first set of progeny records, through adjusting the initial records by theoretical regression factors. The genetic change is then estimated as a function of the difference between the adjusted first progeny records and the records of subsequent progeny groups.

Separate estimates of the genetic change in backfat thickness were calculated in this way from the progenies of sires and of dams at each of the three stations. These were in general agreement and indicated that there was some genetic improvement in backfat thickness in the Danish Landrace from 1952 to 1960 but that not all of the observed change was genetic change. The overall estimate of the genetic change was —0.15 $\pm$0.10 mm per year and this repre-

sented about one-fifth of the observed change. Other less critical results also lead to the same general conclusion.

14.53. Longevity in Sardinian Sheep. PIETRO DASSAT and DOMENICO BERNOCO (Turin, Italy).

The objective of this research was to study the longevity of Sardinian sheep and to investigate the relationship between longevity and age at first lambing. The analyses were made using milk records collected in the Sardinian flock at Barumini (Cagliari) during the period of 15 years beginning in 1943.

The data comprised 2670 lactations of 678 ewes disposed of; their average age was 4.8 years. This represents an average productive life of 3.3 years and a replacement rate of about 30 per cent.

Of the 678 ewes of the sample 505 (74 per cent) first lambed at one year and the rest not until two years of age. The age at disposal of the former group was 4.7 years and for the rest 5.1 years, with a average productive life of 3.4 and 3 years respectively.

In estimating the average productive life in terms of times lambed, results showed 2.48 lactations for the whole sample: 2.55 lactations for the ewes first lambed at one year compared to 2.27 lactations for the animals first lambed at two years. The difference is statistically significant ($P < 0.01$) indicating that early lambing increases the length of the productive life even if it reduces longevity.

The data will be pusblished in full on the Italian magazine *La Nuova Veterinaria.*

14.54. Contributions to the Histologic Study of the Thyroid Gland in Tzigaia Lambs with Hereditary Ophthalmy. N. TEODOREANU and LUCIA GURĂU (Bucarest, Rumania).

Previous studies (Gh. Radu *et al.*, 1947; N. Teodoreanu *et al.*, 1952; and N. Teodoreanu, 1958) have stated the occurrence of hereditary semilethal ophthalmy for the first time in lambs of the black-headed Tzigaia breed, at the Slobozia experimental research station. The disease appears between the 2nd and the 60th day of life, ending with the death of the lambs at the age of 2-3 months.

The present note describes the histologic changes of the thyroid gland in five ophthalmic lambs as compared to one normal lamb.

The current methods of fixing, of inclusion and of staining of the samples were used in this experiment:

The prepared samples were examined at the following magnification.

The form of the follicles in the ophthalmic lambs was between oval and round, with an irregular cornered outline.

The mean height of the follicular epithelial cells was 8.84 μ.

The mean frequency of the various epithelial cells was of 52.08 per cent cylindrical cells, 46.42 per cent cubic cells, 1.25 per cent flat cells. Resorption vacuols existed in almost all the follicles of the thyroid gland. In many follicles the resorption of the acidophil colloid was almost total.

In the normal lamb the proportion of the epithelial cells was different: 42.00 per cent cylindrical cells, 54.00 per cent cubic cells and 4.00 per cent flat cells. The mean height of the epithelial cells was 8.07 μ.

In the ophthalmic lamb, the thyroid gland was in a state of hyperfunction. The weight of the lambs decreased with the increase in intensity of the disorders (symptoms).

14.55. (F). Science and Sheep-breeding. H. N. TURNER (Glebe, Australia).

The film deals briefly with current methods of sheep selection, wool classing and wool buying for Merinos in Australia, all of which rely heavily on hand-and-eye methods of appraisal. The theory behind the use of measurement, the estimation of repeatability and heritability and their application are then demonstrated, and current C.S.I.R.O. selection experiments are described and filmed, including methods of wool-sampling and detailed measurement. In these experiments, wool weight has been raised by 1 lb per head; the absence of any important correlated change is discussed.

The film then moves to a commercial shed and shows how simply fleece-weighing can be introduced in practice.

The film was made for general use to demonstrate the experimental work which lies behind a widespread extension campaign in Australia for the introduction of objective methods in sheep selection.

14.56. Current Progress in Three Selection Experiments with Hill Sheep. A. F. PURSER (Edinburgh, Great Britain).

Three separate two-way selection experiments started by the Animal Breeding Research Organization in 1953-54 with hill sheep are described briefly. In one experiment, selection for cannon bone length at eight weeks of age has resulted in marked changes in cannon bone length at all ages from birth onwards. Other skeletal and body weight changes have also occurred. In the second experiment selection for the average degree of fibre medullation in the lamb fleece has altered adult fleece type and the timing of the waves of shedding of kemp fibres. The third experiment involves selection for two extremes of birthcoat type. Correlated changes have occurred in birth weight, lamb mortality and in adult fleece grade and fleece weight, with the intermediate birthcoat type being generally better than either extreme type. The heritabilities of all three characters are 45-55 per cent and response to selection in each line has been approximately as predicted. However, evidence of a decline in genetic variance in each of the selected characters will be presented.

14.57. The Effect of Two Associated Biochemical Polymorphisms on Red Cell and Production Traits in Sheep. J. H. WATSON, A. G. H. KHATTAB and R. F. E. AXFORD (Bangor, Great Britain).

Two genetic loci governing haemoglobin type and erythrocyte potassium concentration, show evidence of association in the College flock of Welsh Mountain sheep. In 1777 sheep departure from randomness of association stems from an excess of haemoglobin B homozygotes among high potassium animals. This inbalance extends to the three remaining phenotypes containing the haemoglobin B gene but not to haemoglobin A homozygotes.

Differences in potassium concentration between the three haemoglobin types are accounted for by their packed cell volumes. At both high and low potassium levels haemoglobin A homozygotes have higher blood potassium concentration and packed cell volume than haemoglobin B homozygotes. AB heterozygotes are close to mid-parent values. Red cells containing haemoglobin A, although more numerous than those containing haemoglobin B, are also more fragile.

Differences in production records of these sheep are small, rarely significant, but consistent. Low potassium types are marginally superior to high potassium types in all liveweight measurements and first fleece weight. Haemoglobin A homozygotes are marginally better than BB homozygotes, with AB heterozygotes intermediate.

14.58. Some Genetic Studies on the Buffaloes in the U.A.R. A. A. ASKER, L. H. BEDEIR, A. A. EL-ITRIBY and I. A. AHMED (Cairo, Egypt).

Records collected on two herds of buffaloes maintained by the Ministry of Agriculture were analysed to study the inheritance of some dairy traits and to investigate the interrelationships between these characters. More than 400 buffaloes having 1614 records were included in this report. The repeatability of 305 day milk yield, total milk yield, maximum weekly production, intensity, persistency and lactation period were 0.40, 0.41, 0.34, 0.39, 0.18 and 0.27 respectively. The corresponding heritability estimates were, 0.26, 0.27, 0.43, 0.38, 0.33 and 0.27 respectively. The phenatypic as well as the genetic correlations between characters studied were high which indicate that most of these characters are affected by the same sets of genes.

SECTION 15

HUMAN GENETICS

15.1. Biological and Genetical Foundations of Great Historical Features. MARIO TIRELLI (Rome, Italy).

A brief exposition of the phenomena, observations, criterions which may permit previsions on the evolution of human groups, and thus on the evolution of the reciprocal relations between the groups themselves, from which depend precisely the development and the succession of the great features and historic events.

15.2. Phylogenetic Relationships of the Forms of Erythrocyte Carbonic Anhydrase in Primates. RICHARD E. TASHIAN and DONALD C. SHREFFLER (Ann Arbor, U.S.A.).

Characterization of the non-heme erythrocyte proteins from a number of representative primate species of the families Lorisidae, Cebidae, Cercopithecidae, and Pongidae has revealed the presence in some species of two distinct molecular forms of carbonic anhydrase (CA). These forms are designated as CA-I and CA-II. In addition to carbonic anhydrase activity, these enyzmes also exhibit esterase activity toward α and β-naphthyl acetate, with CA-I possessing markedly greater esterase activity than CA-II. The electrophoretic separation patterns and degree of esterase activity for CA-I appear to be species specific. However, two variants of carbonic anhydrase-I (CA-Ib and CA-Ic) have been described from human hemolysates and demonstrated to be under genetic control; a variant of CA-I from an orangutan hemolysate has also been observed.

Additional evidence that the CA-I of different species are homologous enzymes and that CA-I is distinct from CA-II has been obtained from immunodiffusion studies utilizing specific rabbit anti-human CA-I serum.

Often, both electrophoretic and immunological characterization were necessary to establish tentative phylogenetic relationships among species. For example, although limited immunogical differences could be detected within the species of a particular family, they could usually be readily separated on the basis of their combined electrophoretic patterns and esterase activity. To a lesser extent, species exhibiting similar electrophoretic patterns and enzyme activity were shown to differ immunologically. The application of these methods to the study of primate evolution will be discussed.

15.3. Modern Concepts in Clinical Genetics. ROBERT L. TIPS and G. SMITH (Portland, U.S.A.).

In modern practice clinical genetics can be considered the application of genetic knowledge in an effort to solve the diagnostic, prognostic, and management problems of the patients and their families. Subtle but severe medical emotional, and socio-economic disturbances are universally expressed by these families following the introduction into the family environment of a child with disease of genetic origin. A specifically trained team, coordinating their efforts, can isolate and evaluate these problems, so that the affected family can gain understanding and a healthy adjustment.

The patient, parents, and siblings are evaluated in an appropriate clinic setting by the specialists —the geneticist, who explores the total kindred history with emphasis on reproductive patterns; the physician, who evaluates the impact of the pathological processes on the entire family; the social worker, who relates to the problem extended into the socio-economic milieu of the kindred; the technicians, who perform the battery of genetic tests on each member of the family. The elimination of emotional factors emanates from conference discussion of the team after their interviews and tests are complete.

The discussion is amplified by a review of four families whose problems were resolved by the clinical genetic counseling team.

15.4. A Genetic Discriminant for Diseases of Heterogeneous Origin. BERTRAM L. HANNA, A DONALD MERRITT, CHARLES WM. TODD, JR. and TERRY L. MYERS (Indianapolis, U.S.A.).

Many diseases which segregate within families as expected under a Mendelian model demon-

strate clinical heterogeneity which suggests that all cases may not be of the same genetic etiology. Some have been separated with discriminate function analysis of clinical data or by the demonstration of distinct biochemical differences within diagnoses. These methods have failed to define homogeneous groupings within other diseases, including diabetes mellitus and cystic fibrosis. On examination of the frequencies of occurrence of a given disease in relatives of an affected sibship an unbiased estimate of the allelic frequency in the population may be obtained on the assumption that homozygosity at a single locus is responsible for all cases. The population incidence estimated under this assumption will be less than the observed population incidence if the same gene locus is not segregating in all observed families.

Differences in the survival of affected individuals, age of onset and parental genotypes require that different models be developed for diabetes mellitus and cystic fibrosis on the assumption that both of these are autosomal recessive traits. The problem of reliable sampling in cystic fibrosis families may lead to gross bias in the fitting of the theoretical model. A method is developed to obtain an approximation to the population allelic frequency which deviates by only 0.5 per cent from that value expected under the theoretical model.

From study of 754 cousins of cystic fibrosis probands a population incidence of 1/3824 is obtained. The similarity of this estimate to that obtained from an epidemiologic study of a comparable population (1/3700) suggests that only one gene locus is involved in the etiology of cystic fibrosis.

15.5. (D.) The Application of Automatic Record Linking Procedures. J. H. Edwards, I. M. Leck, T. McKeown, and R.G. Record (Birmingham, Great Britain).

The application of automatic record linking procedures and other methods dependent on advanced computational methods to the Birmingham data. These include detailed notifications of all births, with special reference to malformations, and extensive linking of other data. Births are identified by both mother and child and sibships formed automatically as they arise.

15.6. On the Statistical Significance of One Pair of Monozygotic Twins in Clinical Genetics. Luigi Gedda (Rome, Italy).

A case of one twin pair concordant or discordant as to a given disease is always very important for Clinical Genetics, especially when it is monozygotic. In Clinical Genetics, isolated pairs occur to the physician, and the diagnosis must therefore be based on intra-pair comparison. The statistical treatment of the case must consider the fact that a concordant monozygotic twin pair represents a statistical universe rather than a statistical unit. This entails taking into account many traits concerning the time of onset, the symptoms, development, result of treatment, etc. Suggestions are made for the statistical treatment of such cases.

15.7. First Findings on the Behaviour of Red Blood Cells from MZ and DZ Twin Pairs in Isoniazide-fixation Tests (in Vitro). M. Bolognesi, U. Fantoli and M. Milani-Comparetti (Rome, Italy).

An isoniazide-fixation test, described in the paper, was carried out *in vitro* on red blood cells from 30 female twin pairs, in order to investigate its usefulness in zygosity determination. The zygosity classification of the material was based on several criteria of similarity including blood groups (ABO, NM, Rh-Hr).

The material was divided in two groups, respectively under and above 16 years of age (to guard against the higher frequency of fast inactivators among individuals under 15, denounced by Sunhara).

Several tables contain the findings and statistical analyses; in particular Table V indicates that only the variability due to zygosity exeeds Fisher's F, being ascribable to chance only with one probability out of 1000. The authors conclude that the factor responsible for individual behaviour in the test as applied seems to be under genetic control, thus making it potentially useful as a further test in zygosity determination. Further research tends to:

— determine the correlation between behaviour in this test and the fast or slow isoniazide inactivator trait *in vivo*.

— establish the limits of individual and intra-individual variability for methodological codification.

— extend the study to a wider sample, applying Kallman's twin family method to identify the mode of transmission of the trait.

15.8. Blood-groups and Haptoglobines by 200 Twin-pairs. Hans Niermann (Münster, Germany).

For the diagnosis of twins it can be important

to use blood-groups and haptoglobines. The blood-groups and haptoglobines were determined in 200 twin-pairs. All of 56 monozygotic twins were concordant, while only three of 81 twins with the same sex were concordant.

15.9. Disparity of Colour Vision in Uniovular Twins. J. ZANEN and A. MEUNIER (Brussels, Belgium).

The authors relate two cases of uniovular twins. The colour vision normal for one of them, is wrong for the other and belongs respectively to protanopia or deuteranomaly group.

They add a third case where the twins are both deuteranomalous, but the disparity was so slight that it was not significaat.

15.10. Banks for Frozen-Stored Human Spermatozoa. J. K. SHERMAN (Little Rock, U.S.A.).

Feasible applications of frozen-stored human spermatozoa include: clinical treatment of infertility with donor or husband's semen, maintenance of husband's fertility in absence of testes, in old age and in death; ready availability of spermatozoa bearing desired genetic characteristics for unusual blood types, etc; protection from radiation induced genetic alterations on earth and in space; and eugenically directed population control and selection. Successful banking of human spermatozoa preserved by freezing is dependent upon several considerations. In addition to simplicity and efficiency, methods employed must favor minimal loss of spermatozoal motility, retention of fertilizing capacity, and absence of genetic alteration. Superiority of the author's combined freezing and storage method using liquid nitrogen-vapor([1]) is shown in comparison with earlier dry-ice method. After an average freeze-thaw survival of 70 per cent, no further motility loss occurred during storage at —196°C for three years, the longest period observed, while 10-20 per cent was lost at —75°C. Retention of fertilizing capacity of human spermatozoa preserved by nitrogen-vapor method is reported. Possibility of freeze-thaw induced genetic damage is recognized. Integrity of normal and malignant cells for research is maintained in frozen-storage and frozen bovine semen is used routinely to breed millions of dairy cattle. No alteration in chromosomal structure or reflected cellular function has been reported with mammalian somatic cells preserved by freezing. An increase in abnormalities of progeny has not accompanied the use of frozen-stored bovine and human spermatozoa. The current view is that there is no such genetic damage.

1. *Fertil. & Steril* **14,** 49-64, 1963.

15.11. Selection Relaxation in Man. R. H. POST (Ann Arbor, U.S.A.).

The relaxation of selection pressures against deleterious traits resulting from cultural growth can be demonstrated by the markedly higher frequencies of red and green "colorblindness" among populations in Europe and the Far East, contrasted with the lower frequencies prevalent among populations living in hunting habitats, such as Australian aborigines, Eskimos and certain American Indians. Frequencies are approximately four times higher in the former populations than the latter, i.e. some 6 and 1.5 per cent, respectively, for the mutant alleles at the deuteron locus, and some 2 and 0.5 per cent respectively, at the protan locus.

The demonstration depends upon certain assumptions which appear reasonable, although they are not yet proven. Evidence is presented for the selective disadvantage of "colorblindness" in hunting habitats and its relative unimportance in most walks of life among non-hunters.

The two loci are quite closely linked on the X-chromosome. Diagnosis is fairly facile and reliable at early ages, environmental effects and age changes are negligible, and published data are sufficient to permit comparisons among populations which vary in habitat or cultural history.

Rough estimates of mutation rates can be made by dividing the difference in gene frequencies between hunters and non-hunters by the number of generations which have elapsed since the ancestors of the latter relinquished their hunting habitats, under the assumption that relaxation has been complete since that time.

Analysis of the frequencies of "colorblindness" among geographic areas within several countries together with their cultural histories gives further support to the relaxation hypothesis, viz. Britain and France, Japan and China.

Population differences in vision acuity and hearing acuity offer less satisfactory opportunities for demonstrating selection relaxation. Frequency data for several different populations are presented, with discussion.

15.12. (D). Consanguinity Studies in Italy. A. MORONI (Parma, Italy).

An analysis of consanguineous marriages which have taken place in Italy from 1910 to the present has been started, using consanguineity dispensations available in bishopric archives and at the Vatican.

About 500.000 dispensations have been examined and are being transferred to magnetic tape for (a) statistical analysis with a view to detect what can be learnt on population structure from the study of the frequency of consanguineous marriages, (b) linkage, with other types of investigations on records (e.g. hospital, school records, etc.) in order to estimate the effect of inbreeding on mortality disease, anthropometric characters, etc.

15.13. Recent Investigations about the Direct Estimation of the Mutation-Rate of Autosomal Dominant Genes in Men. WILHELM TÜNTE (Münster, Germany).

The problem of the frequency of spontaneous mutations in men is an important field of research at the Institute of Human Genetics in Münster. The starting point for these investigations is the Genetic Register for the administrative area of Münster having been established in 1957. On the basis of experience hitherto existing, it will be discussed to what extent special hereditary diseases are suitable for estimating the mutation-rate. In this connection investigations are reported that are now in operation or have been closed in the institute mentioned above. Preliminary findings will be discussed for syn- and polydactylie. Complete results will be brought about multiple cartilaginous exostosis and Osler's disease.

15.14. Sex Ratio Shifts among Progeny from Patients having received Therapeutic X-Irradiation. P. J. L. SCHOLTE and F. H. SOBELS (Leyden, The Netherlands).

The material was collected from four hospitals, situated in The Hague, Leyden and Rotterdam. Selection was made for patients who, following exposure to gonad doses of a few hundred rads, had progeny and who at the time of irradiation did not age more than 36 years for women and 41 years for men. Out of a total of 85,000 patients these conditions were fulfilled with only 183 women and 748 men. The material could be expanded by cases which had been collected by E. Kruyff in an investigation on the induction of leukaemia following radiotherapy of ankylosing spondylites. In addition, progeny was scored from a group of males having received low gonad doses of 1-10 rads. Data on the sex ratio, expressed as the percentage of males in the progeny, were extracted from birth records in the municipal registration offices. Following maternal irradiation with 70-270 rads the sex-ratio decreased from 0.541 (242 births) before irradiation to 0.485 (230 births) after irradiation. The shift is not significant, but the data show no heterogeneity with those obtained by Turpin, Lejeune and Rethore (1957) in France. For the combined sets of data the decrease of the sex-ratio after exposure to radiation of women is significant ($P = 0.023$). Since any of the well-known factors influencing sex-ratio, such as birth order, paternal age and annual fluctuations, including war, cannot account for the observed decrease in sex ratio, we feel justified in concluding that it was brought about by radiation-induced genetic changes. Non-disjunction would have resulted in an increase of the sex ratio. The consistent decrease in this and similar studies suggests then that the frequency of non-disjunction in irradiated women, is negligibly small relative to the frequency with which recessive lethals or loss of the X-chromosome are induced. Following paternal irradiation (25-370 rads) the sex-ratio increased significantly ($P = 0.035$) fiom 0.470 (1258 births) before irradiation to 0.525 (932 births) after irradiation. Here, however, the sex-ratio before irradiation was abnormally low and differed significantly from the population average, thus no definite conclusions can be drawn. In contrast to results of Lejeune, Turpin and Rethore (1960), no change in the sex-ratio was observed after paternal exposure to doses of 1-10 rads.

Subsidized by the Organisation For Health Research T.N.O.

15.15. Investigations on the Low Sex Ratio in a Human Population. I. LESTER FIRSCHEIN and DIANE SANK (New York, U.S.A.).

A further statistical analysis of the sex ratio within families will be made in a population known as the "Black Caribs" of British Honduras, Central America. Previously it was shown

that in a sample of mothers with sickle-cell trait (Hb^A Hb^S) the sex ration among their children is normal whereas in the sample of Hb^A Hb^A mothers, the sex ration was low. The observed equilibrium values for sex ratio in this population are very close to the calculated equilibrium values based on the observed frequency of the sickle-cell trait in adult males and females. The relationship between sex ratio of a population and level of malarial immunity will be explored.

15.16. Does there Occasionally Exist an Incompatibility of Mothers with Their Male Offsprings? K. O. RENKONEN (Helsinki, Finland).

The known phenomena of incompatibility are enhanced by repeated pregnancies or transplantations. Accordingly, in a study of incompatibility of mothers with their male offsprings the first question should be: how are the sex ratios (m/f) of live births influenced by repeated pregnancies. It is well-known that the sex ratio is steadily decreasing by birth order. As such it is not a sufficient argument for incompatibility, because the primary sex ratio could also have been decreasing by birth order. That would imply a decrease also in the sex ratio of stillbirths by birth order, but there is a remarkable increase instead.

If the decrease of the sex ratio by birth order is due to incompatibility, it should be manifested not only by repeated pregnancies but also by repeated births of boys. The families that have already got both boys and girls should then have the highest sex ratio of the next child, if there is only one boy among the precedent children; lower, if two boys were already born, and lowest, if there were three of them. The observed statistical data are in agreement with this prediction.

Let us suppose that some of the mothers in one type of families are incompatible with their male offsprings, but all are compatible in the other. Then the birth interval to the next child should be lengthened among the families including incompatible mothers. The observed data suggest that it is so. In addition, the lengthening of the birth interval should be caused only by those intervals terminating with a girl, just as it is, for, if the birth interval is terminated with a boy, the mother can hardly be incompatible. In conclusion I should like to interpret the statistical data to support the assumption of an occasional incompatibility of mother with her male offspring.

15.17. Studies on Colour Blindness among the Tribals and Non-tribals of Andhra Pradesh, India. K. R. DRONAMRAJU (Orissa, India) and P. MEERA KHAN (Andhra Pradesh, India).

