A GENETIC INVESTIGATION OF THE
AD9 CISTRON OF ASPERGILLUS NIDULANS

A Thesis submitted to the University of
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by

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ABSTRACT

The purpose of this work was to construct a fine genetic map of the ad9 cistron in *Aspergillus nidulans* and to discover if any correlation exists between the genetic and functional relationships of some of the ad9 alleles. Preliminary results suggested that certain of the ad mutant strains investigated carried inversions spanning part of the ad9 cistron, and further experiments were therefore carried out to elucidate the nature of these inversions.

It had previously been shown by Calef (1956) that, among the four alleles ad9, ad13, ad15 and ad17, only ad15 and ad17 complement one another. In the present investigation ad32 and ad33 were identified as alleles of ad9. All possible pairs of the six mutants were tested for complementarity, and one new complementing pair was discovered, namely ad32 and ad17. Analysis by means of mitotic and meiotic recombination gave the sequence of mutant sites as ad33 - ad13 - ad9 - ad32 - ad17 - ad15 (in non-inverted strains). There was no apparent relationship between the genetic and complementation maps. Complementation was inhibited *in vivo* in the presence of mercuric ions, suggesting that S-S linkages may be involved

in the complementation mechanism, possibly in the formation of a hybrid polymer of polypeptide chains, as proposed by Crick and Orgel (unpublished).

It was established by analysis of mitotic and meiotic recombination that the ad13 strains used most probably contained an inversion spanning the sites of ad13, ad9 and ad32. Recombination studies suggested that the ad17 strains contained an inversion identical with the ad13 inversion and that ad15 strains carried an inversion for part of the ad9 cistron which apparently differed from the ad13 inversion. The ad13 inversion (and the ad17 inversion) does not apparently give rise to a detectable mutant phenotype. Because of the location of the mutant sites analysed in the present studies, it was not possible to establish whether the suspected ad15 inversion also determines a non-mutant phenotype. The significance of the "wild-type" inversion in relation to gene function is discussed.

Localised negative interference was found, in agreement with results of previous workers. Inversions increased negative interference in mitotic and possibly also in meiotic recombination. It was concluded that the effects on recombination of intra-cistronic inversions are compatible with the "effective pairing" hypothesis of Pritchard (1955).

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GENERAL INTRODUCTION

The results obtained in genetic and allied investigations over the past twenty years (especially during the last decade) have made necessary a reappraisal of genetic theory with respect to the nature and structure of the genetic material, gene function and the dynamic aspects of gene structure (e.g. recombination and replication).

(1) Complex structure of the gene.

In 1940 Raffel and Muller suggested in connection with their breakage analysis of the 'scute' gene in *Drosophila melanogaster* that the apparent size of a gene may vary, depending upon the criterion used to define it. This prediction has been amply verified by genetic fine-structure studies (see reviews by Pontecorvo, 1958a; Demerec and Hartman, 1959; Carlson, 1959b).

(2) Chemical nature of the gene.

The discovery of capsular-type transformation in *Pneumococcus* by isolated deoxyribonucleic acid (DNA) (Avery *et al.*, 1944) first prompted the now widely held view that DNA is the genetic material in all DNA-containing organisms. This contention was supported by the finding that DNA is most probably the genetic material of bacterial viruses (Hershey and Chase, 1952) and that the amount of DNA in the various differentiated cells of an organism is constant for cells of the same ploidy (see review by Taylor, 1957). Ribonucleic acid (RNA) is thought to play a similar role in the plant and RNA-containing animal viruses (e.g. Gierer, 1960).

(3) Genetic coding.

X-ray diffraction analysis of DNA (Watson and Crick, 1953a, b & c) led Watson and Crick (1953c) to suggest that the genetic information resides in the sequence of purine and pyrimidine bases of DNA. Subsequently, many workers have attempted to discover the nature of the genetic code (see review by Levinthal, 1959b). Several authors have put forward hypotheses to bridge the gap between the Watson-Crick model for DNA and the more complex structure (cf. Ris, 1957) at the chromosome level (e.g. Taylor, 1958a; Freese, 1958 ; Schwartz, 1955 and 1958).

The view that the function of at least some genes is the specification of the amino-acid sequence of proteins has been encouraged by investigations of gene-enzyme relationships in micro-organisms and by protein studies of human haemoglobin abnormalities (see reviews by Fincham, 1959a; Catcheside, 1960a; Beadle, 1960; Yanofsky and St. Lawrence, 1960).

(4) Complementation.

A new aspect of gene function has come to light with the discovery by Calef (1956) of complementation between alleles (see also Catcheside and Overton, 1958; Case and Giles, 1958b, 1960; Catcheside, 1960a & b; Ahmad and Catcheside, 1960). The term "complementation" is used when two mutants, a and b -- both allelic to a third mutant -- nevertheless give rise to a wild-type (or near wild-type) phenotype in an (a +) + (+ b) heterokaryon or in an a + / + b heterozygote. The underlying mechanism of this phenomenon, which is at present unknown, is of basic importance to any theory of gene action.

(5) Recombination and replication.

Studies of the dynamic aspects of gene structure in micro-organisms have revealed two characteristics of the recombination process which were not apparent previous to the development of selective techniques for genetic fine-structure analysis and to the widespread use of tetrad analysis. The first was intense negative interference associated with recombination between very closely linked markers (e.g. Pritchard, 1955, 1960a & b; Chase and Doermann, 1958). That is, when selective techniques were used to map a series of allelic mutants, the progeny of a cross that were recombinant for the selected interval were also recombinant for adjacent intervals more often than expected (on the basis of the linkage relations determined for an unselected sample of the progeny from the same cross).

The second was the discovery of "gene conversion" (e.g. Lindegren, 1955; Mitchell, 1955a & b, 1956; Case and Giles, 1958a & b; Roman, 1956 and 1958; Strickland, 1958b) -- i.e. the occurrence of tetrads with 3:1 or 4:0 ratios with respect to one or more loci. The non-reciprocal nature of "gene conversion" has led some investigators to postulate two different recombination mechanisms, one which would give rise to reciprocal recombinants and a second which would not (Lindegren, 1955; Beadle, 1957; Bonner, 1956; Mitchell, 1957; St. Lawrence, 1956; St. Lawrence and Bonner, 1957;

Glass, 1957; Freese, 1957a; Roman, 1956).

A copy-choice mechanism of recombination at the time of replication was proposed by Lederberg (1955, see also Levinthal, 1954) to account for the apparently non-reciprocal nature of recombination in bacteriophage and also to provide a model applicable to bacterial transformation and transduction. A copying-choice at the time of replication is finding increasing favour over the breakage-and-reunion hypothesis as a general model of recombination (see reviews by Pontecorvo, 1958a; Pritchard, 1960b). Pontecorvo (1958a) has pointed out that the divergences between recombination in bacteriophage and the conventional reciprocal recombination found in other organisms could be explained by assuming that replication is not always synchronous. Other authors (e.g. Roman, 1956; Freese, 1957a; Chase and Doermann, 1958) have suggested non-synchronous replication as the basis of "gene conversion." Pritchard (1960a & b) favours a single copy-choice mechanism for both reciprocal and non-reciprocal recombination. He suggests that recombination is generally a reciprocal process which becomes non-reciprocal when the switching between templates does not occur at exactly the same place for both replicating strands. However, any mechanism of recombination based exclusively on switch of copy-choice fails to explain the occurrence of three-strand multiple recombinants as detected by means of tetrad analysis and by other suitable systems (see, for example, Pontecorvo, 1958a; Pritchard, 1960b).

Purpose of research reported in this thesis.

The purpose of the work described in this thesis was threefold: -

(a) to construct a fine genetic map of the *adenine-9* cistron* in *Aspergillus nidulans*,

(b) to elucidate the nature of the intra-cistronic inversions carried by some of the adenine-mutant strains studied and

(c) to discover if any correlation exists between the genetic and functional (i.e. complementation) relationships of some of the *adenine-9* alleles.

The first chapter of this thesis comprises a description of the methods and materials used. It also

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* Hereafter the more precise term "cistron" (Benzer, 1957, see also introduction of chapter II) will be used in place of "gene" wherever practicable.

gives the results of some preliminary experiments that were required before the main problems could be investigated. In the second chapter experiments are described which investigate the functional relationships of the adenine-9 mutants, and various hypotheses relating to the mechanism of complementation are discussed in the light of these experiments and those carried out by other workers. The final chapter is concerned with experimental evidence relating to the linear arrangement of the adenine-9 mutant sites and to the nature and effects of the inversions. The negative interference found in selection experiments and the effects on recombination of intra-cistronic inversions are discussed in relation to current genetic theory.

CHAPTER I.

MATERIAL, METHODS AND PRELIMINARY EXPERIMENTS

A. Life Cycle of Aspergillus nidulans

As the life history, genetics and cytology of Aspergillus nidulans (Eidam) Winter have been described in great detail by Thom and Raper (1945), Pontecorvo *et al*. (1953) and Elliott (1960a) it will suffice to give a brief resumé of the principal characteristics of this homothallic ascomycete.

The vegetative mycelium, which forms a compact colony on solid media, consists of branching, septate hyphae, the "cells" of which contain many nuclei. Hyphae anastomose readily, producing heterokaryons when fusion takes place between hyphae containing genetically different nuclei.

The conidia (asexual spores) are uninucleate and are produced in chains on differentiated hyphal cells (conidiophores). In heterokaryons, different chains on the same conidiophore may be genetically different, but the conidia borne by a single sterigma usually have identical nuclei. The diameter of a haploid conidium is *ca*. 2.9μ.

Diploid conidia are also produced during the asexual phase of the life cycle of A. nidulans. Heterozygous diploid conidia, which can be recognised in a heterokaryon (Roper, 1952), are found among the conidia of a heterokaryon at a frequency of 10^{-6} to 10^{-7} (Pontecorvo, 1958a). Homozygous diploid conidia probably occur in both heterokaryons and homokaryons, but they are not easily detected. Diploid conidia are roughly twice the volume of haploid conidia and have a diameter of ca. 3.8µ (Pontecorvo, Tarr Gloor and Forbes, 1954).

Colonies established from heterozygous diploid conidia (or ascospores, vide infra) do not remain homogeneous as they undergo recombination and haploidisation (Pontecorvo, Tarr Gloor and Forbes, 1954).

The sexual cycle occurs in perithecia (or, more accurately, cleistothecia) in which large numbers (ca. 10^5) of eight-spored asci are formed. The mature dark-brown perithecia must be crushed to make them release their ascospores. The ripe asci have very fragile walls, and the ascospores within them are unordered. Two mitotic divisions following meiosis give rise to eight haploid binucleate ascospores because the second mitotic division takes place after the spores are delimited. Diploid (non-reduced) ascospores are also produced occasionally; Pritchard (1953, 1955, 1956) found that heterozygous diploid ascospores occur at a frequency of ca. one per cent.

Perithecia produced by heterokaryons are usually either selfed (i.e. contain selfed asci of one parent) or hybrids. "Twin" perithecia (i.e. having a mixture of selfed asci of both parents or a mixture of selfed asci of one parent and hybrid asci) are apparently rare occurrences (Pontecorvo et al., 1953; Strickland, 1957).