The Polavaram Agency area of Andhra Pradesh in southeastern India has, according to the 1961 census, a population of 22,461 non-tribals and 23,810 tribals. The non-tribals are largely Hindus, but there are a few Christians and Muslims also. The tribals include Koya Doras, Koya Kammaras, Musaras, Konda Reddis, Konda Rajus, Sugalis and Pandava Nayakas. Tests using Ishihara (1960) Plates, showed that 6.5 per cent of 569 non-tribals and 2.5 per cent of 1155 tribals were colour blind. 1.5 per cent of non-tribals and 0.34 per cent of tribals were Protans. The tribes in which one or more colour blind men were discovered are Koya Daras, Musaras, Konda Rajus and Sugalis. The low frequency of colour blindness found among these tribals is in general agreement with that reported in Navajo Indians, Australian aborigines, and some African tribes. The difference between the frequencies among the tribals and non-tribals in Andhra Pradesh is highly significant ($X^2 = 16.40$) and is explained as due to the relaxation of selection against colour blindness in civilized communities.

15.18. The Incidence of Cuna Moon-Children. CLYDE E. KEELER (Milledgeville, U.S.A.).

The incidence of Cuna Indian Moon-child albinos is more than 60 per 10,000 population, being reduced from about 69 per 10,000 in 1925. Stout's 1940 estimate of 47 per 10,000 is probably in serious error. Our 1962 data suggest infanticide is uncivilized towns and possibly selective interbreeding of heterozygotes in the town of Nargana. Absolute population figures cannot be obtained, due to census methods and unrecorded absentees. Our figures show albinos to be less viable than normals, in keeping with general Cuna belief. Since albinos seldom reproduce, the gene frequency in one generation without mutation should have been reduced to 57 per 10,000, so there is evidence that mutation does take place. It is estimated that about 398 mutations per generation per 10,000 population are added to the gene pool in order to provide the 5 new albino homozygotes per generation necessary to keep the incidence at equilibrium. Examination of our 1950, 1960 and 1962 data all show Moon-child albinism to be due to a single pair of recessive, Mendelian, mutant genes.

15.19. Contribution to the Study of Isolates in Hight-Normandy (France). P.-Y. ROUSSEAU, C. ROPARTZ and R. KHÉRUMIAN (Rouen, France).

In this data, the study of the isolates has not been performed with the use of the bias of the consanguinity coefficient but by the direct study of populations' movements.

Investigations among 9700 blood donors living in Seine-Maritime (France) allow us to study and precise the migrations of weak amplitude inside a french department.

The following distances have been analysed for each subject (from 18 to 60 years old).

Distance between the birth-places of its parents
between the birth-place of the subject and these of its parents
between its birth-place and that of its spouse.

Many biological characters, with a well known genetical determinism, such as the blood groups A. B. O and Rhesus, the dyschromatopsia, and the curling have been gathered up in view to define the best criterium for an anthropological sampling.

It has appeared to us that if the coefficient of consanguinity has lowered is a very important rate, the whole population geographically stand very stable.

The mean estimation of the amplitude of the movements is about 8 to 10 km. Nevertheless, notable differences are found following the geographical position = (sea-side for example or presence of a river).

These phenomenon when studied in function of the ages of the subjects allow to sketch the chronological variations.

15.20. Demography and Genealogy of Different Types of Isolates. ROBERT GESSAIN (Paris, France)

The Anthropology Research Centre (Musée de l'Homme), the establishment of which was announced at the Rome Congress, has now become a laboratory of the third section (biology) of the Practical School of Higher Studies. Its research workers are now engaged in the study of several endogamous human groups from the anthropological, demographic and sociological view points. These groups, whether monogamous or polygamous, patrilineal or matrilineal instructive, show *different* characteristics.

The Angmassalik eskimos are a closed isolate for whom registry office records are available since 1900, in addition to which we have census lists for the entire population for 1884, the data of their discovery, and for 1935, the date of our study.

In Brittany, the two villages under study form what can be called "open" isolates for which are available all relevant Registry Office records since 1800.

In Senegal, we are studying two groups, without any such records, on the basis of incomplete lists taken at the official census and genealogical data collected directly from the inhabitants. One of these groups is an almost completely closed isolate whose patrilineal inhabitants are strongly attached to the soil; the other is an isolate "open" on neighbouring groups, matrilineal in structure, with frequent comings and goings between villages near and far.

In Angmassalik only one migration, affecting one third of the population, male, female and children, has taken place at a precise date, while in Brittany emigration, which affects 25 per cent of the inhabitants, is continuous and difficult to situate at any given time; in Senegal the societies being studied have only been exposed very recently to migration.

Thus the varying state of these isolates, some more open than others, the completeness or lack of population statistics and the different state of migrations in each condition the difference in methods and lead to fruitful comparisons, although these isolates are far removed from each other in space and in their degree of endogamous evolution.

15.21. Human Genetic Studies on an Isolated Population in Åland (Finland). A. W. ERIKSSON and H. FORSIUS (Helsinki, Finland).

In Kökar, an isolated island in the Åland archipelago lying in the Baltic Sea between Sweden and Finland, investigations on population genetics have been made. The well-preserved church records going back over 300 years have provided an invaluable source of information on pedigree, sex ratio at birth, twinning rate, consanguinity, etc. Medical, and especially haematological and ophthalmological examinations, blood and serum grouping, serum cholesterol, serum B_{12} vitamin, P.T.C. taste tests and other biochemical and anthropological tests have been performed on the *ca.* 550 registered Swedish-speaking residents.

The investigations are still in progress but among the results that have already emerged, the following may be mentioned: endogamous marriages have been very common; the rate of multiple confinements has been very high; the

dizygotic twinning rate has decreased significantly during an observation period of 200 years (similar results have also recently been observed for Åland as a whole and in Sweden).

Not only the inhabitants shown by the genealogical analysis to be of pure native descent for at least 4 generations but also the population as a whole, have some extreme blood groups frequencies. The A-blood group percentages 67-70 (gene frequencies: $p_1 = 0.27\text{-}0.28$, $p_2 = 0.17\text{-}0.18$) are to our knowledge the highest hitherto reported for a white population. The frequency of D-negative individuals is very high. The frequencies of the following genes are low: q in the ABO-system, M in the MN-system, Hp^1 of the haptoglobin serum groups, Gm^x of the gamma globulin groups and Gc^1 of the group specific components ($Gc^1 = 0.615$ is the lowest yet recorded in the literature).

Some hereditary diseases, such as recessive autosomal tapetoretinal degeneration and the Åland Islander bleeder syndrome (v. Willebrand's disease, Thrombopathia v. Willebrand-Jürgens) have a noteworthy frequency.

15.22. The Blood Groups of Ashkenazic Jews. F. Ottensooser, N. Leon, M. Sato and P. H. Saldanha (São Paulo, Brazil).

The genetical diversity of Jewish people and the persistence of their communities as isolates within other populations pose interesting evolutionary problems.

From 586 B.C. to modern times there have been several Jewish dispersal movements which resulted in four main Jewish groups: *Ashkenazic* or Eastern European Jews, *Sephardic* or Mediterranean Jews, *Oriental* or Asiatic Jews and *Yemenite* Jews. Before and during dispersal, Jewish populations acquired various non-Jewish components and gene flow into Jewish communities appears to have been strong during captivity in Egypt and in the Roman Empire.

Several polymorphic traits have been studied among Jewish populations of various origins and blood groups proved to be especially useful for comparing Jewish populations with their neighbours.

The ABO, MN and Diego blood groups of 100 Ashkenazic Jews in São Paulo, Brazil were investigated. All subjects were Diego-negative. The ABO and MN frequencies were similar to those in previous Askhenazic samples. Rh frequencies presented typical Mediterranean distributions, Ashkenazic Jews showing higher *B* and R^0 gene frequencies and lower *r* frequency than their neighbours. Probably these characteristics were inherited from ancient Hebrews in Palestine.

The high *B* values of the Ashkenazim and their low values of non-tasters and Rh negatives are not derived from intermarriage with Mongoloid people but a Mediterranean heritage as evidenced by absence of Diego-positives and high R^1 values. The incidence of R^2 gene also suggests that an African component similar to that found today in Egyptians was present before the diaspora. The Jews that preserved a small but constant Negro component during thousands of years are an example of social isolate comparable to endogamous groups in India.

15.23. A Comparative Study on Blood Groups Distribution among Normal and G6PD Deficient Subjects in Israel. A. Adam, L. Dressler, C. Sheba and A. Szeinberg (Tel Hashomer, Israel).

A recent investigation demonstrated significantly lowered frequency of the E gene (Rh system) among glucose-6-phosphate dehydrogenase (G6PD) deficient American Negroes, as well as a tendency toward differences in the B and O blood groups distribution, between them and the normal controls (1)

In a similar study, carried out in Israel, about a thousand normal and 350 G6PD deficient subjects were tested simultaneously for G6PD activity and for the distribution of the Rh and ABO blood groups. The investigated population included representative samples of several communities (Jews from Kurdistan, Iraq, Iran and Yemen and Israeli Arabs) in which G6PD deficiency is frequent.

No significant differences were detected between the distribution of the various Rh genes among C6PD deficient and normal subjects in each of the communities investigated.

Similarly no deviations from homogeneity was found in the ABO distribution except for the Kurdish community. In this sample (102 normals and 112 G6PD deficients) a significantly lower frequency of 0-group and higher frequency of A group was found among the G6PD deficients ($P<02$).

A larger population sample is being studied in order to examine the significance of this observation.

---

1. Tarlov, A. R., Brewer, G. J., Carson, P. E. and Alving, A. S. Primaquine sensitivity, *Arch. Int. Med.* **109,** 209 (1962).

This investigation was supported by grants

A-2740 (Hema) from the U.S.A. Public Health Service and R/00014 from the World Health Organization.

15.24. The Problem of the Associations Between Blood Groups and Disease. ANGELO CRESSERI and ANGELO SERRA (Milan, Italy).

It has been frequently stated that research on the associations between blood groups and disease would have ensured an important contribution to the understanding of the workings of natural selection, and given the chance of opening up new methods of research on important diseases.

Of course both are goals that suppose (1) a detailed knowledge of all the blood groups of a given system effectively associated with a particular disease, and of what is the degree of association; (2) a definite demonstration of the constancy of the phenomenon of the association between blood groups and a disease.

With a view to get consistent information on these to fundamental points, an analysis has been done—according to classic models and with the help of an electronic computer—of most published data since 1953. 1162 samples in all were analysed; 115 of them ought to be excluded, because the frequency distribution of the blood groups in the control subsamples deviated significantly—the significance level having been fixed at the probability point 0.01—from that expected in the hypothesis of panmixia, thus suggesting non-randomness in the sampling. The other 1057 samples are unevenly assignable to 273 diseases; subdivisions by age, sex and clinical criteria increase up to 330 the independent classes.

The detailed results will be published elsewhere. A few general conclusions seem worthy to be outlined here.

(1) No clear-cut statement, relevant to the two fundamental points mentioned, may at present be done for the majority of the diseases studied up tonow, because of scarcity of samples available for the analysis.

(2) The relative risks of morbidity of given blood groups, estimated from various samples of the same disease, are often significantly heterogeneous, even though either each one or most of them deviate significantly from unity as the combined estimate also does. That happens, for instance, for the risk A : O in the cases of gastric cancer (58 samples), peptic ulcer (35 samples), duodenal ulcer (31 samples) and of the cancer of uterus (cervix) (16 samples). This fact would suggest that, when the existence of a peculiar association may be considered as statistically proved, one should take in account also the possibility that systematic factors affect differently the degree of correlation.

(3) Many more researches at the biometric level are still highly desirable, in order to reach reliable and meaningful conclusions.

15.25. The Genetics of Insensitivity to Pain: Studies on Two Families. N. LEON, W. BEÇAK, M. L. BEÇAK, B. J. SCHMIDT, F. OTTENSOOSER and P. H. SALDANHA (São Paulo, Brazil).

Congenital analgesia is a very rare disease. About sixty cases have been reported in pertinent literature but its etiology seems rather obscure mainly because of the variable expressivity of the disease. Most propositi are sporadic cases which are identified by their indifference to painful traumatisms. They frequently have sequels of bone fractures and tongue cuts. Both sexes seem to be equally susceptible and occasionally members of the same sibship present the same condition. The parents of the affected subjects are frequently normal and sometimes consanguineous.

Two families, including various members with congenital analgesia have been examined. Both families live in country towns of the State of São Paulo. In the first family, the propositi are children of normal and unrelated parents. The sibship is represented by 8 living and two deceased sibs. Two brothers, 8 and 2 years of age, are affected, one of them suffering from bilateral cryptorchidism; a third affected brother died with six months of age. A girl belonging to the same sibship has *heterochromia iridis* and a cousin of the propositi, on the paternal line, has club foot.

The second family includes ten living and one deceased sibs. Two of them, 6 and 11 years old, present analgesia. The parents of the propositi are normal and display multiple consanguinity ($f = 0.0703$). It is noteworthy that the father is the only son in a sibship of 11 individuals. Cleft palate occurred in two cousins of the propositi.

It should be emphasized that in the two families examined, the parents are normal, one of the families presenting a high inbreeding coefficient. The rate of normals to affected members in both families is 5 : 16. These findings suggest recessive monogenic inheritance.

The ABO, MN, Rh blood groups and salivary secretion have been analysed in all members of both families. No close linkage between these "markers" and analgesia congenita is apparent.

Cytologic studies of peripheral blood from affected as well as normal members were performed. A defect involving an extra chromosome was found to be present.

15.26. Deviations from the Mendelian Laws in Human Families. The Action of Natural Selection on Blood Groups in Twins. L. E. Nijenhuis (Amsterdam, the Netherlands).

Previous population studies gave evidence for strong action of natural selection on blood groups, which varies more together with the genome composition than with environmental factors[1]. The explanation could be that certain genomes provide more viability than others. Other indications for this phenomenon might be found in family studies, and especially in twin studies, because the less viable partner may be superseded by the other. Dizygotic twin partners are more often double concordant of discordant for factors belonging to two different blood group systems than would be expected.

As to the MN and Rh groups an excess of 10 per cent double discordance and concordance is observed for the single gametes transferred by the parents, independent of the way of combination, i.e. MN and Rh alleles from the same parent or not. This gives rise to the conclusion that selection takes place after fertilization. If in the parents combination heterozygosis exists for both systems, the excess is 22 per cent. This is only 12 per cent if both parents are homozygous for MN or Rh, which indicates that the selection is exercised on genes linked with the blood group loci. Excess of double discordance and concordance can also be observed, though in a lower degree, in non-twin family studies.

1. Nijenhuis, *2nd Intern. Conf. Hum. Genet.*, Rome, 1961.

15.27. Genetics and Linkage Relations of the Lp Serum Type System. Jan Mohr and Kåre Berg (Oslo, Norway).

The discovery of a serum type system concerning human β-lipoproteins is reported.

From a material of Norwegian unrelated individuals and families a hypothesis of determination by a gene pair Lp^a and Lp^a is proposed, the estimates of gene frequencies in the Norwegian population being about 0.19 and 0.81 respectively.

Linkage relations with other marker systems are considered.

15.28. Linkage Studies in Families with Pathological Dominant Traits. Walter Scholz (Münster, Germany).

Linkage studies in man are made more promising by the availability of an increasing number of well-suited and well-defined marker genes, especially in serology. Families showing regular dominance of pathological traits are best suited for such investigations. The following antisera have been used in the present investigation, anti-A, -B, -AB, $-A_1$, $-A_2$, -M, -N, -S, -s, -P, -D, -C, $-C^w$, -c, -E, -e, -K, -k, $-Fy^a$, and Jk^b.— Haptoglobin types and gamma globulin groups Gm(a) and Gm(x) were used as additional markers. Linkage data were received for families with dominant characters as hereditary multiples exostoses, telangiectasia hereditaria (morbus Osler), polydactyly, syndactyly, ectrodactyly and others. The results of these investigations will be given in particulars and discussed.

Details will be published in the *Z. f. menschl. Vererb.-u. Konst.-Lehre*, 1963.

15.29. Scoring for Linkage Between Several Loci. Cedric A. B. Smith (London, Great Britain).

The detection and estimation of linkage between two loci is most simply done by using Morton's z scores. However, when tests are made on three or more loci on the same chromosome, as already happens on the X chromosome, new problems arise. Families may be investigated because of the presence of one sex-linked character, and then used to determine the strength of linkage between two others. This will require the use of new types of score correction.

15.30. Male and Female Recombination Fractions in Man. J. H. Renwick (Glasgow, Great Britain).

The linkage data from 27 pedigrees of the nail-patella syndrome have been analysed by the computer programme of Renwick and Schulze (1961). A search has been made for inconsistencies in the *nail-patella:* ABO linkage of the

type (1) that might indicate the existence of a mimic locus and of the type (2) that might indicate differences (a) between recombination fractions in men and women or (b) between recombination fractions in persons of different ages. The findings will be discussed. It is hoped that the analysis will be published in the *Annals of Human Genetics*.

15.31. Hereditary Factors in Longevity. M. HAUGE, B. HARVALD and B. DEGNBOL (Copenhagen, Denmark).

The influence of hereditary factors on the life-span of the individual has been estimated by comparing the intra-pair difference of age at death in 239 monozygotic, 414 dizygotic, same-sexed and 387 dizygotic, different-sexed twin pairs. The average differences of age at death in these groups were 14.5 years, 18.7 years and 20.4 years; the difference between monozygotic and dizygotic pairs was significant ($0.01 > P > 0.001$), thus proving that heredity plays a role as a determinant of the life-span. The relative importance of heredity compared with that of the sum of environmental factors turns out to be increasing within modern society, where the environment is nearly optimal, and environmental differences are unimportant.

15.32. Family Patterns of Mortality and Longevity. BERNICE H. COHEN (Baltimore, U.S.A.).

Two studies wil be discussed: (1) a community based population study and (2) a special group study.

The first investigation is designed to determine whether there are family patterns of mortality and, if so, the nature of those patterns in terms of age at death, cause of death and cause-age interaction. For this purpose, a sample of 550 deaths occurring in Baltimore city to U.S. born white residents has been selected and is being matched with living individuals (controls) of the same sex, race and neighborhood and U.S. born within 5 years prior to the deceased probands. The mortality experience of relatives will be compared with that of the corresponding family members of the matched living controls. Interfamilial comparisons of probands dead at different ages and of different causes will be examined.

The second investigation deals with "Test Responses, Aging and Family Mortality Patterns" in a group, comprising 500 living white males from 24 to 100 years of age who have volunteered to participate in a longitudinal study of aging being carried out by the National Heart Institute, with periodic physiological, biochemical, psychological tests and anthropometric measurements. In addition, this investigator is obtaining PTC tests, smoking histories and detailed family and personal data similar to that being collected in the community study. The purpose of this study is to determine whether there is a correlation between family mortality patterns and differences in test responses of living individuals, the latter possibly being precursors of a genetically determined "death syndrome" to follow.

15.33. Dominant Inheritance of a Metric Trait in Man. ITALO BARRAI (Pavia, Italy).

The mode of inheritance of stature and chest girth in man has been investigated using measurements taken from 3202 males of average age of twenty years, whose immediate inbreeding value was known.

Environmental factors were found to play an important role in the final phenotypic expression of the trait stature.

Chest girth shows less sensitivity to social and environmental factors; the data agree quite satisfactorily with the hypothesis of an average dominance of factors determining "large" size.

15.34. Familial Correlations in Human Obesity. R. F. J. WITHERS (London, Great Britain).

Obesity has been defined as being 20 per cent overweight. Parent-child and sib-sib correlations have been calculated for several populations of individuals. These include a group of adopted children. This enables the amount of environmental variance to be calculated. A study of the body build of the people investigated and familial correlations of the components of their somatotype, show that overweight by itself does not appear to be a reliable phenotype for use in the genetical study of human obesity.

15.35. The Effects of Parental Blood Groups on Birth Weight of the Offspring. HENRY GERSHOWITZ, ATMARAM H. SONI and MILLICENT W. PAYNE (Ann Arbor, U.S.A.).

From among a sample of 742 married couples

with 1820 children, selected for completed reproduction (wife age 40 or over), complete birth weight information was secured for 1715 children. Tests performed on the parents of these children included blood type determinations for the ABO, MN, Rh, P, K, and Fy^a systems and secretor status for ABH blood group substance. Birth weights for all children were as reported by the parents.

Since the usual weight differential for the two sexes (892 males, 823 females) was found ($P<0.001$), all analyses were performed on male and female children separately. The following significant effects were noted for male children only, after correcting for the effect of parity: (1) paternal main effects: (a) ABO ($P<0.025$)—type B fathers have heavier sons, type A fathers have lighter sons; (b) MN ($P<0.05$)—type N fathers have heaviest sons with M and MN fathers following in that order; and (c) Rh ($P<0.005$)—the paternal gene *r* seems to contribute most to an increase in birth weight, while the R^2 gene seems to be associated with a below average weight, and (2) maternal main effects: confined to the Kell system ($P<0.05$)—Kell positive mothers have heavier sons than Kell negative mothers. Probability values of 5 per cent may be subject to some uncertainty in view of the number of tests of significance performed.

The data for female children are being analyzed and will also be presented.

Supported in part by U.S.P.H. Grant H-4145 from the National Institutes of Health and in part by AEC Grant AT (11-1) 405.

15.36. A Study of Seven Blood Group Systems in Sterility and Child Mortality. T. Edward Reed, Henry Gershowitz, and Atmaram Soni (Ann Arbor, U.S.A.).

A sample of 742 married couples, the wife being 40 or more years of age, from a town in Michigan, U.S.A., was studied. All couples were of European ancestry and the only selection was for willingness to cooperate. For each couple a personal, social and reproductive history was obtained and blood group phenotypes in the ABO, Rh, MN, Kell, P, and Duffy systems, as well as ABH secretion, were determined. The results for two reproductive indicators, (1) proportion of couples (first marriages, married for 10 or more years) who were sterile, and (2) proportion of liveborn children born to these couples who died non-accidentally under 5 years of age, are reported here.

Two-way analysis of variance (husband's group one way, wife's group the other) for each of the 14 blood group-indicator combinations yielded the following significant items—Sterility analysis: MN group of the wife ($P<0.005$, N women most sterile), Kell group of the wife ($P<0.025$, Kell + wives most sterile); Child mortality analysis: ABO group of father ($P<0.001$, O fathers highest), P group of mother ($P<0.005$, P— mothers highest), P group of father ($P<0.05$, P— fathers highest), Rh group of mother ($P<0.05$, R_1R_2 mothers highest), and MN group of couple ($P<0.05$, interaction item).

This research was supported by a grant from the U.S. Atomic Energy Commission. A complete report of this study will be submitted to the *American Journal of Human Genetics*.

15.37. (F). **Teratology.** P. H. Saldanha (São Paulo, Brazil).