Recent cytological work by Elliott (1960a) indicates that in haploids the diplophase is limited to the fusion nucleus in the young ascus. Diploids, although producing many perithecia, are largely sterile. The few asci which are formed contain haploid ascospores (a single diploid nucleus in each ascus having undergone meiosis, followed by a mitotic division, to produce eight spores). The haploid chromosome number is eight (Elliott, 1960a), which is in agreement with the eight linkage groups found by means of mitotic haploidisation (Käfer, 1958).

B. Methods of Culture.

The procedures described by Pontecorvo *et al*. (1953) for the maintenance and purification of strains, for plating, incubation, etc. were followed unless it is otherwise specified.

1. *Media*: - Details of the media used have been given by Pontecorvo *et al*. (1953). The minimal medium (MM) consists of mineral salts and glucose. The complete medium (CM) is composed of MM plus yeast extract, casein hydroylsate, hydrolysed nucleic acids, peptone and vitamin solution. Media are solidified with 1.5% agar when required. Solid media were employed in this work except where otherwise indicated.

Nutritional mutants were grown on either CM or MM supplemented with the necessary growth factors. When adenine-requiring mutants were grown on CM, the medium was further supplemented with 0.5 ml. adenine-HCl (0.05 M) per 100 ml. CM; this medium will be designated CM + ad. MM for crosses between non-complementing (see Chapter II) adenine-requiring mutants was supplemented with 4-5 ml. adenine-HCl (0.05 M) per 100 ml. medium.

The concentrations of acriflavine recommended by Roper and Käfer (1957) were generally used, although it was necessary to determine the amount of acriflavine required with each new batch of CM, since sensitivity to acriflavine varies with the nucleic acid concentration of the medium (McIlwain, 1941; Roper and Käfer, 1957).

2. Preparation of ascospore suspensions: - In addition to the two methods for preparing ascospore suspensions described by Pontecorvo et al. (1953), viz., single perithecial and pooled perithecial suspensions, a third procedure was followed when large numbers of ascospores for selective platings (see section D: methods of genetic analysis) were desired from relatively sterile crosses.

In this procedure thousands of perithecia, with adhering conidia and debris, are harvested from one or more plates of the same cross and transferred to a screw-top bottle containing saline. The bottle is shaken vigorously to separate the perithecia and then centrifuged at low speed. After removal of the supernatant, the contents (including material adhering to the walls of the bottle) are crushed with a glass rod. The resulting ascospore suspension is washed and centrifuged several times to remove as much remaining debris and conidia as possible. Further debris can be removed by wiping the walls of the bottle with a sterile cotton swab. After this cleaning routine is completed the ascospore suspension is ready for counting and plating.

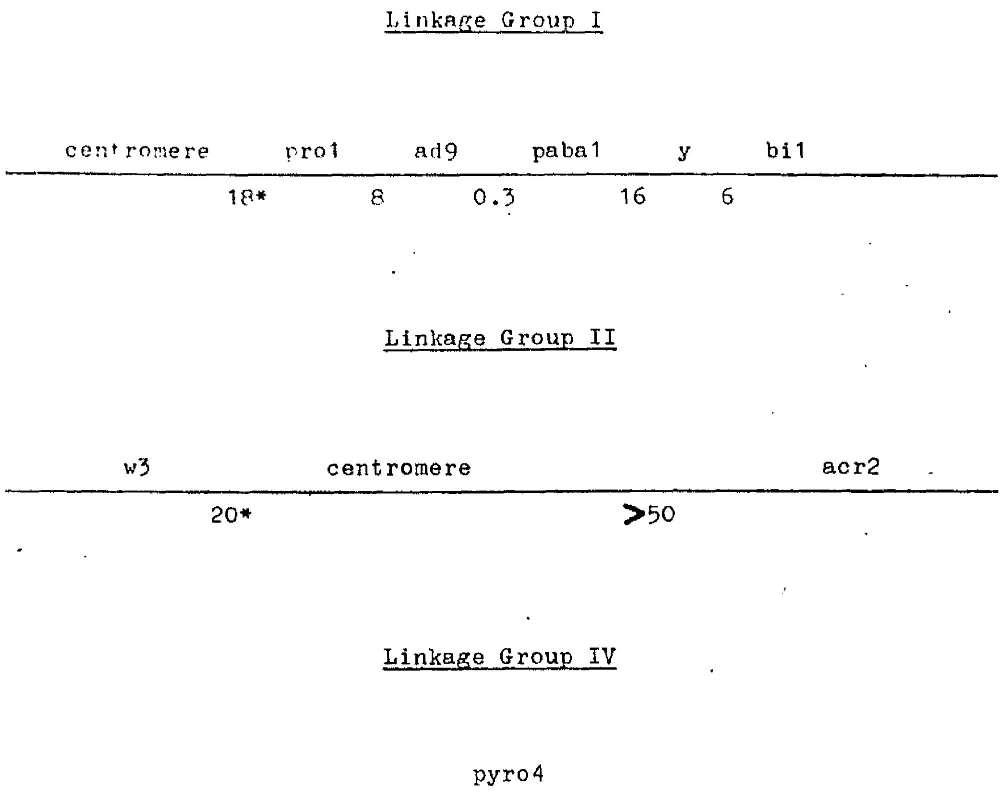
3. Heterokaryon formation and isolation of diploids: - For 'balanced' heterokaryon formation the second technique given by Pontecorvo *et al.* (1953), viz. preincubation of the mixed inoculum in liquid CM, was used exclusively. Diploids were isolated from heterokaryons by plating conidia at high density on media which would not support growth of the component haploids (Roper, 1952; Pontecorvo *et al.*, 1953).

4. Methods of crossing: - The following standard procedure was used. Conidia from the strains to be crossed were streaked together across the

diameter of a Petri dish of MM, and 3-4 drops of liquid CM were spread along the streak to ensure germination.

When crosses were made between allelic (see Chapter II) adenine-requiring mutants, the MM was supplemented with adenine. These crosses were incubated for four to six weeks, and in the case of crosses between non-complementing alleles the plates were sealed with cellulose tape (Pritchard, 1955) in an endeavour to lessen the sterility of the crosses and to reduce conidial formation. (Crosses made solely for the purpose of obtaining adenine-requiring strains with new combinations of markers were incubated for two to three weeks.)

5. Testing of isolates from crosses and from diploids: - When large numbers of isolates were to be tested for their nutritional requirements, the wire replicator of Forbes (unpublished) was used. Conidia from the isolates were first inoculated at marked positions on CM or on fully supplemented MM 'master plates'. A set of test plates, in which each plate was deficient in a different growth factor, was used



(The map units are per cent meiotic recombination)
* per cent recombination calculated from tetrad data (Strickland, 1957).

Linkage Map of Markers Used
(after Käfer, 1958)

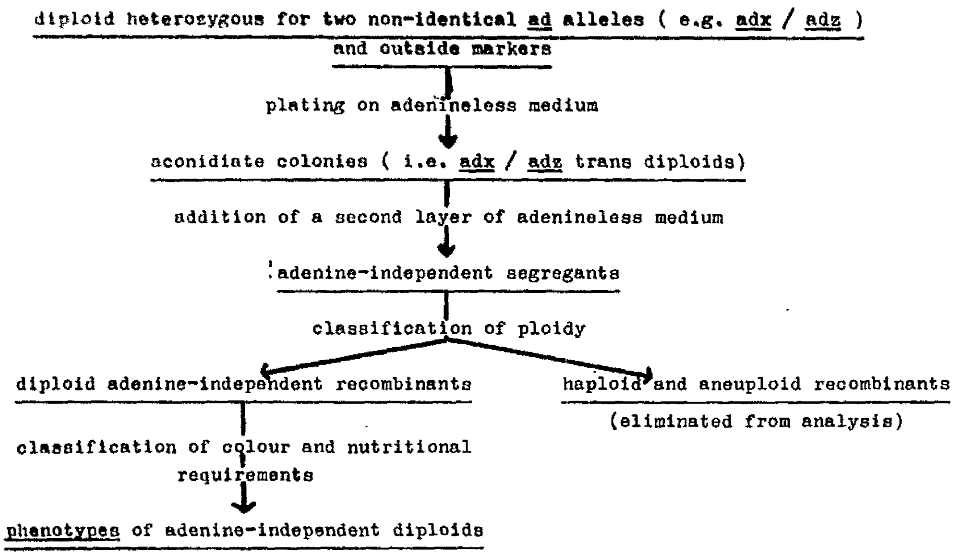
FIG. 1

with each master plate. The test plates were inoculated with conidia from the master plate by means of the wire replicator. Test plates were classified after 24 hours; those few colonies that were difficult to assess were again scored after about 48 hours or re-tested, if necessary.

C. Strains.

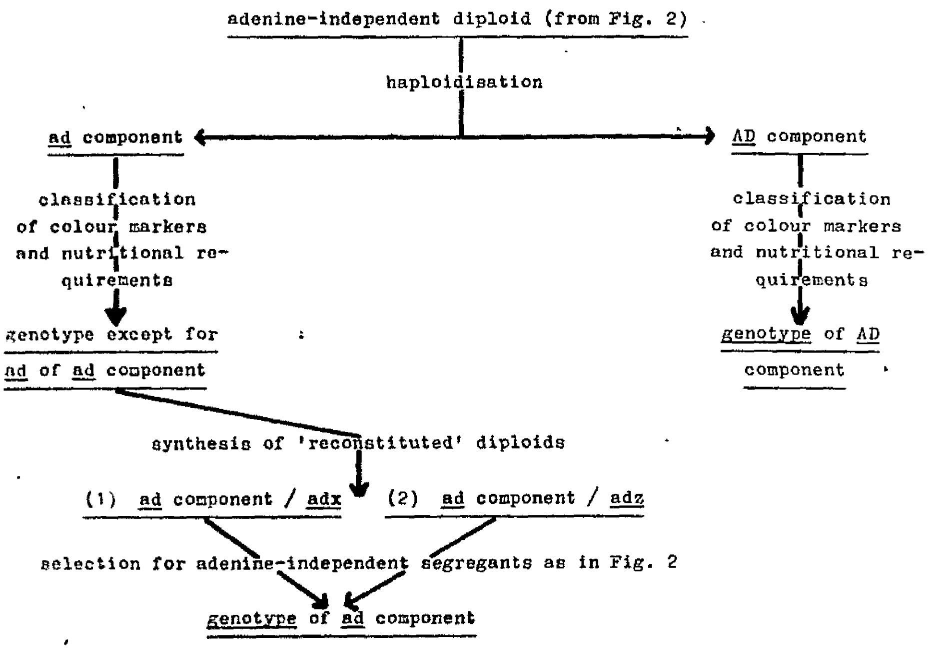
1. Notation: - The notation used is that of Pontecorvo *et al.* (1953), revised by Käfer (1958). An explanation of the mutant symbols employed in this thesis is given in Table 1. (Table 1 and succeeding tables have been placed in Appendix A.)