1st part: Genetical teratology

The first part of the film describes simple rules of dominant and recessive inheritance. As examples of these hereditary patterns, pedigrees of achondroplasia and the Laurence-Moon syndrome are presented.

The chromosomal constitution of the normal human cell and the classification of chromosomal types are analysed. The well known chromosomal aberrations found in some clinical entities are presented, including mongolism, Turner and Klinefelter syndromes. As an example of rare disease believed to be associated to a 17-18 chromosomal trisomy, a case of Klippel-Feil syndrome is shown.

The most characteristic clinical aspects of the patients affected with the syndromes mentioned above are indicated in detail.

2nd part: Descriptive teratology

The second part of the film intends to classify and characterize the most representative anomalies associated with embryonic development. Malformation patterns found in anomalies due to retarded, excessive and faulty development are exhibited. Anencephaly, cleft lip, heart defects, sirenomielia, cyclops, xiphopagus and other monster types are shown as examples.

Surgical correction of hare lip is presented as a clinical recovery.

15.38. The Torus Palatinus. C. MAXIMILIAN, IOANA POPOVICI and R. POPESCU (Bucarest, Rumania).

Investigations concerning the incidence rate of the *torus palatinus* in various races have led to the conclusion that this structure is a racial character. This would account for the different figures recorded in the populations studied. Hereditary transmission is considered to be either of the autosomal dominant or of the sex-linked type.

We have investigated the incidence rate of this character in our country during the course of history, from the neolithic age up to the present. In order to determine the figures prevailing at present, we investigated a number of 1000 skulls and two additional series, one intensely exogamic, and another endogamic one formed of 20 families comprising 156 subjects from an isolate in the Carpathians. The incidence rate of the torus was found to range from 26 to 46 per cent, in all the series investigated, with the exception of the isolated group, in which it attained 75 per cent. These figures are similar to those prevailing in peri-arctic groups. The formation is already apparent during the first years of life. Its development is genetically determined. In the isolate it was often found to be of considerable size (3rd degree) and clearly detectable in 7- or 8-year children. It is not yet clear whether this large size is determined by a homozygotic condition or by other causes. The type of transmission appears to be autosomal dominant, the incidence rates of the character being almost the same in both sexes.

The part played by heredity was further confirmed by the investigations of 7 pairs of monozygotic twins who were all found to be concordant, and of 2 pairs of dizygotic twins one of which was discordant. Similar observations have already been published by other authors.

15.39. The Epidemiology of Spina Bifida Cystica. J. LORBER (Sheffield, Great Britain).

In the Sheffield Children's Hospital a large unit has been established for the care and study of infants born with spina bifida cystica and over 100 new cases are seen each year. A detailed and prospective family survey of over 500 unselected families revealed a very much higher incidence of multiple cases in sibships than hitherto reported. In completing the family pedigree one must not rely on a single interview or on the evidence of non-medical persons not trained in genetics. Frequently parents did not know the cause of stillbirths or of neonatal deaths which occurred prior to or after the birth of the "index case". Repeated questioning, enquiry from hospitals and family doctors often disclosed such additional cases. Further, a continuous follow-up study of our patients allowed me to study prospectively the condition of children born after the index case. Finally, a detailed history is taken of any congenital malformations which occurred among cousins and in previous generations. Data are available now for thousands of children. These indicate a strong, possibly recessive genetic tendency. Among many interesting features I have seven families in which more than 2 siblings were affected. In these families over 50 per cent of infants born after the second affected child had either spina bifida cystica, anencephaly or hydrocephalus.

15.40. A Study of Mental Deficiency on Some Causes. YASUHIKO KOBAYASHI, K. KISHIMOTO, M. MATSUI, H. TSUBOI, Y. SHIRAKI, and K. NAKAI (Nagoya, Japan).

We presume that mental deficiency is only a "symptom name" due to its etiology. Mental deficiency may be preferably considered to be a collective name given to independent diseases from the nosological point of view.

The following points may be considered:

(1) Race
(2) Sex
(3) Polygenes (subcultural group)
(4) One gene (pathological group-which is less frequent in Japan than in Europe)
(5) Chromosome aberration
(6) Incompatibility of normal genes
(7) Germinal injury
(8) Foetal injury
(9) Birth trauma
(10) Injury in childhood

Mental deficiency is not necessarily due to only one cause. Mental deficiency may be in consequence of the dynamic combination of many causes.

Here, we only make reference to consanguineous marriage, whose frequently in Japan is 10-20 times higher than in Europe, and incompatibility of blood types (ABO-type, MN-type and Rh-type: Incompatibility of ABO-blood type in Japan is possibly more frequent than in Europe, because O-type in Japan is less frequent than in Europe).

For the same purpose we also studied electroencephalogram and amino acid in urine both on patient and parents to find a few special forms of amino aciduria, e.g. histidinuria, etc. We de-

monstrate a few points on which consanguineous marriage and incompatibility of blood types revealed to be the causes of mental deficiency. Nosology of mental deficiency is discussed with further investigation from the point of physiological and biochemical genetics.

15.41. Juvenile Amaurotic Idiocy in Sweden. STURE RAYNER (Lund, Sweden).

During the years 1950-1959 I have collected and personally investigated 37 cases of juvenile amaurotic idiocy together with their relevant family data.

The incidence rate for the disease was estimated to be 0.023 per cent, i.e. about 1 per 50,000 with an approximate heterozygote frequency of 1.0 per cent. The incidence of first cousin marriages among the investigated parents of juvenile amaurotic idiots was 9.1 per cent. This, together with the observed incidence of juvenile amaurotic idiocy among the sibs of the *propositi* ($p = 0.26 \pm 0.05$) confirmed the earlier hypothesis of a simple recessive and autosomal type of inheritance.

Hematological investigation of this, the largest material of juvenile amaurotic idiocy collected so far, showed that lymphocytic vacuolation is an early and constant sign in this disease. The average frequency of vacuolated cells in the patients was 20.6 ± 1.6 per cent.

Of the parents 95 per cent had vacuolated lymphocytes when 500 cells were counted. The frequency of vacuole positive individuals among the sibs of the *propositi* was 65 per cent. The mean frequency of vacuolated lymphocytes per individual was about 1 per cent.

These results, together with other findings in the examined relatives and in various groups of control materials, strongly suggest that the occurrence of lymphocytic vacuolation in healthy relatives of patients with juvenile amaurotic idiocy is an expression of the gene for this disease in heterozygous condition and that, in the patients (homozygotes), it is a pleiotropic effect of this gene when present in "double dose".

15.42. A Test for the Detection of Latent Carriers of the Duchenne Type of Pseudo-hypertrophic Muscular Dystrophy. J. VAN DEN BOSCH (Amsterdam, The Netherlands).

In 1958 a Dutch, seventeen-year-old sister of several brothers, affected with the Duchenne form of muscular dystrophy of the sex-linked, pseudohypertrophic and progressive type, was examined by means of electromyography, in connection with the genetical advice she had asked about her progeny. The electroneurologist (Dr van der Most van Spyk, Utrecht) found signs of myopathic changes in both her EMG and in that of her mother. This finding resulted in a wider-scale research into the significance of this phenomenum: during 1960, 1961 and part of 1962 the author visited and examined "possible" and "assumed" carriers for this disease in the United Kingdom and made tape-recordings of the electromyographic responses obtained by a specially for this purpose designed portable EMG apparatus. The recordings were subsequently replayed at the laboratory (The Galton Laboratory, U.C., London) and the output of the taperecorder was displayed on the screen of an double-beam oscilloscope and photographs of these visible patterns were taken. The results of the statistical analysis of these recordings form the subject of this communication.

15.43. An Unusual Pedigree of Hurler's Syndrome. L. P. CHIASSON (Antigonish, Canada).

This study is based on a pedigree of Hurler's syndrome for which the previously published explanations—sex-linked genes, and recessive autosomal genes—appear inadequate. The affected individuals are children of normal brothers whose normal wives are unrelated to one another or to their husbands. The results of investigations designed to detect heterozygous individuals and/or low expressivity will be presented, and the genetic implications of these results will be discussed.

15.44. The Zollinger - Ellison Syndrome as a Partial Phenocopy of Adenomatosis of Endocrine Glands. PAUL WERMER (New York City, U.S.A.).

The Zollinger - Ellison syndrome consists of the simultaneous development of severe peptic ulcers and of solitary tumors of the islets of Langerhans. In adenomatosis of endocrine glands peptic ulcers of the same character occur together with isletcell tumors, which are always multiple and exhibit a specific microscopic structure: several other endocrine glands also show excessive growth. The Zollinger-Ellison syndrome occurs only sporadically. Adenomatosis

is a familial disease with autosomal dominant inheritance.

The tumors in adenomatosis and in some other inherited deseases always grow in multiple fashion in the affected organs. In view of the great regularity of this finding the presence of multiple tumors in one organ and/or of symmetrical tumors in paired organs can be used as a priori evidence of their genetic origin. It is assumed that tumor-multiplicity comes about by necessity: it presumably results from the action of a growth-promoting gene endowed with pleiotropism of cell-reaction (Hadorn). The insulomas in the Zollinger-Ellison syndrome are not inherited and are therefore solitary like other not genetically determined tumors. Their microscopic picture likewise sets them apart from the inherited tumors of adenomatosis, which is true of solitary isletcell tumors in general.

From the genetic point of view the Zollinger-Ellison syndrome appears as a partial phenocopy of the inherited syndrome of adenomatosis of endocrine glands.

15.45. Hereditary Hyperparathyroidism. CHARLES E. JACKSON (Bluffton, U.S.A.).

The purpose of this paper is to present four families with hyperparathyroidism seen at our institution in the past six years—the first with 9 members affected in two generations, the second with a father and son affected, the third with a woman and her great nephew affected and a fourth with 5 members affected in two generations. These pedigrees and those in the literature suggest an autosomal dominant type of inheritance.

The finding of other endocrine adenomata in members of 2 of these families has emphasized that hereditary hyperparathyroidism is most likely a part of the syndrome termed hereditary endocrine adenomatosis.

The ascertainment of several asymptomatic cases in these family studies led to the speculation that hyperparathyroidism might be more common than had been realized in the past. To test this possibility, routine serum calcium determinations were performed on 12,000 consecutive patients coming to our general medical clinic with the finding of 10 cases of hyperparathyroidism (an incidence of almost 1 in 1000). This again illustrates how genetic studies help provide data on the true incidence of certain diseases.

During this 6-year period only 10 other index cases have had parathyroid adenomas removed at our institution. Since only 11 other families have been reported in the literature, the finding of these 4 families in our small series suggests that heredity is a more important factor in the etiology of parathyroid adenomas than has been recognized previously.

15.46. Albright's Hereditary Poly-osteo-chondrodysplasia (Pseudo-pseudohypoparathyroidism). LUC GOEMINNE (Ghent, Belgium).

In 1942 Albright described a syndrome with biological and clinical findings characteristic of hypoparathyroidism but refractory to parathyroid extract. The syndrome was labelled pseudohypoparathyroidism (PHP) because the target-organ (renal tubule) fails to respond adequately to parathyroid hormone.

The patients show a typical morphologic syndrome, various dyschondroplastic anomalies, tissue-calcifications ossifications, exostoses and trophic disturbancies (teeth, eye-lens, skin) (67 cases reported in 1960, first familial cases published in 1950).

In 1952 Albright described a syndrome without symptoms or biological findings of tetany, but with the same somatic features, named pseudo-pseudohypoparathyroidism (PPHP) (50 cases known in 1962, first familial cases reported in 1957).

Symptoms are: short size, obesity, round face, brachydactyly.

We describe a family with

1. 1 case of PPHP, diabetes, hypertension, polyarthritis, arteritis (femoral artery ectopie calcifications).
2. 1 case of PPHP with hypertension.
3. 1 case of PPHP with polyarthritis.
4. At least 4 other cases of PPHP and 2 PPHP "formes frustes".

This is the second observation in Belgium after the 3 cases described by Nagant and Hoet, in 1960.

Our pedigree is the first in the medical literature where more than 5 cases of PPHP are found in 4 successive generations.

The association of PPHP with diabetes (25 per cent), hypertension (25 per cent), hypothyroidism (15 per cent), polyarthritis (15 per cent) and gonadal dysgenesis (25 per cent) is probably significant.

Hypertension and diabetes might arise from diffuse calcifications and clerosis of renal and pancreatic arteries (?).

McKusick, Uhr and Bezahler (1961), Mann (1962) and Schwartz (1963) suppose a sex-linked incomplete dominance. Our extensive pedigree suggests, (yet with one exception of a clear male-to-male transmission), this mode of inheritance

with a varying expressivity and weak specificity (polypheny).

15.47. An Apparently Sex-linked Heredo-degenerative Disease of the Central Nervous System combined with Leber's Optic Atrophy. L. N. Went and G. W. Bruyn (Leyden, the Netherlands).

The index case of the family to be reported here was suffering since his 7th year from a heredodegenerative disorder for which he has been admitted 6 times and which has been diagnosed on several occasions as spastic paraplegia. In addition he experienced on acute bilateral decrease of vision when 36 years old; pale discs, central scotomas and a vision of 1/60 ODS were found.

His family could be studied over 7 generations and revealed in addition 5 persons with both abnormalities, 6 with optic atrophy only and 6 with the neurological disorder only. All patients were males, while their clinical picture was almost identical. Clinically it seems likely that a basal ganglia disease is responsible for the neurological findings. The only female family member affected (who had an affected brother and an affected son) was clinically very different from the others and had actually been diagnosed as a case multiple sclerosis.

The distribution of the affected patients over the family is such that it seems justified to conclude that only one gene, most probably on the X-chromosome, is responsible for both the neurological and ophthalmological abnormalities.

Chromosomal and linkage studies and extensive biochemical investigations have been undertaken or are under way and will be reported upon. Our findings will be published in detail elsewhere.

15.48. (D.). Genetic and Epidemiological Investigations on Pigmentary Degeneration of the Retina in Switzerland. F. Ammann, D. Klein and A. Franceschetti (Geneva, Switzerland).

Since 1959 we are dealing with a genetic and epidemiological investigation on all types of pigmentary degeneration of the retina in Switzerland. We relate here the results obtained until now in about 10 of 22 cantons.

Our material is composed of the following clinical types of pigmentary degeneration: typical and atypical retinitis pigmentosa out of which some cases belong to Bardet-Biedl syndrome, congenital tapeto-retinal amaurosis of Leber, retinitis punctata albescens and fundus albipunctatus cum hemeralopia. About 90 per cent of those cases are transmitted by the recessive mode of inheritance, and 9 per cent are dominant. One family only presents a recessive sex-linked transmission of retinitis pigmentosa. In this family, the female heterozygotes show the tapeto-retinal reflex which was never observed before in Switzerland. If we keep out the cases of fundus albipunctatus, because of its special clinical character, and the cases of Bardet-Biedl syndrome, we find in 5 cantons 101 living persons suffering from recessive pigmentary degeneration. This fact allows us to estimate the frequency of this affection at 1:7000, the gene frequency at 1/84 and the frequency of the heterozygotes at about 1/42. 10 per cent of these 101 cases are issued from unions between first cousins.

Among the hereditary affections associated with tapeto-retinal degeneration, congenital deafness is known as the most frequent; indeed we discovered this type of association in about 13 per cent of our material, while psychiatric disorders as oligophrenia and psychoses do not seem to be more frequent in the affected people than in the general population. The Bardet-Biedl syndrome was observed in four patients out of a population of 700.000 inhabitants.

The great diversity of clinical forms of the tapetoretinal degeneration raises the problem of heterophenia of tapeto-retinal degenerations. Indeed, the study of an isolate in the canton of Valais allowed us to demonstrate a phenotypical alternation between different types of pigmentary degeneration within several branches of a large family issued from the same ancestor. This pedigree comprises 7 cases of peripheral tapeto-retinal degeneration in 2 sibships, 5 cases of macular degeneration in 5 sibships and 3 cases of fundus albipunctatus cum hemeralopia in 2 sibships.

15.49. A Pedigree of Reccessive Deaf Mutism from Orissa, India. K. R. Dronamraju (Orissa, India).

A pedigree of Bengali Kayastha settlers in Orissa, India was investigated for the inheritance of hereditary deaf mutism. This is a small endogamous community in which consanguineous marriages are scrupulously avoided. The propositus was a female congenital deaf mute aged 17 years and was a student at the School for the Deaf and Dumb in Bhubaneswar,

Orissa. The pedigree contains 182 people in 5 generations and has 5 sibships with at least one affected member. Two of these sibships are doubly related, the parents of each, though not known to be related, being first cousins of the parents of the other. All the 7 female and 4 male deaf mutes recorded in this pedigree are the progeny of non-consanguineous matings between unaffected people. Three marriages of deaf mute females to unaffected males produced six unaffected sons. The evidence suggests that the deaf mutism represented expression in the homozygote of one of the autosomal recessive genes.

15.50. Studies in the Aetiology of Congenital Deafness. GEORGE R. FRASER (London, Great Britain).

A series of 2355 children attending special schools for the deaf in the British Isles was studied. A clinical examination was performed and a full family history obtained from the parents. On the basis of data thus collected together with a consideration of audiometric patterns, the children were classified into aetiological categories.

In approximately 33 per cent of cases the deafness was due to acquired disease in early life, usually meningitis, and in 17 per cent it was due to exogenous pre- and perinatal causes, the most common of which were maternal rubella and neonatal jaundice.

Analysis of the data revealed that simple Mendelian inheritance could account for the deafness of the remaining 50 per cent. Recessive types were most common but dominant and sex-linked varieties were also present. Thus, in 12 per cent of this hereditary group the deafness was inherited in a dominant and in 1 per cent in a sex-linked manner.

Three recessive syndromes were celarly differentiable clinically. Thus, deafness was associated with goitre (Pendred's syndrome) in 10 per cent of this hereditary group, with retinitis pigmentosa (Usher's syndrome) in 2 per cent and with unique ECG abnormalities in 1 per cent. In the dominant group, Waardenburg's syndrome (white forelock, heterochromia of the irides, dystopia of the medial canthus of the eyelid, congenital perceptive deafness) was frequently seen. In many other families with dominant deafness, lesser anomalies of pigmentation were encountered.

In 2 per cent of the entire group deafness was associated with congenital malformations, in some cases familial. While the Klippel Feil and Treacher Collins syndromes were the most common in this group, some of these malformations were unique.

15.51. Hereditary Hearing Impairment and Related Anomalies. H. W. KLOEPFER, JEANNETTE LAGUAITE, and J. W. MCLAURIN (New Orleans, U.S.A.).

This is a preliminary progress report on genetic, audiological and clinical data collected over a period of five years from approximately 500 individuals who were selected from 2000 persons distributed in some 250 family units in which deafness occurred at least in one parent or offspring. Through a study of 4000 ancestors of these families specific relationships between family units and common ancestors can be traced. Although all individuals have been placed on 126 major pedigree charts, an IBM computer program has been developed which will create the information on a 9-generation pedigree chart, find and evaluate each ancestor in terms of association with family units in common, and group family units according to the most probable common genotype.

At this stage of the study it would seem that at least three independent autosomal recessive genes (one later in life causing in addition retinitis pigmentosa and cataract) account for the dearness in most instances in a three-parish-area of Louisiana in which all known cases of deafness have been ascertained. However, hearing loss was encountered in one kindred which is caused by an autosomal dominant gene associated with ear anomalies, brachial clefts, and ear pits. A more detailed report will be given on this kindred.

Supported by NIH Grant 22586.

15.52. Deafness Associated with Split Hands and Feet in Two Siblings. A New Syndrome? L. S. WILDERVANCK (Groningen, the Netherlands).

Two brothers, pupils of the Royal Institute of the Deaf, Groningen, Holland, are suffering from a perceptive deafness with a hearing loss of 60 to 80 db. An older brother and the parents, who are not consanguineous, are hearing well. There is no family history of deafness. Moreover the two boys show split hands and feet, associated with syndactyly. This anomaly is not known in the family. Two sisters of the mother and a son of one of them show a stiff thumb (radiographs

normal). It is highly improbable that these, apparently fibrous stiffness, is related to the syndrome. We have to take account of a possible recessive transmission of a mutated gene. The most common form of hereditary deafness is a recessive one. Splithand and foot nearly always follows a dominant mode of transmission, only very few (doubtful) recessive cases are known. The incidence of recessive deafness in Holland is 1: 5000, splithand and foot occurs in 1:100,000. These figures make it highly improbable that the association of the two abnormalities is fortuitous. Being an exogene causation of the occurring together of the two anomalies *in two brothers* highly improbable, I think the most reasonable explanation is a rare recessive gene giving rise to the syndrome. Only Birch Jensen saw one sporadic case and an inquiry to the other institutes for the deaf in Holland did not reveal any more cases.

15.53. Inherited Multiple Neoplasia Syndrome. ELDON J. GARDNER (Logan, U.S.A.).

A syndrome including multiple or diffuse intestinal polyposis, osteomas, fibromas, epidermal cysts, and dental anomalies, has been investigated over a period of 15 years in a Utah family group. The most serious aspect from the standpoint of the family under consideration was the polyposis, known to predispose to carcinoma of the colon and rectum.

When the study began in 1948, carcinoma originating in the lower digestive tract had been the cause of eight deaths among the descendants of one woman. All 51 living descendants of the woman were examined. Six were found to have intestinal polyposis and other manifestations of the syndrome.

Two cases of fibrosarcoma have been added to the list of manifestations in people who evidenced other aspects of the syndrome. While the family members have been under observation, two children have developed multiple polyposis and five others have expressed epidermal cysts and/or fibromas. These latter types of lesions appear earlier in life than intestinal polyps and therefore serve as diagnostic traits for identifying children who may be expected to express the more serious aspects of the syndrome later in life.

Among the children who have lived long enough to be identified as having or not having the syndrome and who had one parent who expressed the syndrome, 20 were positive and 16 were negative. This observation is not significantly different from the 1 : 1 ratio expected in cases of single gene dominant inheritance.

15.54. Neoplastic Diseases in Twins. RICHARD H. OSBORNE and FRANCES V. DEGEORGE (New York, U.S.A.).

This first report of a comprehensive study of twins in the hospital and clinic populations of the Memorial Sloan-Kettering Cancer Center, New York, will introduce the methodological problems inherent in twin-cancer studies, and present the variable distribution of twins in this patient population.

In theory twin studies constitute a simple and an effective method for investigating the human cancer problem. In actual practice, however, it is found that the application of a standard twin study method results in untenable assumptions concerning the probable nature of human cancer. The variable distribution of 166 twin-born patients in different benign and malignant diagnostic categories relative to that of single-born patients demonstrates the unique nature of the twin-cancer problem and the possibilities offered by twin study methods for investigating neoplastic diseases.