2. Origin of strains: - All the strains used in this work were obtained by mutation and recombination from a single wild type strain, A. 69 (NRRL 194) (Yuill, 1939, 1950; Pontecorvo *et al.*, 1953; Thom and Raper, 1945). All mutants were isolated by other workers in the Department of Genetics, University of Glasgow; details and references are given by Käfer (1958) and Pontecorvo *et al.* (1953) for all the mutants used except for the *ad* mutants marked with an asterisk (*) in Table 2. The linkage map for the markers



Analysis by Means of Mitotic Recombination: -
Diagram Showing Procedures Followed to Obtain Adenine-independent Recombinants and to Determine the Phenotypes of Those Recombinants

FIG. 2



Analysis by Means of Mitotic Recombination:
Diagram Showing Procedures Followed to Determine the Genotypes of Adenine-independent Recombinants (see Fig. 2)

FIG. 3

relevant to this investigation is given in Fig. 1 and the origin of the ad mutants used, in Table 2.

D. Methods of Genetic Analysis.

1. Analysis by means of mitotic recombination: -

Three steps are involved in the mitotic recombination studies described in this thesis. The first is the production of a diploid heterozygous for two ad alleles. The second is the isolation of adenine-independent diploid colonies from such a diploid. The third is the elucidation of the genotype and/or phenotype of these colonies. These steps are summarised in diagrammatic form in Figs. 2 and 3.

(a) Production of diploids and isolation of adenine-independent segregants: - The procedures used for the production of diploids and selection of segregants were those of Pritchard (1955), modified in some details, as follows:

Diploids with the generalised genotype given in Fig. 4, where adx and adz are two non-complementing alleles of independent mutational origin, were synthesised. Conidia from these diploids were plated (about 10 per dish) on MM supplemented with all the

$$\frac{\text{pro1} \quad \text{adx} \quad \text{ADZ} \quad \text{PABA} \quad \text{Y} \quad \text{bi1}}{\text{PRO} \quad \text{ADX} \quad \text{adz} \quad \text{paba1} \quad \text{y} \quad \text{BI}} \qquad \frac{\text{ACR} \quad \text{W}}{\text{*acr2} \quad \text{w3}}$$

or

$$\frac{\text{pro1} \quad \text{ADZ} \quad \text{adx} \quad \text{PABA} \quad \text{Y} \quad \text{bi1}}{\text{PRO} \quad \text{adz} \quad \text{ADX} \quad \text{paba1} \quad \text{y} \quad \text{BI}} \qquad \frac{\text{ACR} \quad \text{W}}{\text{*acr2} \quad \text{w3}}$$

* In some mitotic recombinant selection experiments the trans diploids were homozygous ACR / ACR .

Generalised Genotype of Trans Diploids Synthesised for Mitotic Recombination and Haploidisation Experiments

FIG. 4

growth factors required by the haploid components except adenine. Adenine was added in limiting amount (0.0005 mg./ml. MM, i.e. 1/200th the normal concentration used for full growth).

After two days' incubation the conidia produced spidery aconidiate colonies. These were marked on the reverse of the dish, and a layer of the same medium was poured over the colonies. The adenine-requiring colonies continued to grow slowly between the layers. Under these conditions, hyphae containing adenine-independent nuclei had a great selective advantage over the rest of the colony and were able to reach the surface of the medium. These hyphae produced adenine-independent sectors which were easily recognised, because after reaching the surface of the medium they continued to grow over it and sporulated profusely.

Only one adenine-independent segregant was isolated from each marked colony in order to avoid picking from the same clone twice. In every instance the first segregant appearing was isolated in order to reduce subjective bias in favour of a particular type of colour segregant. In most experiments about 10 to 20 per cent. of the requiring colonies produced segregants after up to ten days' incubation (cf. Pritchard, 1955, who found ca. 10 per cent of the ad8 / ad16 diploids produced adenine-independent segregants).

(b) Phenotypic classification of adenine-independent segregants: - The isolates were placed on master plates of MM fully supplemented except for adenine, tested for growth requirements and scored for colour and ploidy. Measurement of conidia (Roper, 1952; Pontecorvo et al., 1953; Pontecorvo, Tarr Gloor and Forbes, 1954) is too laborious a method of determining ploidy when large numbers of isolates must be examined. Advantage was therefore taken of the fact that diploids can be distinguished visually from haploids on the basis of the morphology and intensity of colour of the conidial heads. The adenine-independent colonies were also searched for secondary colour segregants, white and yellow in the case of green colonies, white only in the case of yellow colonies. The accuracy of this method was checked by measuring the conidial size of several hundred adenine-independent segregants, and it was found that the determination by the visual method was always in agreement with that reached by conidial measurement.

(c) Haploidisation of mitotic recombinants and determination of genotype: -

Haploidisation. Selective methods for obtaining the haploid components of mitotic recombinant diploids have been described by Pontecorvo and Käfer (1956, 1958). Two types of selection were used here: (i) colour and (ii) a combination of drug resistance and colour. The mitotic recombinant diploids were those obtained by the technique already described.

In haploid selection (i) the colour segregants were either secondary diploid recombinants or haploids; the haploids were distinguished by measurement of conidial diameters.

In haploid selection (ii) a combined selection of two markers on different arms of linkage group II (Fig. 1) was used, viz. *acr2* and *w3*. The method of Roper and Käfer (1957) was modified slightly: - The adenine-independent recombinant diploids, still presumably heterozygous *acr2 w3* / *ACR W* (see Fig. 1 and 4), were plated (about 20-30 per dish) on CM + ad, incubated about 20 hr., and a top layer of CM + ad, also containing acriflavine, was then added. Acriflavine-resistant sectors emerged from many of the colonies after several days' further incubation. In some experiments these were so poorly sporulating that the sectors had to be isolated before classification of colour could be made.

The resistant sectors were either acr2 / acr2 (i.e. diploid) or acr2 (i.e. haploid). Since the chance of mitotic crossing over in each of two arms of one linkage group is very low (Pontecorvo and Käfer, 1958), the selection of white sectors picks out the haploids and 'non-disjunctional' diploids (i.e. diploids in which migration of both acr2 w3 chromatids to one pole during mitosis had occurred). Determination of ploidy eliminated the 'non-disjunctional' diploids from the analysis.

In certain experiments the low frequency of resistant white segregants as compared to resistant green segregants made selection (ii) difficult. However, in these instances an appreciable increase was noted in the incidence of sectoring for colour in the diploid segregants over what is normally encountered when there is selection for drug resistance. (Cf. for induction of high frequency of somatic segregation and haploidisation: Käfer (1960) and Fratello *et al*. (1960) in *Aspergillus nidulans*; Morpurgo and Sermonti (1959) and Sermonti and Morpurgo (1959) in *Penicillium chrysogenum*; Ikeda *et al*., (1957) in *A. oryzae*.) These mainly haploid sectors often grew much more vigorously than the diploids, thus making selection of type (i) a simple matter.

It should be noted that there is a great similarity between the effects of acriflavine treatment of diploids and those of formaldehyde, nitrogen mustard and UV observed by Fratello *et al*. (1960) with respect to 'non-disjunction', haploidisation and the occurrence of small, poorly sporulating colonies (presumably aneuploids, Käfer, 1960).

Determination of genotype. Genotypes of all nutritional markers except *ad* were determined directly by testing the haploid components of the adenine-independent recombinant diploids. Since it was impossible to distinguish phenotypically between the various *ad* alleles (see section E), in contrast to the situation found by Pritchard (1955, 1960a) for the pairs of *ad* alleles he investigated mitotically, elucidation had to be made by genetic analysis.

The adenine-requiring haploid components were used to synthesise new diploids (termed 'reconstituted' diploids for short) by combining them with suitably marked strains having one or the other of the ad alleles present in the original (parental) diploid from which the haploid had been derived by mitotic recombination and haploidisation. The 'reconstituted' diploid was then tested for ability to produce adenine-independent segregants by the procedure already outlined.

For example, if the parental diploid is adx / adz and the ad haploid components of the adenine-independent recombinant diploids ad-a, ad-b ...ad-n, then ad-a / adx, ad-b / adx ... ad-n / adx and ad-a / adz, ad-b / adz ... ad-n / adz diploids are synthesised and selection for adenine-independent segregants is made. Production of segregants by ad-a / adx (but not ad-a / adz) indicates that ad-a = adz, and segregation from ad-b / adz (but not ad-b / adx) demonstrates that ad-b is identical with adx.

Failure of segregation from both *ad-n* / *adx* and *ad-n* / *adz* identifies *ad-n* as the double mutant, *adx adz*. (This is assuming that, by comparison with the number of segregants produced by 'reconstituted' diploids of the type *ad-a* / *adx* and *ad-b* / *adz*, the experiment is conducted on a scale sufficient to make absence of recombination significant.)

There are two possible difficulties involved in the use of this method -- mutation (cf. Pritchard, 1960a) and reduction in frequency of recombination.

If an *ad* chromosome in a homoallelic diploid (Roman, 1956; Pontecorvo, 1958a) colony mutated to adenine-independence and gave rise to an adenine-independent segregant the homoallelic diploid would appear to be heteroallelic on the basis of the foregoing test. That is, in the previous example the finding of a segregant from *ad-n* / *adz* might suggest that *ad-n* is not the double mutant if precautions were not taken to eliminate background mutation-produced segregants from the data. However, this problem did not even arise, as is shown in Section E and in Table 8.

Although it is conceivable that mitotic

recombination between heteroalleles could be reduced to such an extent in certain 'reconstituted' diploids that no segregants would be found, this possibility seems unlikely for two reasons. Firstly, data from experiments involving several different combinations of ad alleles give no indication of any great variation in the frequency of segregants obtained from diploids heterozygous for a given pair of ad alleles. Secondly, even diploids requiring a double crossover to produce adenine-independent segregants gave segregants under the conditions of this test (cf. Table 29 and Chapter III).

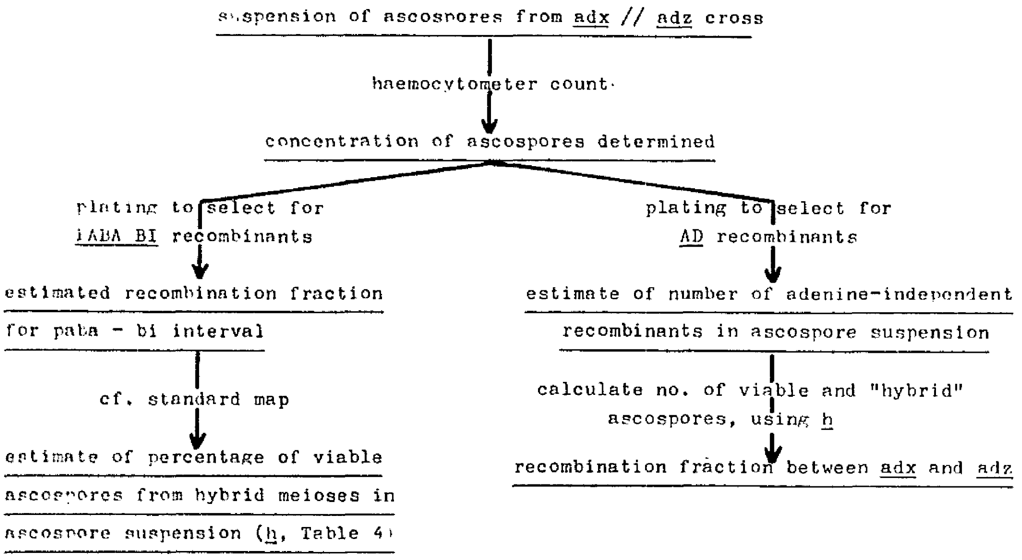
The genetic constitution of white haploid segregants (from recombinant diploids) with respect to the y locus cannot be determined without further investigation, except in the case of those derived from recombinant diploids of y phenotype (i.e. W; y / w3; y genotype). The procedure followed is summarised in Table 3.