15.55. Genetic Factors in Hypophysial Tumours. H. HAMLIN (Boston, U.S.A.).

A statistical relationship between O and A blood group genes and chromophobe adenoma of the pituitary has prompted search for genetic determinants in other types of hypophysial tumor. There is the chromophil (eosinophil) adenoma that produces the extraordinary clinical condition of acromegaly; also the pituitary giant, usually associated with a mixed type of chromophobe and chromophil hyperplasia. Such abnormally overgrown specimens of humanity are counterpoised by the unique pituitary dwarfs who are usually well-proportioned physically and often superior mentally. Genetic factors are strongly suggested in the hypophysial duct tumor (craniopharyngiona) because of its clearly established patho-embryogenesis. Experimental data is cited to suggest that estrogen-induced pituitary adenomata have shown genetic linkage in certain F_1-hybrid mice strains. Examples of human hypophysial neoplasia are presented to indicate that constitutional factors should always be suspected in these disorders.

15.56. Genetic Variation and Leprosy. S. G. SPICKETT (Cambridge, Great Britain).

The causative organism of leprosy is *Mycobacterium leprae*. There is evidence that some human individuals are resistant to parasitism by this pathogen. Susceptibility to leprosy would appear to be determined by a single irregularly dominant gene. The frequency and penetrance of this gene varies between populations.

Leprosy may be manifest in several forms, the relative frequency of these forms shows striking variation between populations. There is moreover a significantly higher concordance between monozygotic than between dizygotic twins in the form of leprosy suffered. This and other evidence suggests that the genotype of the human host is of importance in determining the course of the disease. Evidence of higher concordance between unrelated contacts than random expectation and the failure to find any environmental factor to account for this, is suggestive of an effect of the genotype of the pathogen on the course of the disease.

The history of epidemic leprosy is consistent with there being genetic control of incidence and form of leprosy by both host and pathogen.

Current studies indicate that drug resistance is increasing in pathogen populations with the risk that the control of leprosy may become more difficult in the future. Moreover, leprosy is increasing in some populations that have been free of it for many generations. It is probable that this is associated with a rise in the gene frequency for susceptibility (in the absence of selection pressure against it) coupled with the greater chance of contact with the disease that is associated with the greater mobility of individuals between populations.

15.57. The Role of Genetics in Diabetics. NANCY E. SIMPSON (Toronto, Canada).

Analysis of the genetics of diabetics will be presented from data obtained from a voluntary register of diabetics in Canada sponsored by the Canadian Diabetic Association. For the past two years diabetics have been asked to complete questionnaires which contain detailed information regarding their immediate relatives (parents, sibs and offspring).

The register will be a continuing one in which respondents will be asked to keep the information regarding their families up to date. Ultimately a history for three generations of families in which there is at least one diabetic will be available for genetic analysis. These sort of data have long been needed to establish the genetic role in the etiology of this condition with variable age at onset.

From the register, the proportions of diabetics among offspring of two diabetic parents and one diabetic parent (selected through the matings) are compared with each other and a suitable population control. The ages at onset and treatment of the diabetics are taken into consideration. The data suggest that diabetes is not genetically homogeneous. The results lead to interesting speculations on the role of genetics in diabetes and to discussion of future advantages of the register when complete histories for the generations become available.

15.58. The Natural History of Hyperuricemia and Gout. M. T. RAKIC, H. A. VALKENBURG, R. T. DAVIDSON, J. P. ENGELS, W. M. MIKKELSEN, J. V. NEEL and I. F. DUFF (Ann Arbor, U.S.A.).

To determine with more precision the natural history of hyperuricemia and gout, the families of 19 proposity (a total of 99 relatives) studied in 1938-42 or 1946 were reinvestigated in 1961-62. Nine of the propositi (all still with signs and symptoms of their disease) and 262 relatives (69 from the first study, the remainder seen for the first time) could be examined. In the period of follow-up gout was present in 14 male relatives and 6 female relatives. The mean age of onset gout was in males 39 years and in females 54 years ($P < 0.005$).

Thirty-five male relatives and 34 female relatives were seen in both studies. Eleven of the 26 male relatives with normal serum uric acid levels in the previous study developed hyperuricemia as determined by the colorimetric method and defined as a level in males of 6.0 mg per cent or greater and in females of 5.0 mg per cent or greater. Of these 11 two had developed gout. Of the 9 hyperuricemic male relatives in the first study 1 had developed gout. Five of the 9 adult males with a normal serum uric acid level in the previous study apparently developed hyperuricemia in the interim. Eight of the 27 female relatives with normal serum uric acid levels in the previous study now had hyperuricemia and two of these had gout. Hence gout developed in equal numbers in these 35 male and 34 female relatives. No significant differences existed between the two sexes regarding the incidence and development of hyperuricemia in the previous and the present study. These apparent similar findings could be explained by a significant difference in mean age at the time of the first study, the male relatives

being 21.7 years and the female relatives 36.3 years. No differences in mean values and mean age could be found between the male relatives who stayed normal during the period of follow-up and those who became hyperuricemic.

On a NATO Science Fellowship supported by a grant of the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

15.59. Carbohydrate-Induced Hyperlipemia in Childhood. S. Jakovcic and David Yi-Yung-Hsia (Chicago, U.S.A.).

The present paper will describe the biochemical studies undertaken in a hyperlipemic family, where 3 of 13 children are affected. The parents of these children are second cousins.

The children were first placed on a high carbohydrate diet containing 15 per cent protein, 10 per cent fat and 75 per cent carbohydrate. During this period the triglycerides increased from 1670 to 2700 mg per cent and the lipoprotein lipase remained low with levels of 0.07-0.13 μEq/ml/min. They were then placed on a high fat diet containing 15 per cent protein, 40 per cent fat (half corn oil and half cream), and 45 per cent carbohydrate. During this period, the triglycerides decreased from 2700 to 605 mg per cent and the lipoprotein lipase reverted to normal levels of 0.22-0.33 μEq/ml/min. These data indicate that the children had hyperlipemia of the carbohydrate-induced variety and not of the fat-induced type. Heparin sensitivity was also tested and all three siblings showed more than 25 per cent of clearing of the serum measured as triglycerides in mgm. per cent.

Plasma from the affected children were separated by ultracentrifugation into the chylomicrons (<1.006), the low density lipoproteins (<1.019) and the low density lipoproteins (1.019-1.063). Each of the fractions were analyzed for triglycerides, phospholipids and cholesterol.

Finally, studies were carried out on the parents and unaffected siblings. While all showed lipoprotein lipase within the normal range, both parents showed delayed clearing of triglycerides up to 24-48 hr after a 90 g fat meal.

15.60. A Dual Hereditary Red Cell Defect in One Family: Hypocatalasemia and Glucose-6- Phosphate Dehydrogenase Deficiency. A. Szeinberg, A. De Vries, J. Pinkhas, M. Djaldetti and R. Ezra (Tel Aviv, Israel).

Severe hemolytic anemia with sulfhemoglobinemia developed in an Iranian Jew following contact with a fungicide-zinc ethylene bisdithiocarbamate. The previous history of the patient and his family history did not reveal hemolytic occurrences. Examination of his red blood cells demonstrated a fully expressed deficiency of glucose-6-phosphate dehydrogenase (G6PD), as well as a severe hypocatalasemia (about 8 per cent of mean normal catalase activity).

Investigation of members of three generations in this family suggested that the two enzymatic defects were transmitted independently. The hereditary pattern of the catalase deficiency was compatible with transmission by an autosomal gene of intermediate dominance. In the homozygous state (the propositus) the abnormality resulted in severe hypocatalasemia, but not in complete absence of this enzyme. The presence of slight catalase activity in his red cells was confirmed by starch gel electrophoresis followed by specific staining. The heterozygous state was characterized by intermediate hypocatalasemia (50-65 per cent of mean normal catalase activity). Such intermediate catalase activity was detected in all the children of the propositus as well as in several other members of the family both in those with normal G6PD and with G6PD deficiency. No difference in the degree of hypocatalasemia was observed between those two groups of subjects heterozygous for catalase deficiency, suggesting absence of interaction between these two enzymatic defects.

These investigations were supported in part by grants from the U.S. National Institutes of Health, Bethesda (A.2740), and from the Rockefeller Foundation (RF60101).

15.61. Galactosemia and Mongolism in the Same Family. Brigitte Bahrs and Wolfram Ostertag (Münster, Germany).

A family with four children, one of them with mongolism and the other with galactosemia, have been investigated. Following modified manometric methods developed by Kirkman and Bynum (1959) and by Schwarz, Holzel *et al.* (1961) we were able to distinguish the heterozygous carriers from normal controls by measuring the activity of the enzyme Gal-1-P uridyl transferase. All of the children—with the

exception of the galactosemic child—as well as the parents were heterozygous carriers.

The biochemical as well as the cytological results of this investigation of linkage relations will be discussed.

15.62. Down's Syndrome: A Genetic Abnormality Improved by Medication. HENRY TURKEL (Detroit, U.S.A).

In 1866, both Gregor J. Mendel brought out his theory of heredity and Dr. Langdon Down coined the name, mongoloid, for a particular group of patients, it was not until 1958 that Dr. Jerome LeJeune pointed out their relationship in discovering the Trisomy 21.

And in 1961 Dr. LeJeune said: "Cytogenics must not be restricted to genetic counseling. Even more important than the mapping of various traits would be the establishment of solid relationship between cytogenics and biochemistry, leading to the possibility of chemical correction of inborn errors."

Though various abnormal chemicals have been found in mongoloids, none have been pinpointed as the real cause of the various inborn structural and functional anomalies characteristic of mongoloids.

Mongolism appears to be a complexity of problems, but it is like phenylketonuria, galactosemia, diabetes, etc., in that it is a part of the group of conditions which are inborn. In the case of, for example, phenylketonuria, the mother is capable of protecting the fetus, and the fetus does not begin abnormal development until after birth. However, in the case of the mongoloid, the mother is incapable of protecting the fetus, thus, the mongoloidal fetus is born with a group of abnormalities which have already passed into secondary and tertiary stages, thus, piling abnormalities upon abnormalities.

The treatment for the improvement of the mongoloid is based upon assisting the mongoloid to build through normal means a more normal manner of growth, removing the secondary and tertiary outgrowths of the original inborn anomalies. This should enable the genetist to pinpoint the inborn anomalies more easily with the hope that future findings will dictate a correction.

Though the use of the "U" series of drugs does not enable the physician to change the chromosomal pattern, he can still alter the resultant abnormalities and bring the mongoloid into the sphere of normal society. Life-long medication of maintenance doses may be necessary, but this is true also of diabetics who require insulin.

While the "U" series of drugs do not constitute a cure for mongolism, they do allow mongoloids to lead a more normal life and point the way to a genetic solution of the problem.

15.63. Serum Phenylalanine Levels in the Newborn. DAVID YI-YUNG HSIA, J. D. BERMAN and H. M. SLATIS (Chicago, U.S.A.).

Phenylketonuria is a hereditary metabolic disease caused by a deficiency of the enzyme phenylalanine hydroxylase. The mental retardation in this condition can be effectively prevented if treatment with a diet low in phenylalanine content is started very early in infancy. Two approaches have been proposed for routine screening of newborns for phenylketonuria: (1) testing of urine for phenylpyruvic acid and (2) determining phenylalanine levels in the serum. The first has the disadvantage of not being reliable until 4-6 weeks of age. Methods for the second have until now only been semi-quantitative based upon the inhibition of *Bacillus subtillis* by beta-2-thienyalanine in a minimum culture medium.

The present paper will describe a survey of serum phenylalanine levels in 4000 newborn infants. In each instance, 0.1 ml of capillary blood was obtained from a single heel puncture and the serum phenylalanine level determined by the fluorimetric method of McCaman and Robins(1). The data will be analyzed in terms of sex, age, race, parity, maternal age, and birthweight, and compared with observations on known phenylketonuric infants followed in our own Clinic and from other centers.

Since one laboratory technician can perform up to 100 determinations of serum phenylalanine daily, this approach using a quantitative method should serve as the best means of screening for phenylketonuria during the newborn period.

---

1. *J. Lab. & Clin. Med.* **59,** 885, 1962.

15.64. Detection of Carrier State for Phenylketonuria in Familial Genetic Inquiries. J. DODINVAL-VERSIE, P. DODINVAL, R. MALCHAIR and P. MOUREAU (Liège, Belgium).

For a few years, it has been possible to

avoid or improve phenylketonuria or phenylpyruvic idiocy when the children affected with this recessive hereditary disease are fed with a phenylalanine-poor diet from the first months of life. Therefore it is very important to detect this disease at its very beginning by means of a 10 per cent ferric chloride solution added to the urine. If there is any phenylpyruvic acid, the yellow color of the urine becomes rapidly and intensively green.

Moreover, as soon as the disease is discovered in a family, it would be desirable, on the one hand to determine the other diseased persons and, on the other hand the people who are carrying the deleterious gene (heterozygotes) and are likely to pass on the disease to their children if they marry another heterozygote.

Using the microbiological process, elaborated by Knox and Messinger, to determine the fasting phenylalanine level in blood plasma, the authors were able to find out with sufficient accuracy the gene carriers in the successive generations of three families where a phenylpyruvic idiot was first seen.

The average fasting level of plasma phenylalanine was found to be at 80.33 micromoles per liter, ± 9.67 with normal individuals where as the average level with carrier people amounted to 104.47 micromoles per liter, ± 10.88.

The meaning of the intermediate values between these two figures can be better estimated by an oral phenylalanine load test on some individuals.

15.65. Phenylketonuria: Further Studies on the Heterozygous Carrier. WILLIAM F. ROWLEY, MARGARET E. O'FLYNN, and PAUL WONG (Chicago, U.S.A.).

The present paper will describe a series of studies carried out using more sensitive and accurate fluorimetric methods for the assay of phenylalanine and tyrosine in the serum. Phenylalanine levels were determined on 0.05 ml serum using a modification of the fluorimetric method of McCaman and Robbins.[1] Tyrosine values were obtained from 0.02 ml serum from a procedure adapted from that suggested by Waalkes and Udenfriend.[2]

One hundred parents of phenylketonuric children and an equal number of controls were given 0.1 gm per kg L-phenylalanine by mouth. Blood samples were obtained at 0, 1, and 2 hours after the load and analyzed for both phenylalanine and tyrosine. The data are analyzed in terms of the best discriminant for separating the two groups and the influence of sex, age, body weight, etc., upon the segregation between the two groups.

1. *J. Lab. & Clin. Med.* **59,** 885, 1962.
2. *J. Lab. & Clin. Med.* **50,** 733, 1957.

15.66. Frequency of Phenylketonuria. ROBERT GUTHRIE (Buffalo, U.S.A.).

The principle upon which the "inhibition assay" is based can be applied to the detection of various "inborn errors of metabolism".[1] In one application, a microorganism and a specific inhibitor are incorporated in a layer of agar culture medium. Upon the surface are placed rows of paper discs punched from filter paper containing dried blood spots or urine. After incubation, the diameters of the growth zones for the unknowns are compared to diameters of zones surrounding "control" discs. For example, azaserine and *Bacillus subtilis* can be used in this way to detect histidinemia. For phenylketonuria, B-2-thienylalanine and *Bacillus subtilis* spores are used to test dried spots of blood collected upon filter paper by heel puncture of newborn infants before leaving the hospital nursery. A brief description of this technic will be presented.

With the demonstration by the Massachusetts Department of Health that this method is practical for routine screening for phenylketonuria in newborn infants[2] it will now be possible for the first time to estimate the true frequency of this disease within the populations screened. The first 74,000 tests carried out by forty laboratories in 29 states (including Massachusetts) have detected seven cases. The Massachusetts Department of Health has detected five of these cases in that State among the first 27,000 newborn infants tested. At the present time (February 1963), 25,000 total tests are conducted per month, and the rate of testing is increasing rapidly. A recent progress report of this program will be presented. It appears that the frequency of this disease may be greater in the populations so far screened than the highest frequency usually given, 1/20,000. An estimate of the frequency of this disease will be attempted from the latest results available.

The "inhibition assay" method of testing will detect PKU cases in which the urine does not react to ferric chloride, as in young infants. However, this condition is known to exist also in some adults ("PKU without PKU"), but their frequency is unknown. This type of

PKU case may account, at least in part, for the higher frequency we are encountering with this method. This possibility will be discussed.

Work supported by grants from the National Association for Retarded Children, Association for Aid for Crippled Children, the National Foundation, United States Public Health Service, Grant Number B-1960, and the Children's Bureau.

1. *Proc. London Conf. Scientific Study of Mental Deficiency*, 1962, Vol. 2, pp. 672-677.
2. *New Eng. J. Med.* **267**, 1208, 1962.

15.67. Pharmacogenetic Aspects of Taste. ROLAND FISCHER, FRANCES GRIFFIN (Columbus, U.S.A.) and A. R. KAPLAN (Cleveland, U.S.A.).

Very insensitive ("non"-)tasters of HN-c = S type goitrogens, such as 6-n-propylthiouracil (PROP), are also insensitive tasters[1] of an apparently unlimited series of chemically unrelated compounds[2]. Differential taste profiles for 34 such compounds, mainly drugs, will be presented and the stereospecificity of chemoreception described. Sensitive tasters of PROP can taste 8-times less l-quinine (sulfate, chloride, or free base)—concentrationwise—than d-quinine, whereas the reverse is true only for quinine chloride in the case of very insensitive ("non"-)tasters of PROP who constitute about 10-12 per cent of a Caucasian population. Taste testing practice, extending for months, with mixtures of PROP and quinine—under experimental conditions described by Rubin, Griffin, and Fischer[3]—can abolish this difference and make a "learned" taster from a very insensitive ("non"-) taster. This phenomenon can be related to Beckett and Anderson's model of "footprinting" on stereoselective adsorbents.[4]

If the human oral cavity is regarded as a pharmacological preparation *in situ*, taste thresholds for drugs—alone or in mixture—and drug activity can both be treated as analogous pharmacological responses. By plotting the taste thresholds of certain molar ratios of two drugs—in subthreshold concentrations—isoboles can be obtained which may be interpreted as a characteristic systemic response of a particular subject to a binary mixture of drugs. Sensitive and insensitive taster (drug responders) appear to produce different isoboles for the same pair of drugs.

Another pharmacogenetic aspect of taste is illustrated by the following observation in 40 parents of children affected with Down's syndrome. In the maternal sample the antimode, dividing the age of younger mothers from that of older ones, coincides with the threshold antimode dividing tasters from ("non"-)tasters of PROP. In addition, all but one of the fathers in the sample can be characterized as markedly insensitive ("non"-)tasters of PROP.

1. FISCHER, R., GRIFFIN, F., and MEAD, E.: *Med. exptl.* **61**, 177 (1962).
2. For taste testing procedure and criteria, see FISCHER, R. and GRIFFIN, F.: *Proc. IIIrd World Congr. Psychiat.* June 1961, Montreal, Vol. 1, p. 542).
3. *Nature* **195**, 362 (1962).
4. *Nature* **179**, 1074 (1957).

15.68. Reexamination of Genetic Aspects of Taste Thresholds for Thiourea-type Compounds. ARNOLD R. KAPLAN, WILMA POWELL, ROLAND FISCHER and ROGER MARSTERS (Cleveland, U.S.A.).

Monozygotic and same-sex dizygotic twins have been classified according to their taste thresholds for 6-n-propylthiouracil (PROP) and various other compounds. Zygosity determinations are based on combinations of morphologic characteristics and blood types.

A modified Harris-Kalmus testing procedure has been employed—using distilled water for the serial dilutions, placebo, and between-sampling mouth rinses. Other taste-testing methodologies have also been tested, and the latter yield inconsistencies in the data which did not occur with the modified Harris-Kalmus procedure.

Ninety-four monozygotic twins have thus far manifested 100 percent concordance for the 'sensitive taster' vs. 'insensitive ("non"-)taster' dichotomy. These data contrast significantly with the low concordance observed in same-sex dizygotic twins. Furthermore, using a series of fourteen dilutions, all the above monozygotic twin pairs manifested taste threshold concordance within a single serial dilution. One exceptional and discordant pair includes one twin on hormone therapy, who is an insensitive ("non"-)taster while her co-twin is a 'taster.' Two other variables which evidently affect taste threshold for PROP are aspirin medication, and coincidence within the first three days of menstruation. Threshold discordance coinciding with either of the above variables disappeared in each case upon controlled retest.

The data demonstrate the primary genetic basis of taste thresholds for 6-n-propylthiouracil, when properly tested. They also demonstrate that medication and menstruation are variables which may distort the manifest thresholds.

Supported by U.S. Public Health Service, National Institutes of Health, Grant RG-9885.

15.69. Relation Between the Blood Groups of the ABO System and the Taste Sensibility to Phenylthiourea (P.T.C.). FRANCISCO G. HARO (Caracas, Venezuela).

Relation between the blood groups ABO and the sensibility of taste to P.T.C. is analysed in 372 students of both sexes between the ages of 16 and 23 years.

The method of Harris and Kalmus (1949) was applied in the present investigation to determine the taste sensibility to P.T.C.; and the slide-test with the whole blood and with antisera A and B to determine the blood group.

The distribution of blood groups was: 50 per cent O; 32.25 per cent A; 11.3 per cent B, and 6.45 per cent AB.

The threshold distribution of his age group was 6-7 of the solutions of Harris and Kalmus.

The "non tasters" and "tasters" proportion was 22.6 per cent and 77.4 per cent respectively. 71.42 per cent of "non tasters" were of O Group (50 per cent of the whole group of students was of O group)

31 per cent of O group were "non-tasters" (22.6 per cent of the whole group of students was "non tasters")

To all appearances there are correlations between the blood group O and the ageusia ("non-tasters").

It is possible to explain these correlations as a manifestation of linkage of the genes both recessives?

15.70. Quantitative Variation in Antigen Strength within the A Blood Types of Man and its Possible Significance. F. J. GRUNDBACHER (Ann Arbor, U.S.A.).

In hemolytic disease of the newborn due to ABO incompatibility, the most frequently affected children are of type A_1 of O mothers. Since the majority of ABO incompatible child-mother combinations cause no obvious fetal injury, the question arose whether or not quantitative aspects of the A_1 antigen might be responsible for the differential behavior, i.e. infants with a strong A_1 antigen more likely to be affected with hemolytic disease of the newborn than individuals with a weaker A_1 antigen.

A method was developed for measuring the antigenic strength of erythrocytes which depends on freshly drawn blood for reproducible results. Density of erythrocyte suspensions, reagent, complement, temperature and time of incubation are strictly controlled and the degree of hemolysis obtained gives a measure of the antigen strength.

The data obtained thus far reveal considerable variation in regard to antigen strength between and among the subtypes A_1 and A_2. Individuals whose genotype can be inferred to be AO show, as a rule, little or no difference within family units; there is, however, remarkable variation between families. Thus, the findings suggest that the alleles conditioning the subtypes A_1 and A_2 represent an array of allelic subunits within each subtype, which differ from each other in that they are responsible for various degrees of antigen strength, but are alike in their qualitative property. In AB erythrocytes the A antigen is weaker than in individuals of AO genotypes, and cells of suspected homozygous A genotype appear antigenically stronger than when the same allele is in AO combination.

Results will also be presented of a study of the A_1 antigen in families who have had an infant with hemolytic disease of the newborn due to A-O incompatibility.

15.71. A Family Pedigree Showing the Transmission of Rare and "New" Rh-Hr Genes. LESTER J. UNGER (New York City, U.S.A.).