2. Analysis by means of meiotic recombinant selection: - Details of the general method are given by Pontecorvo et al. (1953), its application to crosses

between non-complementing ad mutants by Pritchard (1955, 1960a), and to crosses between complementing ad alleles by Calef (1957).

Because *Aspergillus nidulans* is homothallic, ascospores can be of either hybrid or selfed origin. Perithecium analysis allows one to select perithecia of crossed origin only, but such a method is not practicable when large numbers of ascospores are required and/or the cross is infertile. Therefore, a method of distinguishing the products of hybrid meiosis from those of selfed meiosis must be utilised when massed perithecia are the source of ascospores.

An automatic selection of recombinants was used in this work. Ascospores from ad // ad crosses were plated on adenineless medium; for ad // paba crosses, the medium used was deficient in both adenine and *p*-aminobenzoic acid (p.a.b.a.). By this method segregation of the non-selected markers can be followed among the recombinants. However, recombination fractions for the selected markers cannot be obtained directly.



Analysis by Means of Meiotic Recombination:
Diagram Showing Procedures Followed to Estimate the Recombination Fractions
in *ad // ad* Crosses (see Table 4)

FIG. 5

In his analysis of the ad8 cistron Pritchard (1955, 1960a) used Roper's technique (Pontecorvo *et al.*, 1953) for estimating the recombination fractions of the intervals spanned by the selected markers, and this procedure has been followed in both types of recombinant selection used in this work. A diagram summarising this procedure for ad // ad crosses is given in Fig. 5. A similar routine was followed for ad // paba crosses.

A sample of ascospores whose concentration was known from a haemocytometer count was plated on supplementing MM lacking p.a.b.a. and biotin. Colonies were counted after two days' incubation and the recombination fraction for the paba - bi interval determined. This was then compared with the standard value of the paba - bi recombination fraction in order to ascertain the percentage of viable ascospores of hybrid origin. Another sample of the same pool of ascospores was plated (by adding aliquots of the spore suspension to molten medium and pouring the mixture as a thin top layer on already prepared dishes) on supplemented MM lacking adenine (or p.a.b.a. and adenine in the case of AD -

PABA selection), and the number of colonies (necessarily adenine-independent) appearing after two days was counted. Crosses between complementing alleles (see Chapter II) and the *ad* // *paba* crosses produced diploids and aneuploids (Pritchard, 1955, 1956; Calef, 1957) in addition to haploid recombinants. Only the haploids were included in the analysis. The formulae for determination of the recombination fraction and its standard error (Pritchard, 1955) are given in Table 4.

The shortcomings of this indirect method of recombination fraction estimation have already been discussed by Pritchard (1955). The most serious source of error is liable to be deviation of the recombination fraction for the *paba* - *bi* interval from the standard 0.22. Estimates for this interval by Calef (1957) and Elliott (1960b) vary appreciably from cross to cross. However, Käfer (1958) found good homogeneity for the *paba* - *bi* interval in the 17 crosses she analysed. As information of only a crudely quantitative nature was sought in the meiotic recombination studies the estimated recombination fractions for selected intervals are taken as rough approximations only.

E. Preliminary Experiments.

1. Phenotypic characterisation of the ad9 alleles: - Experiments by Calef (1957) and Pritchard (unpublished) on ad9, ad13, ad15 and ad17 demonstrated that adenine hydrochloride at an optimal concentration of 0.09 mg. per ml. MM is required to support growth, and that adenosine and hypoxanthine also meet the nutritional requirements of these mutants. Pritchard (unpublished) noted that 4-amino-5-imidazole carboxamidine hydrochloride had a sparing effect on adenosine (and possibly hypoxanthine) in the case of the two mutants tested, viz. ad9 and ad15.

During the present investigation attempts were made to distinguish between the six ad9 alleles (Table 2 and Chapter II) in the following ways: (a) by determination of the minimum concentration of adenine hydrochloride which would support the growth of, and also determination of the optimal concentration required by, each mutant and (b) by examination of the response of each allele to different temperatures with varying degrees of adenine supplementation.

All mutants gave identical results in the supplementation and temperature tests (Table 5).

2. Mutation studies: - A summary of the available data with respect to back-mutation, in conidia, of the ad9 alleles is given in Table 6. No corrections were made for clonal effects. The frequency of "reversions" appears to vary from ca. 10^{-8} to 10^{-9}; no adenine-independent colonies were found after plating about 6×10^{8} conidia of an ad13 strain and about 7.5×10^{8} conidia of an ad32 strain.

All the "reversions" listed in Table 6, except for the one isolated by Calef, were tested in heterokaryons for dominance relations; all "reversions" proved to be dominant over the ad alleles from which they had mutated, indicating that adenine-independence was due either to a dominant suppressor mutation or to true back-mutation. The adenine-independent colonies recovered from ad9 paba1 y and ad17 bi1 (Table 6) were outcrossed. No adenine-requirers were obtained from 467 and 355 colonies tested, respectively. These results suggest that the two "reversions" are either true back mutations or very closely linked suppressors.

Grigg (1952, 1958) discovered that the density of plating may influence the frequency of colonies observed in back-mutation assays. Therefore, the possibility that high concentrations of ad mutants might suppress adenine-independent revertants in back-mutation and recombinant selection experiments was investigated. Two reconstruction experiments (Table 7) gave no indication of the "Grigg effect" at the concentrations used in either the back-mutation studies or in the meiotic recombinant selection experiments.

It thus appears that the reversion rates of the ad9 mutants are low enough not to become a complicating factor in meiotic analysis. However, as the frequency of mitotic recombination is less than that of meiotic recombination by a factor of ca. 10^3 (Pontecorvo, Tarr Gloor and Forbes, 1954; Pontecorvo, 1958a) to 10^4 (Pritchard, 1960a), the spontaneous mutation rate of these ad mutants might approach the mitotic recombination frequency, as is the case with the ad8 mutants investigated by Pritchard (1960a). Data from several diploids, each homozygous for a different ad allele (including ad9 and ad32, which are the most tightly linked mutants investigated by mitotic analysis -- see meiotic map, Fig. 51), give no indication that spontaneous mutation in mitotic recombination experiments presents any difficulty (Table 8) .

CHAPTER II.

ALLELISM AND COMPLEMENTATION

1. Introduction.

The classical functional criterion of allelism, restated in modern terms, is that two recessive mutants are allelic if they do not complement one another when in the *trans* (Pontecorvo, 1950) arrangement. That is, if *a* and *b* are two recessive mutants and *a* / *b* the *trans* heterozygote, then *a* and *b* are allelic (i.e. are non-complementary) if *a* / *b* resembles *a* / *a* or *b* / *b* rather than *a* / *+* or *b* / *+* . If, on the other hand, *a* / *b* is non-mutant in phenotype, or less mutant than *a* / *a* or *b* / *b*, then *a* and *b* are said to complement one another and to be functionally non-allelic.

An extension of the complementation test was made by Lewis (1951) to include the comparison of two mutants which, by the classical recombination criterion, he considered to be allelomorphs of separate genes. If recombining mutants *a* and *b* are functionally allelic (pseudoallelic by Lewis's definition) they will show a *cis*-*trans* (Pontecorvo, 1950) or "Lewis" effect (Pontecorvo, 1955): - The *cis* arrangement, *ab* / *++*, will be phenotypically wild type while the *trans* arrangement, *a+* / *+b*, will have a mutant phenotype. In other words, the wild-type alleles of both _a_ and _b_ must be present in the _same_ chromosome to overcome the disabilities caused by _a_ and _b_. The "Lewis" effect has been observed in many different cistrons* of a variety of organisms (cf. Table 6 of Pontecorvo, 1958a).

The discovery in _Aspergillus nidulans_ of complementation in _only one of the three possible combinations_ of three _ad9_ alleles (Calef, 1956) suggested that the _cis-trans_ test for determining allelism requires qualification (cf. Pontecorvo, 1956). Subsequent to Calef's finding many other investigators, working with an array of different micro-organisms, have shown that "intra-cistronic" complementation of the type first detected in _Aspergillus_ is a widespread phenomenon (see Table 9 for summary of reported cases).

The functional unit is nevertheless a valuable concept and is still the best definition of the gene available, if we make the following qualification: - A cistron is a series of mutants which do not comple-

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* Benzer (1957) coined the term "cistron" on the basis of the _cis-trans_ phenomenon to replace the term "gene" as a unit of function.

ment one another either directly (when tested two by two) or through a third mutant (Pritchard, 1960b). For example, mutants a and b belong to the same functional unit, even though a complements b, if both a and b do not complement a third mutant, c. This definition is applicable only to those cases where it has been shown that c is not a deficiency overlapping the a and b 'mutational sites' (Pontecorvo, 1952).

The functional relations between mutants belonging to a cistron showing the intra-cistronic complementation phenomenon can be represented by complementation maps. For instance, the Gal cistron of E. coli K12 listed in Table 9 is divided into complementation units I, II and III (delineated on the map by vertical dashed lines) on the basis of the five complementation groups (represented by horizontal lines) into which the eight mutants tested fall. The mutants within each complementation group do not complement one another. Only those complementation groups which are shown in the complementation map as non-overlapping complement one another -- i.e. group a of the E. coli Gal cistron complements groups b, c and d; group b complements groups a and c; group c complements groups a and b, and group d complements group a.

The limits of the cistron can be related to the genetic map by means of recombination experiments (cf. Benzer, 1959), and the location of the mutational sites within the cistron can be determined. However, complementation units cannot be so mapped because these represent functional interactions and not positions of defect within the genetic material. It is possible to correlate the position of the defect, as defined by recombination experiments, with the functional relations of the mutant containing the defect, but one must bear in mind the differences between genetic and complementation mapping.

2. Experimental

Heterokaryons, first used by Beadle and Coonradt (1944) to test for allelism, have been widely used in several organisms for complementation tests. Examples, including other methods which are functionally equivalent, are given in Table 10. *Trans* diploids were tested in addition to heterokaryons during the present investigation because of the possibility of "nuclear limited"

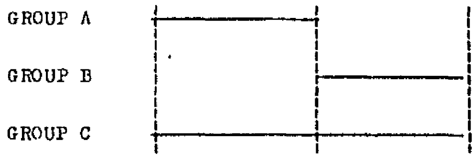
ad13	ad15	ad17	ad32	ad33	
-	-	-	-	-	ad9
	-	-	-	-	ad13
		+	-	-	ad15
			++	-	ad17
				-	ad32

LEGEND: ++ = vigorous growth on MM (indistinguishable from that on MM + ad)
+ = growth on MM (less vigorous than that on MM + ad)
- = no growth on MM

(As the heterokaryon and diploid tests gave identical results the figure represents both experiments.)