This family came from a small village in Puerto Rico so that the circumstances suggest inbreeding. The pedigree covers three generations. The rare gene involved was r^o and the "new" as well as rare gene was r^{od}.

15.72. Lewis Blood Group Haptene in Human Urine. D. A. P. EVANS, R.B. MCCONNELL, and W. T. A. DONOHOE (Liverpool, Great Britain).

It has previously been found:

(a) that there is a pentasaccharide in pooled human breast milk which inhibits anti-Le^a(1)

(b) that human urine from both salivary ABH secretors and salivary ABH non-secretors inhibits anti-Le^a[1]

(c) the blood group substance A, B, H and Lewis (a) purified from human pseudomucinous ovarian cysts have a molecular weight of about 300,000

This present communication presents data including the following:

(a) that the Le^a property in human urine is due to the presence of a freely diffusible oligosaccharide. This is found in urine from both salivary ABH secretors and salivary ABH non-secretors.

(b) this diffusible Le^a haptene is not present in saliva.

(c) the haptene in the urine of salivary ABH non-secretors is capable of "coating" Le (a-) erythrocytes, so changing them to Le (a+) Some implications of these findings will be discussed.

1. WATKINS, W. M. and MORGAN, W. T. J. (1957) Specific inhibition studies relating to the Lewis blood-group system. *Nature*, **180**, 1038-1040.
2. MCCONNELL, R. B. (1961) Lewis blood group substance in body fluids. *Proc. 2nd Internat. Conference of Human Genetics*, Rome (in press).
3. MORGAN, W. T. J. (1954). The chemical basis of blood group specificity in man. *Lectures on the Scientific Basis of Medicine* IV, 92-111.

15.73. Normal Variation in Excreted Amino Acids in Man. HERMAN M. SLATIS (Argonne, U.S.A.).

Paper chromatography has been used in the investigation of amino acids and other ninhydrin-positive substances in the urine of over 2500 persons. Other characteristics that are known for these individuals include height, weight, age, sex, blood type, and ancestry. The statistical analysis of the chromatographic data has been based on the ranking of each spot according to density, rather than by the usual practice of expressing the concentration of each substance in terms of some supposedly standard constituent of the urine. About 20 separate spots were measured on most of the chromatograms.

A number of interrelationships have been observed between the various chromatogram spots. There is no correlation of any of these spots with height or weight but there are correlations with the specific gravity of the urine. The relationships do not seem to shift according to height, weight, age, sex, ABO blood type, or Rh blood type.

Most of the persons observed were of European ancestry. The various ancestry groups within Europe do not appear to differ from each other in significant ways. The non-Europeans were too few in number to permit an adequate analysis of possible differences, although some suggestive trends were observed.

Work supported by U.S. Atomic Energy Commission.

15.74. Genetic and Biochemical Aspects of Sickle Cell Dactylitis. W. G. THURMAN and W. LORRAINE WATKINS (New York, U.S.A.).

Sickle cell dactylitis or the "hand-foot" syndrome is an infrequent complication of homozygous S hemoglobin disease. In the population groups we have observed over 30 cases of this syndrome.

Family studies indicate that this syndrome has a high incidence within the same family group should more than one sibling have homozygous S hemoglobin. Four families have been studied in which three siblings have been affected; one has been studied in which four children have had orthopedic difficulties. Possible gene penetrance as related to male or female "carriers" have been studied in two families in whom siblings have had one common parent with subsequent mating with another parent, also with AS hemoglobin; comparisons of siblings from the first mating with siblings of the second mating that have also developed the syndrome have been made. The relationship of siblings with this syndrome to abortions in the mother before or after the affected pregnancy has also been studied.

Fragilities, serum iron studies, alkaline and acid phosphatase determinations, chloride, calcium and phosphorus levels, fetal hemoglobin studies, relation to infectious processes in other organ systems and incidence of superimposed infection in affected joints have all been tabulated.

15.75. The Chemical Structure of Hemoglobin Lepore and its Interpretation as the Result of Non-homologous Crossing-over. C. BAGLIONI (Naples, Italy).

Three samples of hemoglobin Lepore, ob-

tained from unrelated individuals, have been examined. The chemical findings previously reported[1] have been confirmed. Hemoglobin Lepore appears to be made up of two normal α peptide chains and of two abnormal peptide chains (designated Lepore chains), resulting from the fusion of part of the β peptide chain with part of the δ peptide chain.

A method has been devised to analyze by fingerprinting the trypsin-resistant "core" of the Lepore chain. More information on the chemical structure of the Lepore chain has been obtained by this method of analysis. The region where the δ chain-like portion of the Lepore chain is joined to the β chain-like portion has been localized in the "core". This region is comprised between residue 85 and residue 115 of the Lepore peptide chain. When digested by trypsin, this chain yields peptides identical to either β or δ chain peptides. The entire Lepore chain can now be accounted for; it appears to have the same length as the β and δ chains. These chemical findings suggest that a very specific genetic event is responsible for the formation of the Lepore chain. The fusion of complementary regions of these peptide chains is most likely caused by a non-homologous crossing-over between corresponding points of the β and δ genes.

---

1. C. Baglioni, *Proc. Nat. Acad. Sc.* **48** (1962) 1880.

15.76. Serological Recent Data for the Gm Groups. Their Interest in Sero-anthropology. C. Ropartz, L. Rivat and P.-Y. Rousseau (Rouen, France).

As soon as 1961 we assumed that the Gm(b) factor had not an elementary structure. It is but very recently that such a phenomenon has been highly proved.

Whereas Steinberg found one anti-Gm(b) specific to the whites, we demonstrated that what was called Gm(b) in the yellow Races was in reality a mosaic of numerous unities, specially among the Japanese: sera, specific for the Gm(b) factor in the white people, define 3 different structures among Japanese.

These facts, if complicating the immunological study and the analysis of the genetical determinism, give the gamma-globulins system "Gm" a capital importance in sero-anthropology and human genetics.

In fact, specific anti-Gm(b) reagents have already helped us to review the cross-breeding percentage of the US Negroes. Moreover deep qualitative and quantitative differences in the expression of the mosaic Gm(b) among yellow races highly contribute to study the problem of their paleo-migrations and cross-breeding. So, the Chinese of Macao considerably differ from the Japanese, and important variations are found between different tribes of Central and North American Indians.

Although the genetical determinism of that mosaic which constitutes the Gm(b) factor is still unknown, it is beyond doubt that the facts that we present will help to its study.

Indeed, mind is not satisfied when obliged to accept the presence of the same gene: Gm^{ab}, which should be omnipresent in the melanodermic populations, only very frequent in the xanthodermic ones.

15.77. Studies on the Hereditary Gamma Globulin Factors: Their Relation to Immune Tolerance and Studies on the Antibodies to the Hereditary Gamma Globulin Factors in Man and their Relation to Immune Tolerance. Arthur G. Steinberg and Janet A. Wilson (Cleveland, U.S.A.).

The hereditary gamma globulin factors (Gm and Inv) are detected by means of an agglutination inhibition test. The antibodies which detect these factors may be formed as a result of transfusions of whole blood, injection of gamma globulin, or immunization of the infant by the transplacental "transfusion" of the mother's gamma globulin. It is of interest that the mother's gamma globulin does not induce immune tolerance even though it is present in high concentration before and after birth.

The antibodies are usually carried by β_2M molecules but they may also occur on 7Sγ-globulin molecules. The latter may cross the placenta and be found in the infant. The antibodies may be induced in rhesus monkeys, but it is not yet clear that these are identical with those formed in humans.

The antigens do not appear to cause transfusion reactions when given to recipients who have antibodies to them.

Data collected in this and other laboratories will be presented to substantiate the above statements.

15.78. New Genetic Variants in the Gc-System of Human Serum. HARTWIG CLEVE, R. L. KIRK, W. CAREY PARKER, and ALEXANDER G. BEARN (New York, U.S.A.).

The group-specific components (Gc) of human serum represent an additional protein polymorphism distinguished by differences in relative electrophoretic mobilities. The common phenotypes, Gc 1-1, Gc 2-1, and Gc 2-2 are genetically controlled by a pair of co-dominant, autosomal alleles, Gc^1 and Gc^2. The distribution of the Gc-alleles has been determined in various populations of different geographical and ethnic origin. The population surveys have led to the disclosure of several unusual electrophoretic variants, which were identified by immunoelectrophoresis and starch gel electrophoresis. Two new variants will be described, which migrate more rapidly than Gc 1-1. One was observed in a population of Chippewa Indians and the second in a sample of Australian Aborigines from the Cape York area, and were therefore named Gc Chippewa and Gc Aborigine, respectively. Family data indicated that they are controlled by additional alleles at the Gc locus The distribution of the various phenotypes was in agreement with the assumption of population equilibrium. The incidence of the variants was relatively high ($Gc^{Chippewa} = 0.105$ and $Gc^{Aborigine} = 0.046$). Gc Chippewa has so far been found only in Chippewa Indians. The distribution of Gc Aborigine in a number of aboriginal populations in Australia will be discussed and the results related to observations in populations from South East Asia and Oceania.

15.79. Contribution to the Genetically Determined Transmission of the α_1-Acid Glycoprotein Variants. K. SCHMID, L. MOROZ and K. TOKITA (Boston, U.S.A.).

At the Second International Conference of Human Genetics in Rome we reported on the relative incidence of the different α_1-acid glycoprotein patterns in normal white adults. In the present study, α_1-acid glycoprotein was isolated from plasma of 13 members of a family of Italian origin and analyzed by starch gel electrophoresis at pH 2.9, resulting in most cases in a pattern with six bands. This binding strongly supports the concept that the α_1-acid glycoprotein variants are genetically determined. Further evidence was obtained from the corresponding analysis of identical twins.

In continuing this investigation, α_1-acid glycoprotein was prepared from plasma of 44 white adult patients with various diseases and subjected to the same analytical procedure. The blood level of this protein was elevated in most patients, the maximum increase amounting to 300 per cent. The relative incidence of the α_1-acid glycoprotein patterns was found to be very similar to that of normal white adults and, as judged from the relatively small number of analyses, independent of the plasma concentration of this protein. Thus, the genetic transmission of the α_1-acid glycoprotein variants appears to be well established.

Supported by NIH grants GM-10374-01 and A-3564-C2.

15.80. The Genetically Determined α_1-Acid Glycoprotein Variants. K. TOKITA and K. SCHMID (Boston, U.S.A.).

The α_1-acid glycoprotein patterns obtained by starch gel electrophoresis at pH 2.9 appear too complex for genetic evaluation because of the large number of bands observed (5, 6, 7 and 8, respectively). In an attempt to arrive at simpler patterns, α_1-acid glycoprotein was incubated with neuraminidase and subjected to starch gel electrophoresis at pH 4.8. The enzymatically modified glycoprotein derived from pooled normal plasma revealed a pattern with two main bands of almost equal color intensity and a minor faster moving band([1]).

In the present study α_1-acid glycoprotein was isolated from normal adults, treated with neuraminidase and analyzed by starch gel electrophoresis at pH 4.8. Three types of patterns were noted: the first type exhibited the maximum color intensity at the slowest moving band and the second type at the center band. The third type showed the slow moving and the middle band to be almost equal in color intensity and very similar to that of pooled normal α_1-acid glycoprotein. As judged by the number of analyses carried out (65) the relative incidence of these three types were found to be approximately 10, 50 and 40 per cent, respectively. Additional results obtained from the corresponding study of identical twins supported the concept of the genetically determined transmission of the α_1-acid glycoprotein variants.

Supported by NIH grants GM-10374-01 and A-3564, C2.

1. *Nature*, **190**, 630 (1961).

15.81. Variable Heterozygote Expression in Human Haptoglobins. H. E. SUTTON and G. W. KARP (Austin, U.S.A.).

Two alleles are commonly recognized at the human haptoglobin locus, Hp^1 and Hp^2. In most populations, the heterozygous phenotypes are very similar. However, in Negro populations a variant, Hp 2-1 modified, has been described.

Our studies of an American Negro population indicate that a broad range of heterozygous phenotypes are found, depending on the relative activity of the two alleles. The limited evidence suggests that the differences in allelic activity are not the result of structural differences of the products. Studies of more than 100 matings of various types show that in some families there are factors which influence the activity of the alleles differentially. Ahaptoglobinemia tends to occur in the same families.

Analysis of the mating studies will be presented and the haptoglobin system will be discussed from the viewpoint of newer ideas on gene regulation.

15.82. Possible Influence of Mother's Genotype on the Foetal Haptoglobin Synthesis. M. SINISCALCO, G. LA TORRETTA, C. DEL BIANCO, S. MARSICO and L. BERNINI (Naples, Italy).

The incidence of cordal blood plasma with a detectable haptoglobin pattern has been found to vary considerably in the different haptoglobin types of mating, suggesting the possible influence of the mother's genotype on the foetal haptoglobin synthesis.

Thus the lowest incidence (4.3 per cent) was found among the "incompatible matings" i.e. when the baby must necessarily possess a molecular haptoglobin species not produced by the mother, and the maximum incidence (23.8 per cent) among the full compatible matings, i.e. those involving a 2.1 mother or parents with identical haptoglobin genotype.

Passive transfer of haptoglobin from the maternal to the foetal circulation is excluded on the ground that the detectability of haptoglobin at birth is not positively correlated with the haptoglobin concentration of maternal plasma and also because of the frequent occurrence of cord blood plasma with a haptoglobin pattern unlike that of the mother.

Extensive data on the quantitative haptoglobin levels of the cordal blood plasma with or without detectable haptoglobin pattern, of the blood plasma from the mothers at the end of the gestation period and of those from the fathers and from normal controls, are also reported and discussed.

To be published in full in: *Acta Genetica et Statistica Medica*, Karger, Basel.

15.83. Asynchrony in the Synthesis of the Polypeptide Chain and Carbohydrate Prosthetic Groups in Human Transferrin. W. CAREY PARKER and ALEXANDER G. BEARN (New York City, U.S.A.).

Transferrin C, the specific iron-binding component of human serum, is a glycoprotein of molecular weight approximately 90,000 whose carbohydrate moiety contains glucose, mannose, glucosamine, fucose, and sialic acid. Recent studies on several glycoproteins by various authors have demonstrated the occurence of prosthetic groups of multiple carbohydrate residues attached at various points to the polypeptide chain of the protein; sugar nucleotides have also been isolated which contain similar carbohydrate groups. Stepwise removal of the four sialic acid residues of transferrin can be accomplished by the enzyme neuraminidase; transferrin devoid of sialic acid retains the iron-binding, antigenic, and sedimentation properties of untreated transferrin. Purification and analysis of a primate transferrin reveals that it contains half the human complement of both sialic acid and glucosamine. Cord serum from newborn infants contains, in addition to transferrin C, four additional iron-binding components which migrate slightly more rapidly by starch gel electrophoresis than the corresponding components in the pattern of neuraminidase-treated adult transferrin. The faint components disappear within 6-12 weeks of birth. These observations suggest that the differences in mobility result from alterations in the molecule in addition to the absence of sialic acid and are consistent with the interpretation that a mechanism responsible for the addition of carbohydrate prosthetic units (containing glucosamine and sialic acid) functions imperfectly at birth, so that not all of the transferrin molecules receive the full complement of sialic acid-containing prosthetic groups. A similar phenomenon is present in human cerebrospinal fluid, where two principal populations of transferrin molecules appear to exist, one with the full complement of sialic acid-containing prosthetic groups, the other devoid of such groups.

15.84. Inherited Structural Variation of Human Myoglobin. SAMUEL H. BOYER, DAVID C. FAINER, and MICHAEL A. NAUGHTON (Baltimore, U.S.A.).

Although considerable variation occurs among the hemoglobins of several species, no such variation has been reported for myoglobin. We shall describe examples of inherited structural alteration in myoglobin and indicate simple methods for their detection.

Myoglobin, obtained from autopsy and diagnostic muscle biopsy, was prepared by ultrafiltration of crude muscle extracts. The ultrafiltrate was subsequently examined by starch gel electrophoresis at pH 8.5. These procedures are simple, highly efficient and well suited for characterization of large numbers of samples.

Among 159 individuals, two subjects possessed both normal myoglobin (Mb^{+}) and an electrophoretically distinguishable variant. One of these variants, $Mb^{Aberdeen}$, migrates more slowly than Mb^{+} while the other variant, $Mb^{Annapolis}$, migrates faster than Mb^{+}.

$Mb^{Annapolis}$ occurred in a mother and her son both of whom were free of muscular and cardiac disease. Mb^{+} and $Mb^{Annapolis}$ are similar in molecular weight and spectral absorption but differ in fingerprints of tryptic digests. $Mb^{Annapolis}$ lacks two normally present peptides, viz., 7 and 13, and possesses a new peptide, X. Amino acid analysis of these peptides and homology with sperm whale myoglobin suggest that the peptides are arranged: NH_2 7 arginine/NH_2 13 lysine

The loss of arginine in $Mb^{Annapolis}$ accounts for the altered fingerprint and the rapid migration of the whole molecule but has no apparent influence on function.

Detection and characterization of additional myoglobin mutants may be helpful in the study of certain muscular diseases as well as quite useful in assigning function to various portions of a molecule whose fine structure is particularly well delineated.

SECTION 16

HUMAN CYTOGENETICS

16.1. (D.) Karyological Evolution in Primates (Catarrhina Monkeys). B. CHIARELLI (Torino, Italy).

The developments of the *in vitro* tissue culture methods are providing considerable insight into the mechanism of evolution related to chromosome variations in mammals.

In this respect some recents works on Primate chromosomes seem very promising.

During the last four years I had the opportunity of studing the chromosomes of 112 animals, of both sex, belonging to nearly all the species of the Old World Monkeys and Anthropoid Apes.

The chromosome numbers of these groups of animals show a good deal of variation.

All the species of the genus *Macaca*, *Papio*, *Theropithecus* and *Cercocebus* have a diploid chromosome number of 42. Different species differ in some morphological characteristics in their chromosomes.

All the species I have investigated in the genus *Hylobates*, *Presbytis* and *Colobus* have 44 chromosomes and they differ from the others for the morphology of some chromosomes.

The species *Erythrocebus patas* has a diploid number of chromosomes of 54.

In the genus *Cercopithecus* the different species have a modal number of 54, 60, 66, 72 chromosomes (it is noteworthy that all these numbers are multiples of 6) the morphological differences among the different species are related to the number of acrocentric chromosomes.

All Anthropoid Apes have 48 chromosomes. Some of their chromosomes are different in morphology.

Moreover in the demonstration I have presented some attempts to interpret the variation in number and morphology of the chromosomes of the different species with centric fusion, inversion and translocation mechanisms.

16.2. Multilateral Aspects of Meiotic and Postmeiotic Sex-chromosomes in Different Species. PAUL EBERLE (Göttingen, Germany).

The X- and Y-chromosomes in the male of different species have been investigated during meiotic and postmeiotic stages. In *Locusta migratoria* (XO), *Mus musculus*, *Rattus norvegicus*, *Mesocricetus auratus*, *Cricetulus griseus* and *Homo sapiens* up to pachynema typical sex-vesicle structures in early meiotic prophase stages, which include parts of whole sex-chromosomes, are visible. The heterologous parts and the solitairy X-chromosome of *Locusta pass diplonema*, diakinesis and metaphase-I in nearly mitotic shape. Entirely heterologous sex-chromosomes are kept together by an unilateral and achiasmatic end-connection. In *Locusta*, 54.2 per cent of early spermatids (gynospermatids?) own a large Feulgenpositive interphase-structure, which lies in the center of the nucleus. Comparable structures with regard to the X- and Y-chromosome are found in the spermatids of man. The sex-vesicle is interpreted as a special structure of allocyclic sex-chromosomes or parts of them, corresponding in early meiotic stages to the chromocenters of mitotic interphase-nuclei. So the heterologous parts are protected against crossing-over and allowed to continue in genetic differentiation. As a meiotic attribute an achiasmatic end-connection is formed and the division of the centromeres in metaphase-I prevented. Thus, the meiosis of the heterologous sex-chromosomes may be thought as the scheme of a primitive meiosis, developed secondarily and differentially in the several species. The formation of the sexvesicle is independent of the nucleus-orthoploidy and the DNA-ratio between autosomes and sex-chromosomes. Heteropycnotic behaviour of sex-chromosomes in spermatids is interpreted as a condition, capable to block genetic activity, giving equal chances of fertilization to both types of gametes.

16.3. Studies of Human Pachytene Chromosomes. J. I. VALENCIA, N. CACHEIRO and C. SONNENSCHEIN (Buenos Aires, Argentina).

The chromomere pattern of human pachytene chromosomes is being studied, with the purpose eventually of constructing a cytological map. Several bivalents can already by identified tentatively with relation to the mitotic chromosomes, and some gross chromomere counts have

been determined provisionally. For example, chromosome No. 1 appears to have 57 chromomeres and chromosomes 21 and 22 show 9 each.

No chromocentral region has been identified, even in very well spread pachytenes, but the sex vesicle has been superimposed on one or more chromosome pairs, giving the impression of a chromocenter.

The X and Y chromosome threads have been observed inside the sex vesicle, apparently attached by the tips of their short arms. The long arm of the Y ends in two minute, very deeply staining appendages which protrude from the vesicle. The basis of the X-Y pairing is obscure, but some favorable metaphases reveal a knot-like structure reminiscent of a chiasma at the point of association.

At the end of pachytene or the beginning of diplotene, the sex vesicle begins to disintegrate and the sex chromosomes become as distinct as the autosomes, each showing two chromatids.

Pachytene cells show from one to four nucleoli, none of them associated with the sex pair. Their size depends upon the number of them present. In most cases, they are associated sub-terminally with small or medium-sized acrocentric autosomes, but there are some variations which will be discussed.

16.4. Studies on the Leucocyte Stimulating Factor in Beans. KERSTIN LINDAHL-KIESSLINS (Uppsala, Sweden).

Saline extracts from beans (*Phaseolus vulgaris*) have been fractionated using different procedures. The fractions have been tested with regard to their mitogenic activity, red cell agglutination, serum precipitation and electrophoretic mobility.

16.5. The Non-random Distribution of Specific Chromosomes in Somatic Metaphase Figures from Cultured Human Leucocytes and Their Relation to the Time of Termination of Chromosome Duplication. O. J. MILLER, W. R. BREG, A. C. CHRISTAKOS, B. B. MUKHERJEE and A. VAN N. GAMBLE (New York, U.S.A.).

The chromosomes are not distributed at random in flattened metaphase figures from colchicine, hypotonic sodium citrate treated human leucocyte cultures. The Y chromosome, one of the X chromosomes, and pair number 13 tend to occupy a peripheral location. Pair number 21 and the chromosomes in group 17-18 are farther from the center of circular metaphase figures, and more frequently at the periphery of less selected metaphase figures than most of the remaining chromosome pairs or groups. Pair number 3 and group 6-12+X share this same rather peripheral location. Chromosome 1 is closer to the center and less frequently at the periphery of metaphase figures than any other chromosome. Pair number 2, group 14-15 and group 19-20 are almost as near to the center as pair number 1.

The location of a chromosome is not related to its size, but may be related to the time it terminates DNA synthesis, as shown by autoradiographic studies with thymidine-H^3. In general, the chromosomes that terminate their DNA replication later in the DNA synthetic period than do the other chromosomes of the complement tend to have a peripheral location.

16.6. A Possible Mechanism of Dicentric Formation: "Telomeric Binding" in Cultivated Human Cells. P. S. MOORHEAD and EERO SAKSELA (Philadelphia, U.S.A.).

Studies of metaphase chromosome rearrangements induced in cultured human cells by the presence of the simian vacuolating agent SV-40 have led to the interpretation of certain abnormal chromosomes as the product of an end-to-end coupling of morphologically identifiable autosomes into a dicentric which replicates further as an entity. Examples interpreted as resulting from such "whole-chromosome-translocations" have been found within independently transformed cultures, including evidence involving diplochromosome homologues following endoreduplication. Cytologic demonstrations of spontaneous occurrences of this process were also found in cells of a mosaic strain and in the degenerative phase of long term cultivated diploid strains. Interpretations of the origin of various other rearrangements and chromosomes losses resulting from *in vitro* SV-40 virus infection will also be considered in the context of findings from studies employing human diploid strains and current attempts to demonstrate similar effects with human viruses adeno-12 and adeno-18.

16.7. Determination of the DNA Content of Cell Nuclei and Individual Human Chromosomes by Photographic Colorimetry. E. M. DEN TONKELAAR, J. L. G. GAILLARD, P. VAN DUIJN and A. SCHABERG (Leyden, the Netherlands).

The DNA content of cell nuclei and individual chromosomes was studied. The cells and chromosomes were stained by the Feulgen method and the DNA measured by a new microspectrophotometric method based on photographic colorimetry.

A photomicrograph from the stained object is taken with monochromatic light. The negative is enlarged and by a special development a blue colored print is made. In this print the pictures of the object to be measured are cut out, the blue dye is extracted and the extinction of this solution is determined with a colorimeter.

As is postulated in the constancy hypothesis, all diploid cells in an organism should have the same DNA content. When measuring a number of cells, a certain variation in the results is found. Analysis of variance was done to separate the variation in the object from the random measuring error. In this way we established the accuracy of the method and tested the constancy hypothesis.

We found a high constancy of DNA content. The measured ratio between the DNA content of diploid and tetraploid cells also fulfilled the theoretical requirements.

Photographic colorimetry has the advantage that objects of very different forms can be measured by cutting out the pictures from the photograph. This fact and the results with interphase nuclei led us to use the method for the determination of the DNA content of individual human chromosomes. These results will be discussed.

16.8. Further Characterization of the Chromosomal Complement of Man Through Autoradiography. JAMES L. GERMAN (New York City, U.S.A.).

In addition to their structural features, the sequence in which chromosomes complete DNA replication provides further characterization. This sequence has been partially elucidated through autoradiography of metaphase chromosomes in which the DNA replicated during the terminal 1-3 hours of the S-period had been labeled with H^3-thymidine.

Though variability and homologue asynchrony are prominent in nucleated blood cells *in vitro*, intra-group patterns in Groups 4-5, 13-15, 16-18, and Y-21-22 serve to distinguish members of the group. Two in Group 4-5 are among the latest of the complement to complete replication, while in the other two there is early completion in the long arms. In Group 13-15 two are very late, while two are among the earliest of the complement to cease the uptake of thymidine. Pair No. 17 completes replication very early, pair No. 18 late. Of chromosomes of Group Y-21-22, in most male cells the Y is last; two of the group complete replication very early.

The late replicating X of cells derived from the normal female is clearly distinguished from others of Group 6-X-12. Its characteristics include: 51 per mille of the total length of the haploid complement; arm ratio of 1.75; extreme peripheral location in 33 per cent of c-metaphases (expected in 32 per cent); secondary constriction in the long arm in 3 per cent of cells; length in relation to the average length of a Group 4-5 chromosome $\left(\frac{\text{average 4-5}}{\text{X}}\right) = 1.25$; inordinate shortening in relation to chromosomes of Group 4-5 in cells having had exposure to colcemide for 3 hours.

16.9. Autoradiographic Studies of Normal and Abnormal Human Karyotypes. WERNER SCHMID (Houston, U.S.A.).

Chromosomes replicate segments of their deoxyribonucleic acid (DNA) strands in nonrandom sequences. The use of tritiated thymidine and autoradiography permit the study of these replication sequences in metaphase chromosomes, a technique which was introduced into cytogenetics by Taylor, Woods and Hughes in 1957 (1).

In short-term cultures of human leukocytes and in embryonic fibroblast cultures, continuous labeling with tritiated thymidine (specific activity 1.9 C/mmol) at a concentration of 1 μc/ml medium, was begun 6 hours preceding fixation of the cells in metaphase. Colcemid was added for the last two hours, collecting cells in metaphase from different terminal DNA replication stages. The chromosomes were photographed before and after exposure (4-6 days) to autoradiographic film. The often stepwise sequences in which DNA replication is completed in individual chromosome pairs will be demonstrated. Homologous chromosomes go through the same final replication sequences, usually synchronously, sometimes slightly out of phase. Five very marked, late replicating regions in chromosome pairs Nos. 1, 4, 9, 16 and in the Y, coincide with secondary constrictions which, by means of special techniques, have been demonstrated in these chromosomes by Saksela and Moorhead(2). So far, no significant differences

have been found between the terminal replication patterns of lymphocyte chromosomes versus chromosomes of embryonic fibroblasts grown in tissue culture. Results of the application of the labeling technique to a number of abnormal human karyotypes, including trisomic and translocation mongolism and chronic myeloid leukemia, will also be demonstrated.

1. *Proc. Nat. Acad. Sci. U.S.* **43**, 122.
2. *Cytogenetics*, in press.

16.10. Structural Variability of Human Chromosomes. THEA LÜERS and EVA STRUCK (Berlin-Dahlem, Germany).

Studying the chromosomes in human diseases sometimes it seems difficult to decide between structural abnormalities, natural variants, and artfacts. A collection of karyotypes from leucocyte cultures is presented and discussed showing atypical chromosomes such as size differences between the two homologues, achromatic regions, elongated centromere region, translocation with partial trisomy, ring chromosomes, dicentrics, variability of the Y chromosome and of chromosomes nos. 21 or 22.

16.11. Evidence from Six X-autosome Translocations Bearing on the Single-active X Hypothesis. LIANE BRAUCH RUSSELL (Oak Ridge, U.S.A.).

Recent results confirm our original thesis that V-type position effects from X-autosome translocations in the mouse (first reported by us in 1959) are found when the translocated X can act heterochromatically. This occurs only when there are at least two X's present, one being required for gene action. Influences from X heterochromatin suppress near-by autosomal genes, allowing the recessives on the standard autosome to express themselves. Lyon later suggested that one or the other X is *entirely* inactive and that the X-translocated autosome behaves completely like the X to which it is attached. This would imply that, for any given autosomal locus, variegation characteristics should be *independent* of the position of the X and autosome break points. In work with six translocations—one T(X;8) and five T(X;1)'s—we have obtained evidence to the contrary.

(1) Apparently, not the entire X has inactivating functions. Thus, the *c*-locus, which shows typical variegation in four T(X;1)'s, fails to do so in the fifth, even though breakpoint is <0.2 c.o.-units from $+^{c}$. (2) Inactivating portions of the X exert their influence along a gradient. Thus, the *amount* of variegation for a given locus differs for different translocations, one factor being distance of locus from rearrangement point. Furthermore, we observe a typical spreading effect for three of the translocations.

The finding that part of the X has no ability to inactivate translocated autosome suggests that this part may itself never become inactive and therefore not fit the single active X hypothesis.

16.12. The Frequency of Sex-chromatin-positive Cells and the Number of Nucleoli per Cell in Cultures of Human Tissues. A. J. THERKELSEN (Aarhus, Denmark).

The number of sex-chromatin-positive cells was counted in tissue cultures of human cells from bone marrow and from embryonic and adult human skin. The frequency of sex-chromatin-positive cells is low—40-60 per cent—in the logarithmic growth phase, but rises to about 95 per cent in the post-logarithmic phase. The variation was found in normal females as well as in sex-chromatin-positive males. The cause of the variation will be discussed. In the same type of experiments, cultures from males and females were grown simultaneously, and the number of nucleoli per cell was counted in specimens stained with Feulgen-light-green. The average number of nucleoli per cell was shown to be significantly higher in normal females than in normal males.

This difference between the two sexes may be explained on the assumption that the female X-chromosome which forms the sex-chromatin is nucleolus-organizing, whereas the Y-chromosome in males is not.

If this explanation be correct, the average number of nucleoli per cell in Klinefelter patients should be significantly higher than in normal males, whereas the number in Turner patients should be the same as in normal males. Preliminary experiments with Klinefelter and Turner patients have been performed. The results are discussed.

16.13. Iso-chromosome X in Man. M. FRACCARO and J. LINDSTEN (Pavia, Italy).

In a group of females selected because phenotypically similar to XO individuals, we found

46 chromosomes instead of the expected 45 and a structurally abnormal X chromosome. Measurement of this metacentric chromosome showed that its arms were approximately equal to the long arm of the X. These individuals were consistently sex chromatin positive and measurements of the DNA content of Barr bodies in their cells (in collaboration with H. Klinger) revealed that these Barr bodies had higher DNA content than those from suitable controls. When labelled with ^{3}H-thymidine (in collaboration with S. Muldal) late in the synthesizing period the abnormal chromosome was heavily labelled (i.e. it behaved as one of the two X chromosomes usually does in this system), and symmetrically so along its length. We tentatively interpret this abnormal chromosome as an iso-chromosome for the long arm of X.

If the cytological interpretation is correct, it follows that the carriers of these iso-chromosomes are monosomic for the short and trisomic for the long arm of X. In one family in our material there was informative segregation of the genes for deutan colourblindness and the sex-linked blood-group Xg. Analysis of this family (in collaboration with Ruth Sanger and R. R. Race) gave evidence that both genes are located on the short arm of the X chromosome. The origin of the iso-chromosome is probably meiotic and likely paternal. There was an apparent deficiency of females among the sibships of the index cases.

16.14. DNA Replication of X-chromosomes in Cultured Leucocytes from Presumptive XXX, XXXXY and XO/X Iso-chromosome-X Mosaic Human Subjects. Barid B. Mukherjee, J. Orlando Miller, W. Roy Breg and Saul Bader (New York, U.S.A.).

An autoradiographic study of DNA replication in X-chromosomes was carried out by exposing the cultured leucocytes to tritiated thymidine for 3-9 hours in the presence of colchicine and then harvesting the cells directly from the isotope-containing medium. Two chromosomes in group 6-12+X in metaphase figures from three chromatin two positive presumptive XXX females and three chromosomes of the same group in metaphase figures from a chromatin three positive presumptive XXXXY male were consistantly the last chromosomes in the whole complement to complete DNA synthesis. Two sex chromatin masses were observed in only two-ten per cent of the interphase nuclei of the three XXX females and three sex chromatin masses were present in only seven per cent of the interphase nuclei of the XXXXY individual. These results support the concept of a one-to-one correspondence between the maximum number of sex-chromatin masses and the number of late-replicating X-chromosomes.

The isochromosome-X was always the last chromosome to terminate duplication in cultured leucocytes from the XO/X iso-chromosome-X female, and the normal X chromosome never terminated duplication late. According to the Lyon hypothesis, random differentiation of the paternally or maternally derived X chromosome should occur in individual cells during mammalian embryogenesis. In this case, however, the structurally abnormal X chromosome always becomes the heteropycnotic, metabolically inert member of the pair.

16.15. The Incidence of Drumsticks in Normal Women and in Patients with Chromosomal Abnormalities. Ursula Mittwoch (London, Great Britain).

The demonstration of sex chromatin has become an important aspect of human genetics, both for theoretical and for practical reasons. Although it is now generally accepted that the drumstick of the polymorphonuclear leucocyte bears a direct relationship to the X-chromosome and is therefore comparable to the Barr body, the interpretation of findings on drumsticks has not always been straightforward. For this there are three main reasons: (1) The low incidence of drumsticks, which is on the average less than 3 per cent of polymorphonuclear leucocytes; (2) the wide variation in frequency encountered in different women; (3) the fact that incidence of drumsticks is to some extent determined by the degree of segmentation of the polymorphonuclear nuclei.

An investigation has been made relating the incidence of drumsticks to the number of nuclear lobes in apparently normal women and in patients with various chromosomal abnormalities. The following individuals were tested: 12 apparently normal women; 27 girls with mongolism (Down's syndrome) in association with 27 non-mongol mentally defective girls for control purposes; 12 patients with Klinefelter's syndrome (XXY), 3 patients with XXXY chromosome constitution; and 3 patients with XXX chromosome constitution.

It was found that in the apparently normal women the correlation coefficient between the incidence of drumsticks and the average number of nuclear lobes was +0.44. In all women there

was an increase in the incidence of drumsticks with an increase in nuclear lobe number from 1 to 5, but the frequency of drumsticks in cells of any given lobe number showed wide variations in different women. This difference could be demonstrated on repeated occasions.

In the patients with chromosomal abnormalities, but not the mentally defective non-mongol girls, there was a decrease in the average number of polymorphonuclear lobes and a lower incidence of drumsticks. Moreover, the frequency of drumsticks per cell of constant lobe number was less than in the normal women.

It is suggested that chromosomal abnormalities tend to lower the segmentation of the polymorphonuclear nuclei and that this is associated with an even more marked reduction in the number of drumsticks.

16.16. (D.) Monozygotic Twins of Different Sex. T. Dent and J. H. Edwards (Birmingham, Great Britain).

A case of Turner's syndrome, presenting with amenorrhoea and dwarfism, was noted to have a twin brother. The brother appeared to be a normal male. Blood and serum groups showed no evidence of dizygosity, and the palm prints were strikingly similar. Chromosome analysis from lymphocyte and fibroblast cultures showed that both twins were preponderately XO in constitution.

The male twin had marked difference in eye colour, one eye being blue-green, the colour of his sister's eyes, and the other brown. This suggests the possibility of a locus on the Y chromosome related to eye colour.

The origin of this chromosomal error was presumably mitotic non-disjunction at a stage before the cell lines determining either twin were defined. The remarkably normal phenotype of the boy suggests that, if his tissues are preponderately XO, then the stigmata of Turner's syndrome may be related to endocrine abnormality rather than to defective responses of structural tissues to directives relating to growth.

16.17. Lethal Chromosome Constitutions in Man. K. Patau, S. L. Inhorn, and E. Therman (Madison, U.S.A.).

The word "lethal" is here restricted to conditions that invariably prevent birth of a live child. Evidently, polyploidy is lethal. In a study of placental and fetal tissues from early abortions a new case of triploidy was found, the first with XXX and no Y. Among mechanisms that may cause triploidy, fertilization by a giant sperm should also be considered. Precise measurements showed these to contain twice the DNA content of haploid sperm. Nullosomy for the X is undoubtedly lethal, and so is monosomy for any autosome. Viable trisomy is known only for three autosomes; D_1, 18, and the mongolism chromosome. D_1 and 18 trisomics may have a high pre-natal mortality, but it is at least as likely that their rarity, compared with mongolism, reflects much lower non-disjunction rates. There are reasons for believing that numerous genes are involved in trisomy effects and that trisomy for any human autosome interferes in many ways with development. It is concluded that trisomy for all, or almost all, but the three above mentioned autosomes is lethal. In the abortion study, no trisomics have turned up as yet, but at least two cases of monosomy for a C chromosome were found. In one, there was an admixture of female diploid cells, but in both cases the sex chromatin count was so high that the lacking chromosome must have been an autosome. In a third case of C monosomy no sex chromatin data could be obtained.

16.18. Abnormalities of the Acrocentric Chromosomes. Kurt Hirschhorn (New York, U.S.A.).

A significant number of reports have appeared during the past few years dealing with various abnormalities of the acrocentric chromosomes. These have included conditions recognized as definitely associated with pathological states (trisomies, deletions), as potentially pathological (translocations), and finally, as of purely morphological interest (for example, satellite structure, distribution, and inheritability). We would like to discuss several new morphological variants. Three patients and members of their family were found to possess additional material in the short arms of either the long acrocentrics, the short acrocentrics, or both.

The first patient, a 19-year-old unmarried female with primary amenorrhea, no other clinical abnormalities, and an excellent response to exogenous hormonal therapy, had one long acrocentric chromosome whose short arms were 2-3 times as long as those of the other long acrocentrics. The same chromosome has been found in the patient's asymptomatic brother, sister, mother, and uncle. The second patient, a one-year-old male child with congenital heart dis-

ease (interventricular septal defect) had two acrocentric chromosomes involved, one long and one short. In both chromosomes a prominent segment was apparent between the short arms and the satellites, therefore showing two secondary constrictions in the short arms. The inserted segments were a bit longer than the normal short arms which were also present. The third patient, a 35-year-old female with chromatin-positive primary amenorrhea and very short stature, showed gonosomal mosaicism (isochromosome-XX/XO). In addition, one small acrocentric chromosome was noted to have a very prominent fragment inserted between the short arms and the satellites, appearing exactly like the chromosome described for case 2. Only in the last case was there a possible source for the fragment; for example, part of the short arms of one X. The parents of the other two cases are being investigated to see if the unusual chromosomes found in the propositi might be present in their karyotypes as well as some obvious deletion in another chromosome. Our working hypothesis is that this additional bit of chromosomal material may be responsible for a partial trisomic state in the propositi.

16.19. Chromosomal Break in Autosome No. 2, in a Patient with Hypogonadism. H. VAN DEN BERGHE, H. VERRESEN, W. DE LOECKER, O. STEENO, and P. DE MOOR (Louvain, Belgium).

A break of the two short arms of one autosome no. 2 has been discovered in a 47-year-old male patient. Numerous chromosomal preparations were obtained from leucocyte cultures at different time intervals. The scission of the two short arms of one autosome no. 2 occurred in the direct neighbourhood of the centromere, these two separated arms staying attached to each other in all the cells.

This chromosomal material, initially belonging to the autosome no. 2, was found to be transferred and attached either to autosome no. 21 or 22, or even existing freely between the other chromosomes. This phenomenon was found to exist in all the examined cells. All the cells have 46 chromosomes.

The clinical picture of this patient showed hypogonadism of testicular origin, disturbed anabolic features, and a series of secondary abnormalities. The sex-chromosome-complement is XY in all the cells, and Barr-chromatine in buccal smear being negative. The karyotype in one parent and seven sibships was equally studied.

The incorporation of thymidine-^{3}H and glycine-2-C^{14} into the chromosomes of the cells of this specific case were performed. The effect of scission of one chromosome and the degree of incorporation of radioactive precursors into the different sections of the chromosome involved as well as in the free existing fragments was examined.

16.20. Chromosomal Abnormalities in a Familial Syndrome Involving Labial Fistulae with or without Hare Lip and Cleft Palate. ADRIANA DE CAPOA, M. SINISCALCO, and D. TARSITANI (Rome, Italy).

Among the types of autosomal trisomies hitherto described and confirmed, cleft palate and hare lip are often present though accompanied by other congenital malformations.

On the other side, in individuals showing cleft palate and hare lip unaccompanied by other congenital anomalies, chromosome studies have failed to show significant abnormalities.

The authors have recently observed a family involving a mother and eight children, seven of which show labial fistulae associated in five cases with hare lip and cleft palate. Chromosomal studies performed on peripheral blood of the mother and three of the affected children showed the existence of an autosomal mosaicism, involving an additional chromosome, apparently belonging to group 4-5. In all the patients, besides the cells with the extra chromosome, another abnormality was found in all mitoses observed: i.e. a small centric chromosomal fragment seemed to be constantly present, while one chromosome of group 18-20 was missing. This fragment seems to pair with the long arm of one of the chromosomes of group 18-20, and it could therefore be interpreted as the missing chromosome, carrying a deletion on its short arm. The father was also studied and his chromosome complement was found to be normal.

The interpretation of these findings is, for the time being, very difficult and indeed hazardous. However, even at this stage, a few conclusions can be tentatively drawn:

(i) On the basis of nuclear sexing, an involvment of the X-chromosome in the abnormal cells can be excluded;

(ii) It is very unlikely that the two chromosomal abnormalities are independent of each other, and probably the delegation affecting the small submetacentric chromosome, being present in all the cells, is likely to be the primary defect.

(iii) The absence of severe malformations and of mental retardation makes plausible the hy-

pothesis that in the patients referred to there was no loss or duplication of genetic material, the primary defect being possibly due to a (reciprocal?) translocation followed by somatic non-disjunction in some cell lineage.

(iv) The pattern of inheritance of the present syndrome (transmission from a parent to all her children but one) suggests the possibility of a non random segregation of the aberrant chromosome pairs during maternal gametogenesis.

To be published in full in *Annals of Human Genetics*, London, England.

16.21. Neonatal Hemoglobin Abnormalities in Trisomy 13-15 Syndrome. Frederick Hecht, Ernst R. Huehns, and Arno G. Motulsky (Seattle, U.S.A.).

Linkage of genes to specific chromosomes was attempted by examining hemoglobin from individuals with autosomal trisomies by starch gel electrophoresis. No hemoglobin abnormalities were found in newborns with trisomy 18 or trisomy 21. In two newborns with trisomy 13-15 syndrome an excess of Hb-γ_4 and a minor hemoglobin with slow mobility (Hb-X^F) were found. No such abnormalities were found in two older children (age 20 and 49 months) with trisomy 13-15.

Hb-γ_4 was identified by electrophoresis, ultraviolet spectrum, and alkali denaturation. The following studies of Hb-X^F have been done. On starch gel electrophoresis the mobility of Hb-X^F resembled that of Hb-Gower 2. On agar gel electrophoresis at pH 6.2 Hb-X^F migrated with Hb-A. The alkali denaturation rate of Hb-X^F was between that of Hb-A and that of Hb-F. The ultraviolet spectrum of Hb-X^F was similar to that of Hb-F. Sedimentation and recombination experiments suggest that Hb-X^F has the structure of $\alpha_2^A X_2^F$.

The net charge difference of the X^F-chain from the β^A-chain makes it likely that there is more than one amino acid discrepancy between these two chains. A hemoglobin similar to Hb-X^F has been found in some small embryos (e.g. the Gower embryo) and rarely in cord blood. It may therefore represent a normal embryonic hemoglobin. Hb-X^F might thus be a physiologically and chemically distinct hemoglobin with its own non-α-chain: the epsilon chain.

The finding of Hb-X^F and excess Hb-γ_4 in trisomy 13-15 syndrome raises the possibility that some of the genes controlling hemoglobin synthesis during fetal life are located on the triplicated chromosome.

Aided by the U.S. Public Health Service.

16.22. A New Form of Trisomy 13-15. St. M. Milcu, C. Maximilian, V. Stănescu, I. Florea and M. Augustin (Bucarest, Rumania).