Complementation Tests of ad9 Alleles and Suspected Alleles

FIG. 6



LEGEND: Group A comprises ad17
Group B comprises ad15 and ad32
Group C comprises ad9, ad13, ad33

Complementation Map of the ad9 Cistron (see text)

FIG. 7

complementation (cf. Pontecorvo, 1950). Some combinations of alleles were also tested for the "Lewis" effect.

ad32, ad33 and the known ad9 alleles (Table 2) were tested in all combinations in both balanced heterokaryons and diploids. The results are shown in Figs. 6 and 7. Two of the possible combinations were partially or fully complementing. The finding of Calef (1956) that ad15 partially complements ad17 has been verified, and another pair, ad17 + ad32, has been shown to be fully complementary.

The ad15 / ad17 diploid responded to adenine supplementation, giving wild type growth when the degree of supplementation was at least 0.01 mg. adenine hydrochloride per ml. MM. Growth of the ad17 / ad32 diploid was wild type on MM and was not enhanced by adenine supplementation.

Both complementing diploids were tested for temperature sensitivity. ad17 / ad32 grew as well as wild type on MM at the three temperatures tested (25°, 37° and 43°C). The ad15 / ad17 diploid achieved completely wild type growth on MM only at 43°.

Several diploids were tested for cis-trans effect. In all cases (Table 11) the cis arrangement was wild-type with respect to adenine-requirement.

3. Discussion

Six mutants -- ad9, ad13, ad15, ad17, ad32 and ad33 -- belong to the same cistron by the complementation criterion. Recombination experiments (see Chapter III) indicate that they are all closely linked and occupy different sites in the genetic map between pro1 and paba1.

The complementation map of the ad9 cistron can be divided into two complementation units, with ad17 belonging to one unit, and with ad15 and ad32 in the other unit. A comparison of the complementation map (Fig. 7) with the genetic map (Fig. 51) indicates that the two representations are not co-linear.

Before going on to consider possible mechanisms of complementation, it is as well to review the factual basis of current hypotheses of gene function and also to outline various aspects of complementation which must be taken into account.

The nature of gene action: - Where the metabolic basis of mutant phenotypes has been determined, it has usually been found to be the virtual absence or alteration of an enzyme or protein (see reviews by Catcheside, 1960a; Yanofsky and St. Lawrence, 1960). For example, in sickle cell anaemia (Ingram, 1957), one of the peptide residues of normal haemoglobin has been altered; in some adenine-requiring mutants of Neurospora, no detectable adenylosuccinase has been found (Giles, Partridge and Wilson, 1957), and in P^- mutants of E. coli inability to dephosphorylate p-nitrophenyl phosphate is correlated with the absence of alkaline phosphatase (Garen, 1960).

In the past few years attempts at relating genetic fine structure and protein structure have resulted in the "one cistron - one polypeptide chain" hypothesis (e.g. Crick, 1958; Pontecorvo, 1958a and b; Benzer, 1959), a modification of Beadle's (1945) one gene - one enzyme hypothesis (cf. Horowitz, 1948; Horowitz and Fling, 1956; Horowitz and Leupold, 1951). Additional to this hypothesis is the tacit assumption that the linear sequence of sites within the cistron should correspond to the linear sequence of amino-acids in a polypeptide chain. It should be emphasised that, while there is some circumstantial evidence in support of the main hypothesis, no test has been made of the additional assumption of co-linearity. There would not appear to be plausible reasons of a theoretical nature which would lead one to expect that this additional assumption should be true (cf. Levinthal, 1959b). Indeed, the recent report by Fresco *et al*. (1960) on the secondary structure of RNA might suggest that co-linearity is not a feature of the translation of information from cistron to amino-acid sequence.

The essential point to be considered here, leaving coding problems (see for example Crick, 1958) aside, is that the cistron is supposed to determine the amino-acid sequence of a single type of polypeptide chain of a protein and that a mutation in the cistron will result in either (a) no polypeptide-chain formation or (b) the production of an altered chain.

Direct evidence in favour of this hypothesis must come from correlated genetic fine-structure analysis and amino-acid sequence determination. Thus far the only relevant investigations are concerned with the various amino-acid substitutions associated with inherited human haemoglobin abnormalities (*vide infra*).

There are, however, several findings which suggest that enzymes differing from the wild-type enzyme are at least sometimes the consequence of a mutation or "reversion" within those cistrons which are *presumed* to determine the enzyme's amino-acid sequence (or, as in the case of tryptophan synthetase of *E. coli* -- *vide infra* -- determine the sequence in one type of polypeptide chain). As examples one can cite (1) thermolabile tyrosinases (Horowitz and Fling, 1953; Horowitz *et al.*, 1959), (2) the qualitatively different "revertant" glutamic dehydrogenases (Fincham, 1957; Fincham and Pateman, 1957a; Pateman, 1960a), adenylosuccinases (Woodward *et al.*, 1960) and tryptophan synthetases (Yanofsky and Crawford, 1959; Stadler and Yanofsky, 1959), (3) the metal-sensitive mutant tryptophan synthetase (Suskind and Kurek, 1957, 1959) and (4) the mutant proteins antigenically related to the wild type enzyme (DeMoss and Bonner, 1959; Yanofsky, 1956; Yanofsky and Stadler, 1958; Suskind and Jordan, 1959).

The one cistron - one polypeptide hypothesis should not be taken to mean that all cistrons specify amino-acid sequences of polypeptides. The existence of "regulating" cistrons in addition to "specificity" cistrons cannot be excluded with the discovery of "repressors" (e.g. Pardee *et al.*, 1959; Horowitz *et al.*, 1959; Gorini, 1960), which, in some cases at least, may be non-protein in nature (Pardee and Prestidge, 1959).

No examples have yet been found of a single cistron determining more than one polypeptide chain or of more than one cistron coding for a single chain. In this connexion the tryptophan synthetases (TSases) of *Neurospora* and *E. coli* should be mentioned. It appears that one cistron determines the TSase of *Neurospora* (Lacy and Bonner, 1958; Ahmad and Catcheside, 1960; Catcheside, 1960a), while two cistrons specify the *coli* enzyme (Crawford and Yanofsky, 1958; Yanofsky and Crawford, 1959). The latter enzyme consists of two protein components (Crawford and Yanofsky, 1958). In each case three reactions in the tryptophan biosynthetic pathway are catalysed by the TSase (Yanofsky and

Stadler, 1958; DeMoss and Bonner, 1959). The postulate that the reactions all proceed on a single enzyme surface is favoured for both systems (Yanofsky and Stadler, 1958; DeMoss and Bonner, 1959). Tests to discover whether non-identical polypeptide chains are present in the *Neurospora* enzyme are urgently needed. However, it is assumed in the ensuing discussion of complementation mechanisms that *td* of *Neurospora* makes one type of polypeptide chain only.

The best evidence in support of the one cistron - one polypeptide chain hypothesis comes from the haemoglobin studies (see for example Ingram, 1957; Hunt and Ingram, 1958; Itano and Robinson, 1960a,b and c; Itano, 1957; Ingram and Stretton, 1959; Murayama and Ingram, 1959; Benzer *et al.*, 1958; Ingram, 1959). On the basis of protein studies (see for example Schroeder and Matsuda, 1958; Rhinesmith *et al.*, 1957, 1958; Ingram, 1959) and genetic investigations (see for example Schwartz *et al.*, 1957) two unlinked haemoglobin cistrons - α and β - have been identified, the α cistron specifying α chains and the β cistron specifying β chains of this "heterodimeric" (Pontecorvo, personal communication) protein. X-ray analysis and other methods have demonstrated that haemoglobin consists of two identical pairs each made up of two non-identical polypeptide chains (see Perutz et al., 1960, Itano and Robinson, 1960a). "Fingerprint" analysis of several abnormal haemoglobins (cf. Ingram, 1959) have revealed that there is an amino-acid replacement in either the α or the β chain. Hunt (1959) has further demonstrated that the α chains of normal and foetal haemoglobins are probably identical. As the latter consists of two α and two γ chains (i.e. the γ chains are different from both the α and β chains), the α cistron is an example of a cistron which affects two different proteins via their common polypeptide chain.

Aspects of complementation that should be considered in relation to proposed mechanisms of complementation: - Firstly, an acceptable hypothesis for complementation mechanisms must be consistent with the fact that the majority of mutants belonging to a "complementing" cistron do not complement in any combination (see Table 9). These non-complementing mutants are usually revertable mutants (cf. Case and Giles, 1960 and Table 6, this thesis). Therefore, any mechanism postulating that non-complementing mutants are multiple point mutations or rearrangements can be dismissed (see also Catcheside, 1960a).

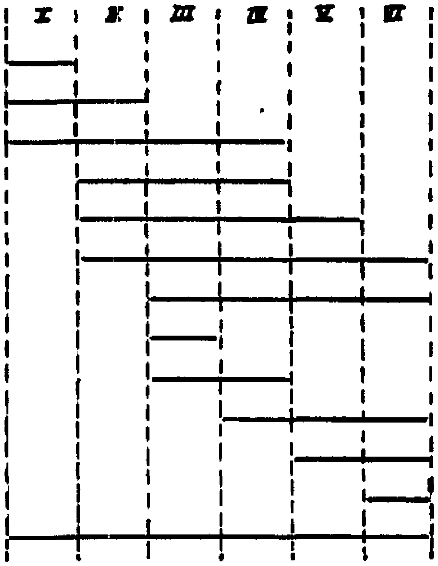
Secondly, any theory of complementation should be applicable to biological systems as diverse as bacteriophage and *Drosophila*. The summary presented in Table 9 suggests that complementation is a wide-spread phenomenon. Although the information from higher organisms is meagre, several cases of complementation have been found in *Drosophila*. In addition, the *lz* gene (Green and Green, 1949; Chovnick *et al*., 1956) may represent another example in *Drosophila*, although more recombination experiments are necessary to exclude the possibility that *50e* and *49h* (assigned to lz^s but complementing all the other *lz* mutants (Green and Green, 1956)) are located in another cistron. The restoration of *S4* antigen in *S2* / *S4'* diploid pollen of *Oenothera organensis* (Lewis, 1958, 1960) might well be an example of intra-cistronic complementation in higher plants. Unfortunately, Lewis's attractive carrier hypothesis (Lewis, 1960) must be tested before it can be assumed that the lack of antigenic activity in such *S* mutants as *S4'* and *S6'* is due to mutation in the "carrier" cistron postulated to govern the expression in style and pollen of the antigen (whose amino-acid sequence is supposedly determined by the "specificity" cistron).