The case of a child, aged 7, presenting Turner's syndrome with a male phenotype, numerous malformations, and a chromosomal trisomy 13-15 with an XY sex karyotype is reported.

The patient had a short stature, a big head with prominent eminences and metopic suture, hyperteloriam, an antimongolian slit, a trilobated nose, ears with a low implantation and asymmetric auricles, retrognathism, a short and palmated neck with a low insertion of the hair in the back, Lyipogenesis of the breast papilla, right umbilical and inguinal hernia, hyperlaxity of the teguments and ligaments, hypoplasia of the genitalia, shortening of the metacarpal and metatarsal bones, clinodactyly. The patient also presented hepatomegaly and the air mediastinography rendered evident an enlarged thymus. The right hypoplastic testis presented a dissociated spermatic cord, and a dysgenetic histologic structure; the right testis could not be palpated. Sex chromatin was negative and the culture of bone marrow tissue showed the trisomy of one of the 13-15 pairs with an XY karyotype.

The reported case differs from the other cases of 13-15 trisomy by the clinical aspect of Turner's syndrome with a male phenotype, emphasizing thus the diversity of the clinical aspects brought about by this trisomy. Nevertheless, in this case an association of the trisomy with submicroscopic alterations of the Y chromosome might exist, but dysgenesis of the gonads might also be an effect of trisomy.

16.23. A B/G (4-5/21-22) chromosomal Translocation associated with Multiple Congenital Anomalies. K. H. Gustavson, S. C. Finley, W. H. Finley and B. Jalling (Stockholm, Sweden).

A case of a male infant with multiple congenital anomalies is reported. The anomalies include scaphocephaly with premature syntostosis of sagittal suture, marked hydrocephalus with dilatation of the ventricular and cisternal

systems, low set malformed ears, slight hypertelorism, bilateral blepharophimosis and coloboma of the iris, micrognathia and macroglossia. There were also malrotation of the small intestine, omphalocele, hiatus hernia, dilatation of the renal pelves, absence of one umbilical artery and anal atresia. The finger and toes were long, and there was a distinct gap between the first and second toes bilaterally. There was marked muscular hypotonia and the deep tendon reflexes were weak. The heart and genitalia were normal. The child died at the age of six months. An EEG was normal. Numerous vacuolated mononuclear cells were found in the peripheral blood and in the bone marrow.

The mother had had five pregnancies. The first child was a male who died shortly after birth. The second pregnancy resulted in a normal male child. The next two pregnancies terminated in early abortions. The present case was the fifth pregnancy.

Chromosome studies of cultured skin and bone marrow cells of the propositus showed a chromosome number of 46 and an XY sex chromosome constitution. There were only three chromosomes in group G (21-22), and there was an odd chromosome which could not be paired. The mother, who was phenotypically normal, had a chromosome complement of 46 in cultured skin cells. In addition to the same abnormalities present in the karyotype of her son, one of the chromosomes in group B (4-5) lacked the short arms. The karyotype of the father was normal.

The most likely interpretation of the chromosome abnormality is a translocation involving a chromosome of group 4-5 and a member of group 21-22.

16.24. Multiple Congenital Anomalies with Autosomal Trisomy amongst the Chromosomes of Group 16-18 and Their Possible Relations to the "Dyscranio-Dysphalangic-Syndromes." M. Tolksdorf, H. G. Hansen, H. R. Wiedemann and W. Lehmann (Kiel, Germany).

We discuss the autosomal trisomy amongst the chromosomes of group 16-18 associated with multiple congenital abnormalities.

In view of the similarity in the clinical pattern of the anomalies in cases so far published we regard some distinctive clinical findings and other features occurring facultatively.

In this connection we give a report of a premature female child with multiple anomalies and a trisomy 17 demonstrated in blood and bone marrow cells.

Moreover, we shall try and find possible relations between the cases with trisomy amongst the chromosomes of group 16-18 and the various types of the "Dyscranio-Dysphalangies" (Günther).

16.25. (D.) Trisomy 17 associated with Multiple Congenital Anomalies. H. G. Hansen, M. Tolksdorf, W. Lehmann and H. R. Wiedemann (Kiel, Germany).

We demonstrate figures of a premature female infant with a relatively paraphased combination of congenital abnormalities. Cytological studies on her blood and bone marrow cells were carried out on short term cultures. The results show a chromosome number of 47 with a trisomy amongst the group 16-18 in the great majority of cells of both cultures.

Some mitoses and karyotypes are demonstrated.

16.26. Discordant Mongolism in Monozygotic Twins? J. W. Bruins, J. van Bolhuis, J. B. Bijlsma and L. E. Nijenhuis (The Netherlands).

In 1956 van Beukering and Vervoorn published a case of negro-twins, one of which suffered from Down's syndrome (mongolism). Investigation of the placenta (plate) proved the twins to be monozygotic. So this would be a case in point of discordant mongolism in uni-ovular twins. It was not possible to examine the bloodgroups or the chromosomes of these negro-twins.

If the general karyotype of the child with Down's syndrome presented 47 chromosomes, this would be a case of non-disjunction which occurred in the fertilized human egg. Instead of a derangement in the meiosis, it would be here non-disjunction in the mitosis.

On October 4, 1962, at Deventer (Netherlands), a pair of twins was born that at first was taken to be dizygotic on account of anthropometrical differences. One of the twins was normal, the other showed nearly completely Down's syndrome. Unfortunately the placenta was not examined but examination of the bloodgroups indicated that the twins were monozygotic. To test this, a small reciprocal skin-transplantation will be made.

The number of chromosomes of the cultivated white blood cells was 47 for the diseased child, and 46 for the normal one. So they are "mosaic-twins", at least if there has been one cell in the

beginning. More investigations will be done with other tissues of the mongoloid child to test the number of chromosomes. It may very well be possible that these tissues will have a mosaic character as a result of the mitotic non-disjunction.

The examination offers possibilities as to the localization of particular bloodgroups on particular chromosomes. The examination is not yet finished and will be extended—if possible.

If congenital dysplasias, whatever they may be, be sometimes discordant in human monozygotic twins and if there be moreover a discongruency in the chromosome-pattern, one may conclude that their origin lies in a very early developmental phase.

16.27. Chromosomal Mosaicism in a Mongol Child with Average Intelligence. P. GIRAUD, R. BERNARD, A. STAHL, F. GIRAUD, M. LEBEUF, and M. HARTUNG (Aix-Marseille, France).

The authors are reporting the case of a sixteen-year-old girl, with the typical features of Mongol disease (Down's Syndrome).

Her mental level is sufficient for a quite normal (on the medium range) scholar activity.

The chromosomic study (including a skin biopsy) shows a mosaicim: Fifty (50) cells have been counted and analyzed. Among these, eight (8) cells were found as being 47 chromosomes, trisomic for the 21 chromosome; the others being normal, with 46 chromosomes.

The authors are supputing about the connections of a such mosaicism with the different features of Mongolism, with particular attention to the intellectual development.

16.28. Familial Mongolism. HANS ZELLWEGER (Iowa City, U.S.A.).

Thirty-two families with more than one case of mongolism are under investigation:

A. In fifteen families more than one mongoloid has been found in one and the same sibship (4 times both twins, 9 times two siblings, 2 times three siblings were affected).

B. Seventeen families were found with two to six cases of mongolism in a wider kindred with no single sibship (except one) having more than one mongoloid.

The average maternal age at the time of birth of the affected children was 29.1 years in Group A and 34.4 years in Group B. So far chromosomal analyses have been performed in eight families of Group A and six families of Group B. The parents of the mongoloid children had a normal karyotype in 13 families. Some normal siblings were found to have a normal karyotype as well. All but one mongoloid of these thirteen families has 47 chromosomes with a trisomy G_m. Translocation of a G_m chromosome as cause of familial mongolism was found in one family only, where the mother and one mongoloid child had 45 and 46 chromosomes respectively with a 15/21 translocation. The father has a normal karyotype. The other mongoloid child died before the chromosomes were studied. Evidence will be presented that several causes may lead to familial mongolism and that familial translocation is not its main cause.

16.29. An Extra Chromosome in Patients with Pelger-Huët's Abnormality of Leucocytes. H. SIEBNER, F. HENI and D. KLAUS (Tübingen, Germany).

In several patients of different sibships with a Pelger-Huët's abnormality of the polymorphonuclear leucocytes a small extra chromosome with subterminal centromere was found in cultures from peripheral blood and in a higher percentage in cultures from bone-marrow. Classified by its size it belongs between the pair of autosomes 20 and 21 following the Denver-classification.

A trisomy 21 appears to be unlikely, because the leucocyte alkaline-phosphatase activity was not increased in the studied patients, although there is a well-known defective lobulation of polymorphonuclear leucocytes in mongols. Relations to the Ch^1-chromosome and the Philadelphia-chromosome are discussed.

The extra chromosome may be regarded as the result of a deletion with nondisjunction of a larger chromosome. There is also the possibility of the same mechanism which is assumed by Pätau about the marker chromosome of Waldenström's Macroglobulinaemia. Chromosomes of one culture were labelled with tritiated thymidine.

16.30. Pyknotic and Binucleate Cells in Irradiated Peripheral Blood Cultures. J. WEIJER, DOROTHY L. WEIJER and H. E. DUGGAN (Edmonton, Canada).

Irradiated peripheral blood when cultured yields aberrant nuclear forms. Apart from chromosome aberrations the binucleate cells and the pyknotic leukocytes are also important.

In order to estimate damage from various dosages, frequency curves have been compiled for some of these nuclear aberrations (binucleate cells and pyknotic leukocytes) using normal leukocyte cultures irradiated with different dose levels of X-rays up to 100 r. In addition, other cultures were supplemented with radiosulphur (S^{35}) and radiophosphorus (P^{32}) (to give accumulated doses up to 200 rad). These experiments were repeated for different culture times.

A correlation was found to exist between the number of these aberrant forms, increasing radiation dosage and increasing culture time. The results with X-ray and S^{35} were comparable but P^{32} gave an enhancing effect due to its incorporation and subsequent transmutation in vital cellular components.

16.31. Chromosome Aberration Rates in Human Somatic Cells Irradiated in Vivo. M. A. BENDER and P. C. GOOCH (Oak Ridge, U.S.A.).

Although a fairly large body of data has accumulated on the induction of somatic chromosomal aberrations in human cells by ionizing radiation, the work has necessarily been done by irradiation of cells *in vitro*. The recent development of a technique for the initiation of mitosis in human peripheral leukocytes, however, permits the examination of the chromosomes of irradiated humans in the first division following irradiation. We have examined such material from a number of normal persons who received whole-body irradiation. One group of three men received doses of mixed gamma and fission neutron radiation estimated at 19, 43, and 110 rem (estimating the RBE for the neutrons as 2). A second group of three men received doses of γ-rays estimated at 17, 22, and 57 rad. The frequencies of aberrations observed in samples obtained immediately after irradiation were consistent with the coefficients of aberration production obtained previously for irradiation of leukocytes *in vitro*.[1] Aberration frequencies remained at essentially the same frequency until about 4 weeks, and the aberrations seen provided evidence that the cells had not divided *in vivo* after irradiation. In later samples, however, aberration frequencies fell, and many of the cells sampled had evidently undergone division *in vivo*. Dicentric chromosomes, symmetrical translocations, and deleted chromosomes continue to be seen many months after irradiation.

1. BENDER and GOOCH, *Proc. Natl. Acad. Sci. U.S.* **48,** 522, 1962.

16.32. Action of X-rays on Transformation of Normal Cells into Malignant in vitro. JU. B. VAKHTIN (Leningrad, U.S.S.R.).

It is known that attempts to accelerate the process of malignant transformation of monolayer cell cultures by means of chemical cancerogens were unsuccessful. The cancerogenic action of ionizing radiation on cell cultures inducing the high percent of mutations and chromosome aberrations has not yet been investigated.

Fibroblasts isolated by tripsinization from subcutaneous connective tissue of newborn rats were cultured in the synthetic medium 199 or 0.5 per cent lactalbumin hydrolysate with 20 per cent of horse or bovine serum. Just after explantation cells of each culture have been divided into five variants. Four of them were treated with 10 r 10, 100 r, 100 r 5 and 500 r of X-rays and control variant was not irradiated. Cultures were maintained for 2-15 months (6-26 passages) and implanted regularly into newborn rats and adults of the same strain (1-5 10^6 cells in each inoculum, subcutaneously and interperitoneally).

No malignization took place in variants of cultures maintained for 2-8 months. All of these cultures consisted mainly of cells with diploid and near-diploid chromosome complements.

Malignant transformation has been found within the only culture in the control variant and the variant irradiated with the dose of 100 r after 12 months of the cultivation. As implanted into rats these variant cells give rise to transplantable sarcomas.

Results obtained are compared with our data of caryological studies of cell cultures and tumours developed. The importance of genetic and epigenetic variations for evolution of cell populations *in vitro* is discussed.

16.33. The Changes in Somatic (Tumour) Cells Induced by Specific DNA and RNA. J. M. OLENOV (Leningrad, U.S.S.R.).

Tumor cells are suitable objects for study of the action of nucleic acids for a selective background can be applied in experiments *in vivo*.

The treatment of the sarcolysine-sensitive variant of the rat sarcoma 45 with DNA from the sarcolysine-resistant variant of the same sarcoma causes the transformation of the former cells (D. Podgajeckaja, V. Bresler, J. Olenov). DNA-ase inactivates the activity of the preparation. The transformants retain their properties in subsequent passages.

Rat liver RNA inhibits the tumor growth in transplantable rat liver mucous cancer obtained by Maljugina (N. N. Aksenova, V. M. Bresler, V. I. Vorobjev, J. M. Olenov). RNA-ase totally inactivates the preparation. The action of RNA from other tissues is less pronounced than the action of liver RNA.

The possibility to transform the nuclear cells indicates the universality of this phenomenon. Simultaneously, the result obtained proves the transgenic origin of sarcolysine-resistant variant. The transformation frequency cannot be determined exactly by studying of populations, but it is likely to be small. The transformability of non-haploid cells indicates the dominant or semidominant nature of the marker. Unlike the transforming activity of DNA, the antitumor activity of RNA affects all or majority of cells in the population—the change of some cells would be ineffective. The paper deals with the nature of normal tissue RNA antitumor action (the templates controlling the protein synthesis or repression and derepression of genes).

16.34. The Dependence of Molecular Weight of DNA and RNA and Its Uptake by Mammalian Cells. T. Wilczok (Gliwice, Poland).

Ehrlich ascites carcinoma cells were used as recipient cells for uptake studies of homologous labeled DNA and RNA. The rate of incorporation was proportional to the molecular weight of tracer nucleic acids; the lower molecular weight, the lower adsorption and incorporation. The highly polymerized polyanions such as polyethylene sulphate compete with highly polymerized DNA and RNA in the process of its uptake by recipient cells. DNA and RNA adsorption on the surface of recipient cells proceeds instantly and this process is not time and temperature dependent. The incorporation of DNA into DNase resistant fraction of host cell is higher at 0° than at 37° and reaches its maximal level after 5 hours. The amount of tracer DNA in DNase resistant fraction does not increase when the exposure is performed at 37°.

16.35. The Change in Chromosome Number of Human Cells in Culture after Heat Shock. W. Ostertag and H. Krüger (Münster, Germany).

The skin (epidermis) of a normal human male was cultivated in modified Puck's medium. There was no change in the normal diploid chromosome count after 4 months of cultivation (93 per cent diploid cells). The cells were then exposed to 56 C for one hour in an atmosphere of 4.5 per cent CO_2. Most of the cultures discontinued their growth, but a few surviving cells were able to form new colonies. The chromosome number of these colonies was determined. Shortly after recovery there were 52 per cent polyploids, some octoploids included. After 6 months cultivation the chromosome number changed again and there were now only 15 per cent polyploids, 9 per cent subtetraploids and 76 per cent diploid cells.

This change in chromosome number from almost all diploid to more than 50 per cent polyploid after heat shock and the slow decrease of tetraploids, that is increase of diploid cells and the almost absent fraction of subtetraploid cells after 12 months of cultivation in Puck's medium will be discussed.

16.36. Alterations in Karyotype of Mammalian Cell Populations Exposed to Antimetabolites. J. L. Biedler (Rye, U.S.A.).

Cytogenetic studies of transplantable mouse neoplasms treated with several cancer chemotherapeutic agents were undertaken in a search for possible specific relationships between antimetabolite, chromosomal alteration, and drug resistance. Karyotype analyses were carried out on sublines of sarcoma 180 and ascitic leukemia L1210 continually exposed to amethopterin, azaserine, 6-mercaptopurine, or 5-fluoruracil, singly or in combination, over periods ranging from a few weeks to several years.

The modal chromosome numbers (70-71) and 4 bi-armed marker chromosomes of the untreated sarcoma remained unaltered for 3 years. In contrast, the modal number was reduced in 7 of 8 treated sublines. In all sublines there were changes in or losses of one or more markers. The disappearance of a small subtelocentric chromosome was prevalent, and thus unrelated to a specific antimetabolite.

The modal chromosome number of the untreated leukemia remained 40-41 for 5 years. Five of 12 variously treated sublines showed a definite increase in number of chromosomes. In early transplant generations of 6 of 13 amethopterin-treated sublines, a small proportion of cells contained 2 characteristic subtelocentric marker chromosomes instead of the usual 1.

These results suggest that the antimetabolites provoked mitotic non-disjunction, resulting in decreases in numbers of chromosomes in the

hypotetraploid populations and increases in the "diploid" populations. The abnormal, bi-armed chromosomes were apparently preferentially involved.

Support by CCNSC Contract SA-43-ph-2445.

16.37. (D.). Some Relationships of Viruses and Chromosomes. WARREN W. NICHOLS, ALBERT LEVAN, REI KATO, and C. G. AHLSTROM (Lund, Sweden).

In a long-term study of the relationships of viruses chromosomes and carcinogenesis, two model systems, Rous sarcoma in the rat and measles, have been utilized. The Rous sarcoma system has been studied from the aspects of zero passage and serially transplanted tumors, both *in vivo* and *in vitro*, and by the addition of the virus to normal cells in tissue culture. Both *in vivo* and *in vitro*, the tumor has shown a progression of chromosomal changes with initially normal karyotypes, or karyotypes with changes in the number of chromosomes around the diploid level, followed by changes in chromosome type, and finally changes in ploidy. The addition of Rous sarcoma virus to normal rat cells in tissue culture produced an increased incidence of chromosome breakage and abnormalities over the control level.

In the measles system, clinical measles was found to be associated with chromosome breakage in the white blood cells. When patients were studied who received live attenuated measles vaccine, breakage was again found but to a lesser extent than in the disease, and there were indications that natural immunity or injection of gamma globulin at the time of immunization offered some protection against the breakage phenomenon.

Chromosome breakage fits well into a somatic mutation theory of carcinogenesis and it is interesting that chromosome breakage is a common denomination in the three classes of material that are known to be carcinogens; namely, irradiation, chemicals and viruses.

This work has been supported in part by grants-in-aid from The National Institute of Health (C4953 and C43845 and CA06415) and The American Cancer Society (EIIIB), The Swedish Cancer Society (62:80), The Swedish Medical and Natural Sciences Research Councils (U212 and T432) and by a Research Career Development Award (PCII-63) from The National Institutes of Health.

16.38. Chromosomal Aberrations Associated with Virus Infections in Man. ULLA GRIPENBERG (Helsinki, Finland).

Leucocyte cultures have been raised at the acute stage of several different infections (1-20 days after getting sick). About 100 karyotypes of each culture have been examined in order to determine the frequency and localization of chromosome breakages. Chromosome preparations have been made from 1-5 patients with each of the following infections: morbilli, exanthema subitum, epidemic parotitis, varicella, infectious mononucleosis, mesenteric lymphadenitis.

An increased incidence of various chromosomal aberrations was found in the different cases. (The final percentage of cells with aberrations, as well as the nature and localization of these will be reported later.)

16.39. Approach to the Cytogenetics of Mammalian Cells cultured in Vitro. L. DE CARLI, J. J. MAIO and F. NUZZO (Pavia, Italy).

Mammalian cells in tissue culture may yet offer the best approach toward initiating true cytogenetic studies which could correlate the data obtained from chromosomal analyses with variations in cellular phenotypes. The possibility would then be offered of locating cytologically the genetic control of some cellular characters recognizable *in vitro*. Although variants may be easily isolated from the heteroploid cell strains, this material is poorly adapted or even useless for many experiments of genetic analysis. However, these same strains could be even preferable to euploid cultures in some instances if one would study the correlation between chromosomal variations and the phenotypic changes in a given character. In fact, the chromosomal variability of heteroploid strains can be usefully exploited since it may give rise to a series of polysomic states for some chromosomes which may correspond to a gradient of expression in the cellular phenotype. Furthermore, particular chromosomal patterns may be stabilized for a sufficient number of cell generations by means of clonal procedures.

As an example of this approach to the cytogenetics of mammalian cells cultured *in vitro*, data are presented concerning the karyotypic analyses of several clonal lines derived from a

human heteroploid cell strain which exhibit marked variations in alkaline phosphatase activity. The karyotypes of the deficient lines showed a loss of a number of chromosomes. The detailed morphological analyses of chromosomes, made it possible to individuate the missing chromosomes, thus indicating the possible site of genetic control of the character.

16.40. Genetic and Biochemical Studies on Mutant Cell Lines in Culture. ROBERT S. KROOTH (Ann Arbor, U.S.A.).

Galactosemia in man is a rare recessive disease. Cell lines developed from the tissue of patients with galactosemia and from the tissues of normal controls have been studied after prolonged growth *in vitro*. When a suitably constituted medium is employed, the galactosemic cells appear to be unable to grow in galactose, whereas the normal cells can. The cultured galactosemic cells do not oxidize galactose-1-C^{14} to $C^{14}O_2$ in short term experiments while cultured cells from control patients perform the reaction readily. These two properties of galactosemic cells have thus far proven to be a stable phenotype of the line. Analogous experiments have also been performed on cell lines derived from patients with another recessve abnormality—acatalasia. Here too the characteristic biochemical abnormality appears to persist in the cultured cells. Preliminary genetic experiments on these lines will be described, and the results of a further search for biochemical markers in cell cultures will be summarized.

16.41. Leucocyte Enzyme Induction and Repression and Human Leukaemogenesis. CHEV. KIDSON (London, Great Britain).

Under certain conditions two catalases, *a* and *b*, may occur in human leucocytes of the myeloid type. Catalase *a* is always present in all leucocytes; catalase *b*, having a different pH optimum, occurs intermittently in normal myeloid cells, never in lymphoid or erythroid cells. In acute myeloid leukaemia catalase *b* is always present, but with altered reactive site, and occasionally altered electrophoretic mobility. Catalase *b* is not induced in normal myeloid cells during *in vitro* phagocytosis, nor by anacrobiosis. It appears that catalase *b* induction in normal human myeloid leucocytes is due to reversible derepression: in the normal cells repression of catalase *b* synthesis can readily occur, but in acute myeloid leukaemogenesis permanent derepression occurs, with subsequent mutation affecting the reactive site and occasionally mutation affecting other points in the enzyme protein molecule. These phenomena have important bearing on gene regulation in mammalian cells, and on aberrations of gene control in carcinogenesis.