Thirdly, the possibility that not all cistrons coding for amino-acid sequences may be capable of showing complementation should be taken into account. It will be recalled that no complementing pairs were detected for the 99 *his-6* mutants of *Neurospora crassa* tested in all possible combinations (Catcheside, 1960b; see Table 9, this thesis). It seems unlikely that a larger sample of allelic mutants would turn up a case of complementation for this cistron.

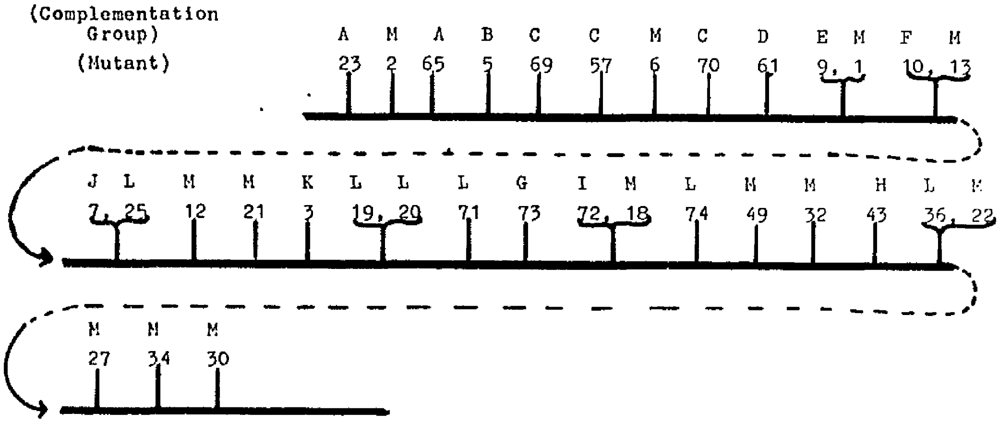
Fourthly, any proposed mechanism should be consonant with both non-linear and linear complementation maps. Three instances of non-linear complementation maps are included in Table 9 -- viz. *his-1* and *lys?* in *Neurospora* (Catcheside, 1960b and personal communication) and *dumpy* in *Drosophila* (Carlson, 1959a). As pointed out by Catcheside (1960b), linear maps based on small numbers of complementation groups are not significant. However, the possibility remains that some of the better-analysed systems actually do have

Complementation Map

Complementation Group	Mutants
A	23, 28, 39, 65
B	5
C	69, 57, 70
D	61
E	9
F	10, 75
G	73
H	43
I	72
J	7
K	3
L	25, 19, 20, 71, 74, 36
M	2, 6, 1, 13, 12, 21, 18, 49, 32, 22, 27, 34, 30



Genetic Map



Complementation and Genetic Maps of the pan-2 Locus of Neurospora Crassa
(after Fig. 1 of Case and Giles, 1960)

FIG. 8

linear complementation maps.

Fifthly, the lack of strict co-linearity between genetic maps and complementation maps must be taken into consideration in any model postulating a co-linear relationship between genetic sites and amino-acid sequence. Of the investigations listed in Table 9, that of Case and Giles (1960) is the most significant. Fig. 8 shows that the genetic and complementation maps of pan-2 in Neurospora are not strictly co-linear. Woodward and Cook (1960) report that further investigation of pyr-3 in Neurospora has led them to revise the original conclusion (Suyama et al., 1959) that co-linearity was established for the pyr-3 cistron. The his-3 cistron of Neurospora (Webber, 1959) and the ad9 cistron of Aspergillus are also not co-linear. The purported co-linearity of genetic and complementation maps for his-D in Salmonella (Hartman et al., 1960a) and me-2 in Neurospora (Murray, 1960b) is not significant because of the small number of complementing mutants mapped.

Sixthly, the occurrence of an active protein differing from the wild-type protein must be allowed by any acceptable complementation model. Fincham (1959b) has shown that the glutamic dehydrogenases produced by heterokaryons between complementing *am* *Neurospora* mutants are qualitatively different from one another and from the wild-type enzyme. Moreover, the observation that enzyme activity in heterokaryons formed between some complementing mutants is apparently temperature-sensitive (cf. Suyama *et al*., 1959; Case and Giles, 1958b, 1960; Woodward *et al*., 1958), in contrast to wild-type enzyme activity, would also indicate qualitative differences.

Mechanism of intra-cistronic complementation: - Since the process of complementation appears to take place in the cytoplasm -- because it can take place in heterokaryons -- clues to its mechanism must be sought at the level of the gene products, in particular, the polypeptide chains composing proteins and enzymes. Unfortunately, nothing is known about either the wild type or mutant enzymes whose amino-acid sequences are presumably determined by the *Aspergillus* *ad9* cistron. However, a recent experiment concerning *in vivo* inhibition of complementation indicates that polymer formation might be required for complementation between *ad9* mutants (*vide infra*).

Three types of mechanisms have been advanced in attempts to explain complementation: -

(1) interaction at the polypeptide-forming level to yield, through recombination, a *wild-type* polypeptide from two differently mutant templates (Woodward *et al*., 1958),

(2) polymerisation of non-identical polypeptide chains to give a *wild-type protein* (Woodward, Partridge and Giles, 1958; Woodward, 1959; Case and Giles, 1960), as in the case of the reassociation between the β chains of haemoglobin I (in which the α chains are abnormal) and α chains of haemoglobin S (in which the β chains are abnormal) to give normal haemoglobin A (in which both α and β chains are normal (Singer and Itano, 1959; Itano and Robinson, (1959),

(3) polymerisation of different mutant forms of the same polypeptide to form an active protein *differing from wild type* (Catcheside and Overton, 1958; Catcheside, 1960b; Woodward, 1959;

Fincham, 1959b; Crick and Orgel, personal communication).

The first two types of mechanism can be rejected because they do not postulate the existence of abnormal, albeit active, protein. Moreover, the *in vitro* complementation observed by Woodward (1959), if really substantiated and shown to occur without protein synthesis, argues against a recombination mechanism at the polypeptide-forming level. The second mechanism can also be rejected on the grounds that the haemoglobin type of complementation would require *all* mutants not associated with deletions or rearrangements to be either α type mutants or β type mutants and *all* those of one type to complement all those of the other type. In other words, the second mechanism is applicable to restoration of a functional protein consisting of two types of polypeptide chain, each of which is specified by its own cistron, and not to a protein consisting of one type of polypeptide chain specified by one cistron.

A specific model for complementation: - Crick and Orgel (personal communication) have advanced a theory of complementation based upon an interaction between differently altered forms of identical polypeptide chains. The essential facets of their model are: -

(1) The polypeptide chain (whose amino-acid sequence is determined by a cistron, some mutants of which show the complementation phenomenon,) exists in its active state as a dimer or polymer consisting of identical subunits.

(2) The active centre of the dimer (or polymer) can be determined by either a single subunit or by the two subunits acting in conjunction, i.e., dimerisation can be regarded as either a prerequisite for maintaining the molecular conformation* necessary for reaction of the active centre with its substrate or as a means of producing the active centre.

(3) Mutants having changes of amino-acid sequence in segments near the dyad axis affecting the foldings of the dimer are potentially capable of complementa-

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* Conformation is defined as "...any arrangement in space of the atoms of a molecule that can arise by rotation about a single bond and that is capable of finite existence." (Dauben and Pitzer, 1956). Configuration is the term applied by the organic chemist to the primary (co-valent) structure of the molecule.

tion. A more general application of this idea is that changes in those segments of the polypeptide chains which are in close proximity in the dimer (or polymer) are potentially capable of complementation. It is envisaged that the mutant amino-acid sequence, producing an inactive protein, in this case indirectly deforms the active centre by producing a misfolding or unravelling in its supporting structure.

(4) Complementation takes place between two mutants which determine such misfoldings in different segments if the monomers of the "selfed" proteins are capable of hybrid formation and if the "hybrid" polymerisation corrects the deformation of the active centre caused by the misfoldings. It is imagined that by crosslinks between differently mutant polypeptide chains the "good" part of one chain can hold at the proper distance the end points of the misfolded segment of the other chain, thus affording a conformation of the active centre sufficiently like, though not necessarily identical with, the wild type to make reactivity with the substrate possible.

Consequences and evaluation of the model: - In its simplest form the Crick-Orgel theory runs into two difficulties when examined in the light of the experimental evidence. In the first place, it assumes that the "unravelling" caused by mutation-produced amino-acid alteration proceeds along the polypeptide chain. Non-linear complementation maps, however, would favour the view that the defect spreads along the surface of the molecule, although Catcheside (1960b) suggests that they indicate "...an interaction between surfaces rather than between linear objects." Secondly, Crick and Orgel (personal communication) assume that the linear sequence of amino-acids is directly related to the linear sequence of the genetic material. Consequently, genetic and complementation maps are expected to be co-linear if the "unravelling" spreads along the polypeptide chain.

The second contradiction is not insurmountable, for the assumption of co-linearity between polypeptide chain and cistron is not a necessary part of the theory. The first one is more serious, although Crick and Orgel (personal communication) suggest that it can be adapted to fit the facts by assuming that the unravelling can spread not only along the polypeptide chain but also along the surface of the molecule.

The model meets the other requirements already mentioned as necessary adjuncts to an acceptable complementation mechanism -- viz. non-complementation by the majority of mutants from "complementing" cistrons, widespread though not universal occurrence of complementation and the production of abnormal protein by complementing systems.

Polypeptide-polypeptide interaction requires formation of a hybrid polymer. Therefore, the absence of observed complementation in the case of particular well-investigated cistrons could be due to (a) the active protein being a monomer, (b) poor or no in vivo dissociation of the selfed polymers, (c) localisation of the selfed proteins to an extent that "collision" between dissociated strands of the two mutant types are not likely in heterokaryons and (d) the inability to recover complementing mutations for technical reasons.

Crick and Orgel (personal communication) also suggest that the degree of observed enzyme activity restored by complementation (cf. Woodward et al., 1958; Woodward, 1959; Megnet, 1959) is consistent with their polypeptide-polypeptide interaction model. However, before such data can be taken as support for their theory it will be necessary to know (a) the number of subunits in the protein, (b) how the active centres are formed, (c) whether the observed amount of enzyme activity is influenced by assay techniques and (d) whether the rates of polymerisation and dissociation of the selfed and hybrid proteins are limiting factors in the determination of enzyme activity.

It is apparent that the real test of polypeptide-polypeptide interaction models such as the Crick-Orgel proposal must be a combined X-ray crystallographic and amino-acid sequence determination of the normal and "complementing" proteins and their postulated monomeric subunits.

However, some type of model involving polymer formation at the protein level seems the most plausible explanation of complementation. The following survey might suggest that S-S linkages could provide the cross-linkages for polymer formation:

(1) *ad9 mutants in Aspergillus*. In a recent preliminary experiment (see addendum to p. 58), suggested to me by Professor Pontecorvo, it was found that complementation by the *ad17* / *ad32* (fully complementing) diploid was completely inhibited by

ADDENDUM TO P. 58

Let us assume that the complementation mechanism involves the polymerisation of two differently mutant polypeptide chains of the same type and that this process requires S-S linkages. Therefore, it might be possible to prevent in vivo complementation with -SH inhibitors, provided the remainder of the cell metabolism is not too seriously disturbed.

Experimental design

The range of $HgCl_2$ concentrations mentioned below were tested to ascertain whether, at a particular Hg^{++} concentration, one or both complementing diploids were inhibited while diploids either heterozygous ad9/+ or heterozygous in trans for two non-allelic ad mutants grew normally.

Roughly equal amounts of conidia from the following diploids were streaked on each of the MM + $HgCl_2$ dishes:

(1) ad15/ad17 (i.e. partially complementing combination)
(2) ad17/ad32 (i.e. completely complementing combination)
(3) ad8/ad9 (i.e. normally ad-independent)
(4) ad9/+ (i.e. normally ad-independent)

Replicate platings of a range of $HgCl_2$ concentrations from ca 10^{-3} to 10^{-8}M were tested.

Results

At concentrations above 10^{-5}M none of the four diploids grew, and at concentrations less than 10^{-6}M all grew. However, the results of the test dishes containing ca. 5×10^{-6}M and 7×10^{-6}M indicated (a) in this range the ad-independent diploids (3 & 4) grew normally, (b) ad17/ad32 was completely inhibited at the 5×10^{-6}M concentration and (c) ad15/ad17 grew normally at the lower concentration and at 7×10^{-6}M $HgCl_2$ it was still able to produce spidery aconidiate colonies.

Hg^{++} and that the growth of the <u>ad15</u> / <u>ad17</u> (partially complementing) diploid was greatly reduced by a slightly higher concentration of inhibitor. In both cases the controls (diploids heterozygous <u>ad</u> / <u>+</u> or heterozygous in <u>trans</u> for two non-allelic adenine mutants) grew normally. As Hg^{++} is a well-known reagent for blocking -SH groups, these results suggest that -SH groups might be responsible for the cross-linking of mutant polypeptide chains, at least in the <u>ad9</u> complementing system.

(2) <u>Tryptophan synthetase in Neurospora</u>. Investigation of the wild-type enzyme and antigenically related proteins produced by certain <u>td</u> mutants (cf. Catcheside, 1960a for summary) suggests that tryptophan synthetase activity is related to the presence of -SH groups. The enzyme is inactivated by dialysis in the absence of glutathione. Further dialysis reduces the enzyme's affinity for anti-tryptophan synthetase (anti-TSase) to that shown by cross-reacting material (CRM) produced by some <u>td</u> mutants. Dialysis does not reduce the affinity of CRM for anti-TSase (Suskind, 1957). Certain <u>td</u> mutants owe their lack of activity to inhibition of the mutant enzyme by a heavy metal (Suskind, 1958; Suskind and Kurek, 1959).

(3) Glutamic dehydrogenase. Kinetic studies of wild-type, "complementing" and mutant *Neurospora* glutamic dehydrogenases (Fincham, 1957, 1958, 1959b; Fincham and Pateman, 1957a and b) have shown that the enzyme produced by heterokaryons between complementing *am* alleles and by *am*-2^1 (a revertant) are qualitatively different from the wild type enzyme in several respects, thus giving support to a polypeptide-polypeptide interaction mechanism such as that proposed by Crick and Orgel.

Biochemical investigation of pig and beef liver glutamic dehydrogenases (Iwatsubo *et al.*, 1954, 1957; Mitani, 1957a and b) indicates that each "molecule" has about 54 -SH groups, of which 15-23 combine with the substrate. Molecular weight studies suggest that the pig enzyme consists of eight identical subunits held together by hydrogen bonds (Soyama, 1958; Fincham, 1959b).

Thus, it could be envisaged that complementation at the *am* locus in *Neurospora* is brought about by S-S cross-linkage between differently damaged subunits having the substrate-combining -SH groups still intact, assuming that the liver and *Neurospora* enzymes do not differ except in minor details. (It could also be argued, of course, that hydrogen-bonding could be the method of cross-linkage, as the liver enzyme subunits appear to be held together by non-covalent bonds.)

Several proteins have been investigated for species differences (see review by Anfinsen, 1959); these results have bearing on the present comparisons between the various glutamic dehydrogenases. Most of the proteins examined appear to have identical amino-acid sequences for the regions essential for enzymatic activity. Moreover, the great structural similarity between diverse myoglobins and haemoglobins should be noted (Perutz _et al._, 1960; Kendrew _et al._, 1960). However, Anfinsen (1959) mentions that the somatotropins and prolactins appear to be heterogeneous, with molecular weights varying over a twofold range and with differences in the number of chains and the cystine content. As their amino-acid sequences have not been determined it is impossible at this time to say whether this heterogeneity reflects fundamental species differences in these proteins.

(3) _Adenylosuccinase in Neurospora._ Woodward (1959) found that reduced glutathione enhances recovery of

adenylosuccinase activity when extracts of complementing _ad-4_ mutants are mixed _in vitro_. Partial _ad-4_ revertants show increased enzyme activity in the presence of glutathione and are inhibited by _Zn_ and _Cu_; the wild-type enzyme is also inhibited by these metals, but at a higher concentration (Woodward _et al._, 1960). These results again are indicative of -SH sensitivity.

Granted the assumptions made in the foregoing survey, it appears that the Crick-Orgel suggestion that S-S linkages might be holding the damaged parts of the molecule in such a way that an active conformation results, could be responsible for at least some instances of complementation.

The use of -SH and S-S reagents provides a means of studying both _in vivo_ and _in vitro_ the prevalence of complementation dependent upon such linkages. That all the cases of complementation where any information regarding the presence or absence of -SH and S-S groups is available, could involve S-S linkages would suggest that this might be a general characteristic of complementation.

4. Summary.

1. Two new *ad9* alleles were identified, viz. *ad32* and *ad33*.

2. Two complementing combinations of the six *ad9* mutants tested were found, viz. *ad15* / *ad17* -- already identified by Calef (1956) -- and *ad17* / *ad32*.

3. The complementation and genetic maps are not co-linear.

4. Complementation was inhibited *in vivo* in the presence of mercuric ions.

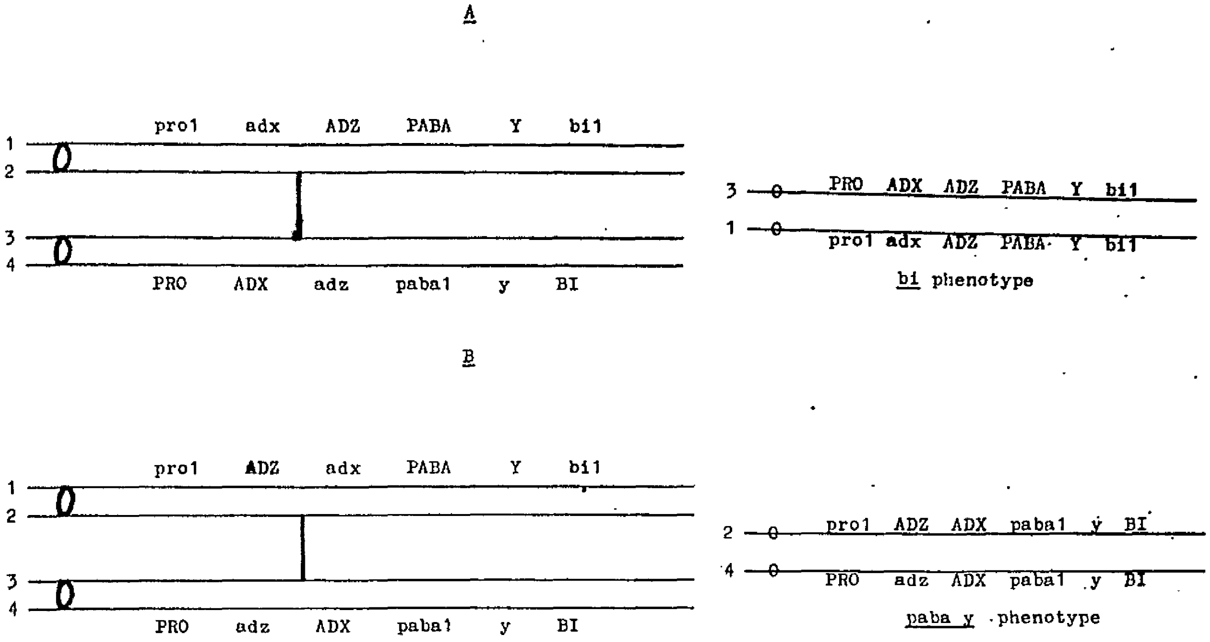
5. A number of authors have suggested that complementation may be due to the formation of a hybrid polymer between differently defective polypeptide chains. As Hg^{++} inhibit the formation of S - S bonds, the results suggest that S - S bonding may be involved in the formation of such a hybrid polymer.

CHAPTER III.

THE LINEAR ARRANGEMENT OF THE ad9 ALLELES

AND

THE OCCURRENCE OF INTRA-CISTRONIC INVERSIONS



In this and subsequent figures the parental diploids are shown having four chromatids held together by centromeres to form homologous chromosomes. The chromatids are numbered to facilitate recognition of segregation types. For convenience the homologous chromosomes of recombinant diploids are represented as single strands.

Consequence of a Single Mitotic Exchange between ad Alleles Followed by Migration of One Crossover Strand and One Non-crossover Strand into the Same Nucleus

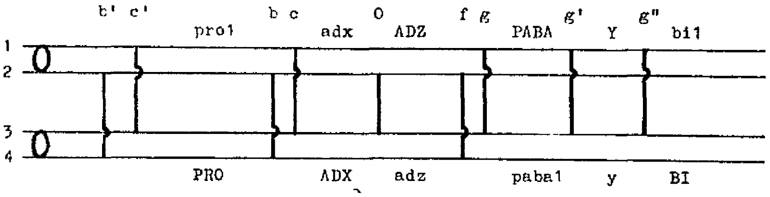
FIG. 9

I. Mitotic Recombination Analyses: -

A. Introduction.

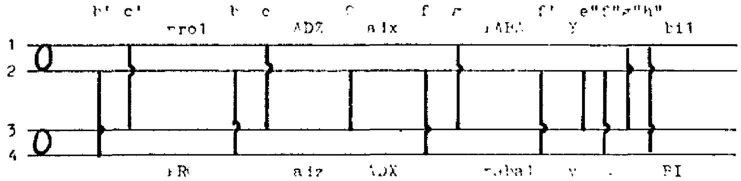
Mitotic recombination experiments, in which adenine-independent segregants were selected (Fig. 2), were carried out for all the possible *trans* pairs of the non-complementing *ad9* mutants. (See Chapter I for experimental details.) Assuming that adenine-independent segregants arise from adenine-requiring diploids of the type *pro1 adx bi1* / *adz paba1 y* (Fig. 4) by a recombination mechanism and that intra-cistron single crossovers are more frequent than double crossovers (cf. Pritchard, 1955, 1960a), homozygosis for recessive markers distal to the point of selected exchange should afford an unambiguous order of the *ad* alleles with respect to the distal markers (Fig. 9). That is, if selection for adenine independence requires a recombination between the two *ad* alleles to produce an *AD AD* chromosome, subsequent segregation of an *AD AD* strand together with a non-crossover strand into the same daughter nucleus will result in a diploid of either *bi* phenotype (A in Fig. 9) or *paba y* phenotype (B in Fig. 9), depending upon the order of *adx* with respect to *adz*.