16.42. Mitotic and Immune Response of Human Peripheral Lymphocytes in vitro. N. HASHEM (New York, U.S.A.).

During the investigation of the mechanism of the mitogenic action of phytohemagglutinin on human peripheral blood lymphocytes, it was noted that mitotic activity is associated with leucocyte agglutination, gammaglobulin production by the lymphocytes and morphological changes of the cells during culture. This immune-like response prompted investigation of the action of specific antigens on lymphocytes from individuals sensitized to the antigens. With the use of tuberculin purified protein derivative and pertussis vaccine it was demonstrated that mitotic activity resulted in cultures of peripheral lymphocytes from sensitized individuals. It was also noted that morphological alterations and gammaglobulin production was limited to only a group of the cells in culture. These experiments have demonstrated the potential ability of "clones" of peripheral lymphocytes to respond to specific antigens with gammaglobulin production and mitotic activity.

16.43. Variation of In Vitro Gamma Globulin Production by Human Lymphocytes. FRITZ BACH and KURT HIRSCHHORN (New York, U.S.A.).

Human lymphocytes, obtained from heparinized peripheral blood by sedimentation and subsequent removal of polymorphonuclear leukocytes, were grown in tissue culture. In the presence of phytohaemagglutinin and other substances, all lymphocytes have the potential to produce gamma globulin. This problem was studied by both radioactive and fluorescent antibody techniques. Further studies were done on the lymphocytes of patients with dysproteinemias including agammaglobulenemia, multiple myeloma, and macroglobulenemia. In cases of hereditary agammaglobulenemia both heterozygotes and homozygotes were examined. Data about the quantitative production of gam-

ma globulin in the whole population of cells as well as production of individual cells will be presented.

16.44. Peroxidase Isozymes in Human Leucocytes. BARBARA Z. SIEGEL and KURT HIRSCHHORN (New York, U.S.A.).

Isozymes, altered but active forms of a given enzyme, have been shown to exist in human tissue and that their presence and intensity may be both genetically and ontogenetically determined. A preliminary report on the technique of preparation and identification of the peroxidase isozymic series from human leucocytes will be presented. Differences between cells from normal and leukemic peripheral blood, bone marrow and tissue cultures will be demonstrated, as well as data on the qualitative and quantitative variations.

16.45. Influence of Steroid Hormones on Mammalian Cells and Culture. DAVID STONE (Shrewsbury, U.S.A.).

Studies on the influence of steroid hormones on Hela cell cultures have indicated that it is possible to select cell sub-lines which are inhibited in growth by particular steroids, but not by others. Chromosome studies of these cultures have indicated that the cell strain which is inhibited in growth by both desoxycorticosterone and testosterone has a chromosome stem line of 68, whereas the sub-lines resistant to desoxycorticosterone or testosterone, respectively, each have a stem line of 74 chromosomes. In order to study the relationship of steroid sensitivity and chromosomal characteristics further, a cell strain having more classic diploid characteristics has been studied. Using cell strains of the Chinese Hamster cultured for varying periods in the presence of certain steroids, it has been shown that the actions of the hormones change with the length of culture over a period of months. Cells which previously grew (at the normal rate of growth) in a particular steroid may become inhibited by the presence of that steroid and later may be stimulated in growth by the same steroid. Chromosome studies have indicated that, compared to control cultures run in parallel, the steroid supplemented cells exhibit altered numbers and morphologies of their chromosomes. Even after one to two days of steroid treatment small but significant changes can be detected. The effects of steroid hormones appear to be related to the age of the culture and the particular batch of serum used in the medium. These results will be discussed.

16.46. Inherited Variation of Somatic Cells to Polio Virus Infection. LAWRENCE N. CHESSIN (New York, U.S.A.).

Studies using primary human amnion cell cultures have indicated that virus resistant clonal variants can be selected from the parental population. These variants are morphologically, karyotypically, and immunologically indistinguishable from the sensitive population

Studies on these resistant and sensitive clonal types haves shown that cellular resistance and susceptibility to poliovirus infection was clonally distributed in a primary human amnion population. Data from virus absorption, penetration and replication experiments will be discussed from the standpoint that somatic cell variation to poliovirus infection may be due to inherent differences in the mechanism for the control of viral replication.

16.47. An X-ring Chromosome in a Turner Patient Without a Specific Peripheral Localization. TH. W. J. HUSTINX (Nijmegen, the Netherlands).

Chromosomal analyses in a Turner patient revealed a ring chromosome in both blood- and skin cultures. Barr-chromatine was detected in part of the diploid skin nuclei.

The ring chromosome is presumed to be a mutated X chromosome. The X-ring seemed to have preferentially a peripheral localization in the metaphaseplates; in 42.5 per cent the ring proved to be totally peripheral. The percentage of metaphase-chromosomes, which are also peripherally localized was 41.1 per cent of all karyograms had a peripheral localization in the metaphaseplates in 45.3 per cent.

The data for these different chromosome classes differed not significantly; the hypothesis of a specific peripheral localization of the X-ring is discarded.

SECTION 17

DERMATOGLYPHICS

17.1. Dermal Configurations: A Study of the Hallucal Area of the Sole in Mongoloids, Non-Mongoloid Mental Defectives and a Control Series. G. F. SMITH and G. M. TURRAL (London, Great Britain).

In the diagnosis of Mongoloids some of the most useful of all dermal configurations are the patterns in the hallucal area of the sole (Ford Walker, 1958). The usefulness of this type of information has been somewhat limited by the lack of information of the distribution of the variety of patterns in different populations. In order to compensate for this deficiency the present study was done on both English and American populations. The dermal ridge configurations and ridge counts in the hallucal area were statistically compared in a group of Mongoloid patients of both sexes. In addition a comparison of pattern frequencies and ridge count distributions were studied in Mongoloids, Non-Mongoloid Mental Defectives and Normal individuals.

17.2. Dermatoglyphics in the Diagnosis of Mongolism and in Family Relations. M. ČERNÝ, M. BARVIČ and B. SEKLA (Prague, Czechoslovakia).

An aid for the diagnosis of mongolism is beeing based on the comparison of dermatoglyphic patterns of mongolic patients and of normal controls. An analysis of extensive samples of Czechoslovak population and of patients with Down's syndrome has shown basic similarities with the Canadian material (N. Walker). At the same time the necessity came out for some corrections; the use of this aid in different demographic regions must be based on standards derived from the populations concerned.

An attempt has also been made of analysing dermatoglyphic patterns of mongolic patients as well as of those with other syndromes connected with karyological abnormalities, in connection of family relations.

17.3. Characteristics of Finger Prints, Palms and Soles in Phenylketonurics. WALTER HIRSCH (Chicago, U.S.A.).

Since the discovery of chromosomal aberrations, various abnormal patterns have been described in finger and palm prints. According to present knowledge, no specific pattern or combination of patterns should be expected. All known patterns are occurring in normal populations too: arch, loop whorl, their borderlines, combinations, accidentals, and defects on fingers, palms and soles, the transversal course of palm main lines, multiplication of achsial triradii, the simian crease and changes in the formation of secondary creases and white lines and dissociations in primary and secondary creases. But the distribution of these patterns is different in pathological conditions and very often this difference is highly significant.

For every one of the possible patterns the percentage frequency in patients and controls have been estimated and the logarithm of these figures has been noted (N. F. Walker). The sum of these logarithms gives a fairly reliable index of the deviation from normal averages.

The results of finger and hand printings in certain groups of mental retardation (Hirsch and Geipel) and in chromosomal aberrations suggested that similar deviations from the normal might also be present in "inborn errors of metabolism". It was the idea of D. Y. Hsia to examine the group of about 50 phenylketonurics under his observation. To these the author could add about the same number of phenylketonurics.

Such a detailed study is also likely to reveal correlations between the dermatoglyphics and creases and some biochemical and enzyme conditions. Preliminary studies of the author have shown such correlations to be present and to be significant. The results will be presented and discussed in detail and a number of slides will demonstrate the essential deviations from the normal patterns.

17.4. Dermatoglyphic Anomalies Associated with Abnormal Sex Chromosomes. SARAH B. HOLT (London, Great Britain).

Striking anomalies have been described in the

dermal ridge arrangements of persons trisomic for autosomes. Anomalies associated with abnormal sex chromosomes are not, on the whole, so marked. Nevertheless, dermatoglyphic peculiarities are discernable. Dermal prints of cases with various abnormalities of the sex chromosomes have been analysed. These include the palm and finger prints of a series of cytologically diagnosed Turners, collected by Dr. J. Lindsten.

In Turner's syndrome, where only one X-chromosome is present in a cell, three genetically controlled quantitative characters, total finger ridge-count, *a-b* count and maximal *atd* angle have been studied. The means of the three distributions differ significantly from those of a control sample. In each case the values are higher than in the normal males and females. Similar differences in means occur in a small samples of Turners with mosaicism (Xo/XX) and in other related conditions.

It is intended to publish a detailed account of the findings in *Annals of Human Genetics*, as part of a more comprehensive survey of the dermatoglyphics of sex chromosome abnormalities.

17.5. Aberrant Dermatoglyphic Patterns and Undifferentiated Mental Deficiency. DICK HOEFNAGEL (Hanover, U.S.A.), JAMSHED MAVALWALA (Cambridge, U.S.A.).

Patients of the State Schools of Brandon, Vermont, U.S.A., and Laconia, New Hampshire, U.S.A., numbering approximately 1500 were examined. Several patients have been found to display unusual dermatoglyphic patterns in that they possess an abnormally low ridge count, as a result of very vestigial patterns on the fingers. This finding was associated with chromosomal abnormalities, not all of which could be definitely established in a specific group or number. All of these patients upon detailed cytogenetic and clinical examination turned out to belong to the group classified as "Undifferentiated Mental Deficiency".

The authors believe that the dermatoglyphic trait of an extremely low total ridge count may be used as a screening device for ascertaining undifferentiated mental deficiency. This technique does not pick up other conditions.

Detailed studies will be reported upon all patients of undifferentiated mental deficiency. Patients with other conditions such as Turner's Syndrome, Klinefelter's Syndrome, Phenylketonuria, etc., are also being investigated.

17.6. Dermatoglyphic Studies at the Anthropology Research Centre, Musée de l'Homme, Paris. MONIQUE GESSAIN (Paris, France).

The Anthropology Research Centre now possesses several thousand finger prints collected

in France, in the Department of Finistère during the Pont-Croix surveys;
from numerous families;
from the Criminal Records Office;

in Senegal from the Tendankés in the Kédougou region (East Senegal).

The material collected during the Pont-Croix surveys relates to more than a thousand subjects from among the 3800 inhabitants of a highly endogamous community, which, for two years, has been the subject of a multi-survey in the demographic, genealogical, ethnological and anthropobiological fields.

The material collected from families includes several hundreds of subjects belonging to 2, 3 or 4 generations.

The material from the Criminal Records Office relates to 8000 offenders charged in Paris, classified according to their ethnic origin.

The material from Senegal covers the larger part of a small isolate of some 900 inhabitants.

The material is now being studied and some of the results will be presented to the Congress by Miss de Lestrange, Miss Pée-Laborde and Mr. Luu. Other studies are being conducted to evaluate genetical, statistical and anthropological evidence from these fingerprints.

17.7. Study of the Palmar Flexion Creases. MARIE-THÉRÈSE DE LESTRANGE (Paris, France).

Genealogical study carried out in French families, certain members of which have particular configurations of creases.

17.8. Multivariate Analysis in the Study of Dermatoglyphics and its Application to the Study of the Fingerprints of 7000 French Offenders (Symmetry, Correlations and Structure). LUU MAU THANH (Paris, France).

The study of finger prints according to form gives rise, generally speaking, to particular difficulties common to all qualitative characteristics.

Various methods provide estimations, for the most part unprecise, of correlations between the different fingers. From canonical analysis, quantitative values can be given to different

types of finger prints (according to form) which until now could only be accorded qualitative values. These qualitative values enable us to establish the matrix of correlations between fingers by the classical method.

The fingers have a particular structure which can be shown by giving that each finger x expresses itself by linear combinations of some orthogonal factors

$$x_i = \sum_{e=1}^{p} a_i e F_e$$

By appropriate rotations, the different groupings of fingers can be located in the space of the factors.

This work has been done from a sample of some 7000 French offenders whose finger prints are analysed according to four types of patterns: Arc, Radial Loop, Cubital Loop, Whorl.

17.9. Some Remarks on the Study of Finger Ridge Count. Lucienne Pee-Laborde and Luu Mau Thanh (Paris, France).

Owing to its quantitative value, the finger ridge count of patterns lends itself readily to statistical analysis.

The utilization of finger ridge count, a somewhat too simple index, can give rise to certain difficulties when an overall study is made relating to fingers, patterns and individuals as a whole. There are, in fact, several different populations following each type of pattern or each finger . . . These superpositions are discernible on the frequency distribution curves.

A sample of finger prints of some hundred French subjects was analysed by counting the ridges and distinguishing between the different forms of patterns, which has enabled us to make a critical study of the two methods.

17.10. R/L Symmetry and Asymmetry of Angles atd, at′d, at″d and at^{P}d and the Corresponding R minus L difference in the Burman Males (400)—A Probable Explanation of Serations. Abhimanyu Sharma (New Delhi, India).

The study reveals "assumed" symmetry (0^0 or $\pm 2^0$ difference between *R and L*) that shows the gradation as t^{P} (230/400=57.50 per cent)— t(116/229=50.66 per cent)—t′(89/240= 37.08 per cent)—t″(12/71= 16.90 per cent) whereas total asymmetry (ranging from —31° to —3° and +3° to +20°) shows it as t^{P} (159/400= 39.75 per cent)—t″(19/71 =26.76 per cent)—t′ (56/240= 23.33 per cent)—t(31/229= 13.54 per cent). These figures are understandable for *t*, *t′*, *t″* but t^{P} ranking number one appears problematic only initially. Its clue lies in the incidence of "indeterminate" cases (N or ?) that show the gradation as t″ (40/71= 56.34 per cent)—t′(95/240= 39.58 per cent)—t(82/229= 35.81 per cent)—t^{P}(11/400= 2.75 per cent) which is indicative of fairly high incidence at t, t′, t″ (from 35.81 per cent to 56.34 per cent) relative to only 2.75 per cent at t^{P}! High incidence of symmetry at t^{P} may be explained by taking example of a person whose right shows only one axial triradius t but located very close to t′-segment (Sharma, 1961a: E 111; 1962a: in the press) while his left shows only one as t′ but located fairly close to t-segment implying thereby that both are near borderline cases between *t and t′-segments*. It is *indeterminate* for two categories, angles atd as well as at′d, but not if the distalmost axial triradius t^{P} (Penrose, 1949, vide Penrose 1954: 10-11) is considered ignoring threefold distinction between *t′ and t″*. Differences of 0 to ± 2 (up to 2 in excess either in rights or lefts) will add to the incidence of "assumed" symmetry while 3 to that of asymmetry. *Angle atd* shows the least extent of morphologically-evidenced fluctuation relative to that shown by *Angles at′d and at″d* that can be explained in geometrical terms.

17.11. The Palmar Dermatoglyphics of a Group of People of Lazio (Italy), and Some Remarks on Methodology. G. Alciati (Rome, Italy).

The results of the examination of the palmar dermatoglyphics of 320 individuals, all born at Pofi, district of Frosinone (South Lazio), are here exposed. The study was performed in compliance with the "revised methods" (Cummins *et al.*, 1929) and is demonstrative of the fact that the palmar epidermal ridges of the above-mentioned group show a behaviour similar to that of other European groups which are already known in literature, both for their mainly transverse direction and for their percentage frequency of patterns in the five palmar areas.

The palmar prints were moreover examined following application of the new method which was introduced by the author during the IInd International Conference of Human Genetics (Rome, 1961); the results thus obtained were compared with those derived from the above mentioned "revised methods"; a close relationship was noticed, wherefrom the author draws the cue for some practical considerations.

17.12. Eurasians Digital and Palmar Dermatoglyphs. GEORGE OLIVIER (Paris, France).

The fingerprints of 160 French-Vietnamese crosses (100 male and 60 female) are studied. They are compared with two new series of 500 Frenchmen and 250 Vietnamese. The characteristics of the female group have been estimated approximately.

In general, the frequencies of the different patterns fall between those of the Vietnamese and those of the French. In males, they get nearer to the Vietnamese frequencies; in females they get nearer to the French frequencies; transmission thus seems to vary somewhat according to sex. The dominance of digital loops (European type) found by Abel, Cummins, Kimura and Mikami, have not been found. The Vietnamese realize at a maximum the dermatoglyphic patterns typical of the xanthodermic populations.

The transverse palmar fold (simian fold) is three times more frequent in Vietnamese than in French populations. Its frequency in Eurasians is intermediate.

The only positive results are:

1. The radial loops of the hypothenarian eminence seem to be recessive (this contradicts Kimura).
2. The thenarian patterns seem to be recessive.

17.13. On the Optimal Extraction of Genetical Information from Fingerprints. FRIEDRICH KEITER (Hamburg, Germany).

Fingerprints (and toeprints) are highly patterned, tenfold, in a complicated manner intercorrelated morphological traits. The tenfinger-ridgecount is a very incomplete indicator of genetical information contained in them. Direct finger-to-finger-comparison on ridgecounts and on degree of similarity in controlled estimation yields 20 (with toeprints 40) single data, whose score can be combined into a simple discriminant system (method of "Trennlogarithmus", separating logarithms). In a sample of Mato grosso-indians (Chavantes) by this procedure the "critical values" (ratio of the frequency of the same finding in related and random pairs) have been found 8.40:1 for pattern similarity, 2.85:1 for finger-to-finger-ridgecount against 1.22 only for ten finger-ridgecounts alone. The same multiplied discrimination effect of parents-children or sibs compared with randomly chosen pairs of nonrelated individuals is well established for Northern Germany since 1955. Agreement with or deviation from multifactorial-additive inheritance can be checked using the same principles.

17.14. Genetics of the a-b Ridge Count on the Human Palm Prints. JOSÉ PONS (Oviedo, Spain).

The present study deals with the distribution of the number of ridges between the triradii *a* and *b* of the human palms. As an individual trait the sum of the *a-b* counts of the two hands is used and, as a measure of bimanual asymmetry we analyze the differences between hands (right minus left). After some brief remarks on racial differences, we consider the heritability of this trait by calculating the intraclass correlation among sibs, as well as the parent-child correlations.

For the study in the general population a series of 412 Spaniards (200 males and 212 females) is used, comprising unrelated persons only. For analysing the heritability we have a series of 307 sibs falling into 123 sibships and of 57 families composed of both parents and at least one child.

17.15. Inheritance of Dermatoglyphic Formulae.—I. Ulnar Loops in Complete or Partial Symmetrical Formulae. C. LAZÁRO (Montevideo, Urugay).

In a previous work we have found that the observed frequency of the ten most common dermatoglyphic formulae for the population of Montevideo departs significantly from the calculated one. Based on this conclusion, we present in this paper the results of the study of ulnar loops in complete or partial symmetrical formulae in all possible combinations in 200 families taken at random in our population.

The results of our data do not show apparently any simple mendelian mechanism of inheritance; they rather suggest the action of a polygenic system, governing the genetics of dermatoglyphic formulae.

17.16. The Hereditary Pattern of the Quantitative Value of Fingerprints from a Critical Aspect. MARGARETE WENINGER (Vienna, Austria).

Among the characteristics of the digital patterns the importance of the quantitative value (QV) has long ago been stressed. In spite of the great difficulties resulting from the sexual dif-

ference of the QV, from its bimanual asymmetry and, above all, from the differences between the finger categories, researches have been made to detect the genetic background of the QV.

K. Bonnevie (1924) developed a hypothesis of 5 pairs of polymeric genes in order to explain the hereditary pattern of the so-called individual QV. This hypothesis was rejected by herself a few years later in favour of another one.

The modern English biometrical school approaches Bonnevie's first hypothesis in that it explains the individual QV, i.e. the value of the 10 fingers combined as the effect of additive genes.

The respective paper is designed to the problem from a critical point of view.

The paper will be probably published in full in the *Mitt.d.Anthropol. Ges. Wien* or in the *Zeitschr. f. Morphol. u. Anthrop.*

SECTION 18

CITATION INDEXING

18.1. Dissemination and Retrieval of Genetics Information Through Interdisciplinary Citation Indexing. EUGENE GARFIELD and IRVING H. SHER (Philadelphia, U.S.A.).

The difficulties in handling the highly interdisciplinary genetics literature by conventional methods led to a study of citation indexing, a method used in legal literature. Citation indexing overcomes the terminological barriers of language-oriented systems and does not require scarce scientifically trained indexers. It uniquely discloses the bibliographic *descendants* of earlier papers rather than their *antecedants*—as in conventional systems. A citation index identifies the *subsequent* papers citing earlier target papers.

For three years methodologies for compiling citation indexes by computer have been studied. Several experimental indexes were compiled and compared to other systems. Over 2,000,000 citations from the published literature have been processed! Invaluable bibliographical, sociological, and historical statistical data have been obtained as by-products and various author, journal, and chronological utilization factors determined.

Citation indexing is based upon *a posteriori* indexing by citing authors, making the literature a dynamic tool for creative research. New or previously unknown correlations and insights are obtained since the citation index integrates the "old" literature with the new, often in dramatic ways. The enormous proliferation of the literature, anathema and disabling in conventional *a priori* systems is highly beneficial in citation indexing. Its self-organizing properties automatically increase specificity as the literature grows. As citation input is increased one approaches critical mass—the coverage necessary to insure a known degree of reliability for a negative search result, an important indicator that new research is needed or justified.

18.2. (D) The Genetics Citation Index. IRVING H. SHER and EUGENE GARFIELD (Philadelphia, U.S.A.).

A citation index is a directory of cited literature references, each of which is accompanied by a list of citing source documents. Typically, the user specifies a target paper in which he is interested. In the citation index he locates the author and specific article first. From there, the index displays the more recent papers that have cited the target paper, thus bringing him forward in time through subsequent interdisciplinary developments. We have prepared several types of genetics citation indexes for comparative evaluation including:

1. Comprehensive interdisciplinary citation indexes compiled from 2,000,000 references.
2. A Genetics Citation Index selectively extracted from a file of 1.4 million references appearing in 550 life science journals published in 1961.
3. Genetics Citation Indexes derived from long runs of "hard core" genetics journals.

Microfilms of the indexes will be displayed and a sample search of the files will illustrate the unique speed and power of this new tool for information retrieval.

In contrast to conventional systems, valuable information is obtained from a Citation Index, even when *no* citations are disclosed, since this implies that the target paper has not been extended. A demonstration of "cycling" will show how a search can be expanded by selecting new targets from the bibliographies of papers which cite the target paper, continuing the process until an optimum list of papers has been retrieved. After the pedagogical demonstration, spontaneous literature searches will be conducted for each visiting geneticist and copies of the search bibliography provided.

INDEX OF AUTHORS