A



CROSSOVER TYPE	CHROMATIDS TO DAUGHTER NUCLEUS	PHENOTYPE	
O	3 & 1	bi	single crossover
b' c' b c	3 & 1 1 & 3 3 & 1 1 & 3	bi bi bi bi	unselected crossover proximal
f g g' g"	3 & 2 3 & 2 3 & 2 3 & 2	paba y bi bi bi	unselected crossover distal

B



CROSSOVER TYPE	CHROMATIDS TO DAUGHTER NUCLEUS	PHENOTYPE	
O	3 & 1	paba y	single crossover
b' c' b c	3 & 1 1 & 3 3 & 1 1 & 3	paba y paba y paba y paba y	unselected crossover proximal
f g f' f" e" f" g" h"	3 & 2 3 & 2 3 & 2 3 & 2 3 & 1 3 & 1 3 & 1 3 & 1	bi paba y bi bi paba y paba y paba y paba y	unselected crossover distal

Possible Origin of bi and paba y Phenotypes from Single and Double Crossovers in Diploids A and B (cf. Fig.

FIG. 10

In order to differentiate between the mitotic recombination experiments in which the analysis was carried only as far as the classification of the diploid recombinant phenotypes (see Fig. 2) and those in which the genotypes of the recombinants were established (see Fig. 3), I have called the latter type of experiment half-tetrad analysis (see also p. 73).

It should be noted that-- even if selection for adenine-independent mitotic segregants is correlated with such an intensity of negative interference that the single (selected) crossover types constitute a minority of the segregants -- a mitotic recombination analysis should reveal the sequence of sites (see Fig. 10), provided the following conditions are met: -

(1) Adenine independence occurs most frequently by means of a recombination mechanism, or -- if an appreciable amount of "conversion" does occur -- it does not show a high correlation with recombination in the _ad_ - _paba_ interval (see Fig. 9).

(2) The products of single and three- or four-strand double crossing-over are viable.

(3) In the case of double crossovers, there is not a restriction of the unselected recombination to the _ad_ - _paba_ interval, concomitant with a very low frequency of the non-reciprocal single crossover type.

If these three conditions hold, it follows (Fig. 10) that if *adx* is in coupling with *pro* and proximal to to *adz*, the diploid segregants will show an excess of *bi* over *paba y*, whereas if *adx* is in coupling with *pro* but distal to *adz*, the diploid segregants will show an excess of *paba y* over *bi*.

B. Results.

Thirteen *trans* combinations of the six *ad9* alleles were tested. (It is not technically feasible to select for adenine-independent recombinants from the two complementing combinations, *ad15* / *ad17* and *ad17* / *ad32*. However, the combination *ad17* / *n-ad13 ad32** was tested and produced adenine-independent segregants, thus indicating that *ad17* and *ad32* represent mutations at different sites.)

Adenine-independent diploid recombinants were recovered in all experiments (Tables 12 and 13).† Nine combinations provided information with respect to the linear order of the *ad* alleles (Table 12).

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* *n* indicates the non-inverted sequence of the *ad13*, *ad9* and *ad32* sites. Details of this recombination experiment are given in Table 36.

† No interpretation has been attempted of the ± phenotypic class in these experiments as (1) it is not known whether chromatid segregation is random and (2) this class would be expected to result from not only reciprocal segregation of chromatids following a single crossover but also following many types of double crossing-over.

Although only one of the two possible trans diploids, viz. pro1 adx bi1 / adz paba1 y, was analysed for each pair of alleles if the probability that there was no departure from equality between the bi and paba y classes was less than 0.01, the reciprocal diploid, viz. pro1 adz bi1 / adx paba1 y, was analysed for the allele pairs where this probability exceeded 0.01. It should be noted that, for the three combinations listed in Table 12 where the reciprocal trans diploids were tested (ad33 / ad13, ad9 / ad17, ad9 / ad15), the excess of the bi class over the paba y class for diploid A (Table 12) is parallelled by an excess of the paba y class over the bi class for diploid B.

On the basis of these results and Calef's (1957) investigation of ad15 // ad17 crosses the following site sequences are established: -

ad33 - ad9 - ad32 - paba1
ad33 - ad13 - ad17 - ad15 - paba1
ad33 - ad9 - ad17 - ad15 - paba1 .

The four combinations ad13 / ad9, ad13 / ad32, ad13 / ad15, ad15 / ad32 (Table 13) gave anomalous results in that the bi and paba y classes were not significantly different from one another for eight of the diploids tested. However, diploid A of the ad13 / ad32 combination produced an excess of the paba y class (signifcant at the 5% level), although the reciprocal diploid also had an excess (not significant) of this class. When the data are pooled, the presumptive single crossover and double crossover classes are not significantly different.

C. Discussion and Conclusions.

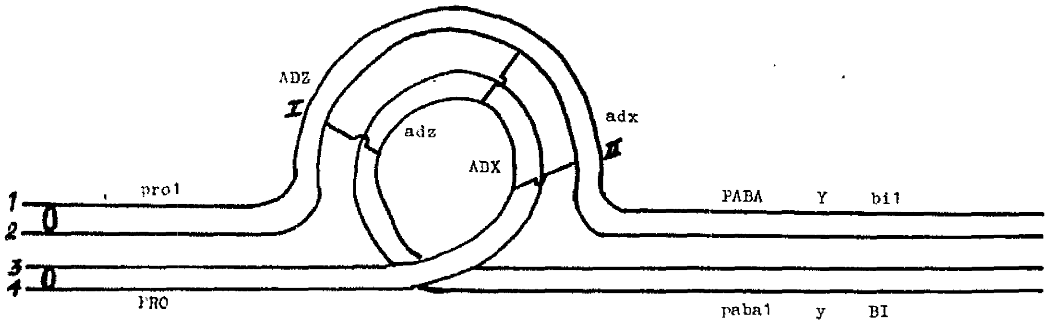
It is apparent that selection for adenine-independence is associated with high negative interference, even in those allele combinations where it is possible to distinguish the sequence of ad sites.

When a non-selective analysis of mitotic recombination is made, or when selection is made for homozygosis of a distally located selector marker, the coincidence of two mitotic recombinations is ordinarily an exceedingly rare event (Pontecorvo, Tarr Gloor and Forbes, 1954; Pontecorvo and Käfer, 1958). Pontecorvo and Käfer (1958) have found that a second crossing-over occurred in about one per cent of the segregants selected because of one mitotic recombination, compared to an expected coincidence of ca. 1/400 (Pontecorvo, Tarr Gloor and Forbes, 1954). However, the coincidence of two recombinations in a single chromosome arm among diploids homozygous for the "selector" marker is still only about 1/400 (Pontecorvo and Käfer, 1958). This contrasts markedly with results obtained when selecting for recombinants between extremely closely linked markers: - i.e. the present results, those obtained by Pritchard (1955, 1960a -- selection of recombinants between ad8 alleles) and those obtained by Käfer (1958 -- selection of recombinants between pyro4 alleles). Pritchard's analysis of 43 adenine-independent segregants from an ad8 / ad16 diploid (Pritchard, 1955) indicated that only 24 of these were the result of a single recombination between the ad alleles.

It should be emphasised that even with the high negative interference found with all the allele pairs listed in Table 12, allelic sequences compatible with all mapping experiments (see section III) can be established. Therefore it seems highly unlikely that these sequences are erroneous.

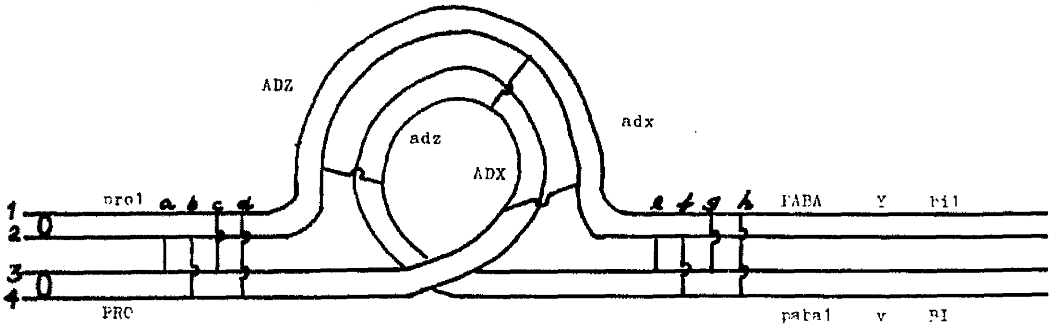
The four combinations for which no allelic order

A. Types of recombination which would give rise to viable adenine-independent diploid recombinants.



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	PHENO-TYPE
I	3 & 2	PRO ADX ADZ paba1 y BI pro1 adz adx PABA Y bi1	+
I	3 & 1	PRO ADX ADZ paba1 y BI pro1 ADZ adx PABA Y bi1	+
II	2 & 3	pro1 ADZ ADX PABA Y bi1 PRO adx adz paba1 y BI	+
II	2 & 4	pro1 ADZ ADX PABA Y bi1 PRO ADX adz paba1 y BI	+

B. Types of recombination in the pro - inversion and inversion - paba intervals which would result in diploid recombinants which have non-wild phenotypes.



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	PHENOTYPE	TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	PHENOTYPE
a, I	2 & 4	paba y	I, e	3 & 1	bi
a, II	3 & 1	bi	II, e	2 & 4	paba y
b, I	3 & 2	paba y	I, f	3 & 2	paba y
b, II	4 & 1	bi	II, f	2 & 3	paba y
c, I	1 & 4	paba y	I, g	3 & 2	bi
c, II	2 & 3	bi	II, g	2 & 3	bi
d, I	3 & 1	paba y	I, h	3 & 1	paba y
d, II	2 & 4	bi	II, h	2 & 4	bi

Mitotic Recombination in Trans Diploids Heterozygous for an Inversion Spanning the Two Sites between which Recombination is Selected: Possible Explanation of the "Anomalous" Mitotic Recombination Results (see text)

FIG. 11

could be determined all involved ad13 and/or ad15, suggesting a special behaviour which might be best explained by the first two possibilities given below (see also introduction, this section): -

(1) An inversion in one of the strands of the parental diploid of such a nature that only a 2-strand double crossover within the inverted region will give rise to a viable adenine-independent recombinant and that there is a high probability of further recombination outside the inversion. In other words, it is suggested that at least some of the "anomalous" mitotic recombination results could be the consequence of the trans diploids being heterozygous for an inversion spanning the ad sites between which recombination is selected and that such inversion heterozygotes show a high probability of further recombination in the pro - inversion and inversion - paba intervals. Consider, for example, the hypothetical diploid pro1 adx bi1 / adz paba1 y, where ADZ is located proximally to adx and ADX adz are inverted with respect to ADZ adx (Fig. 11). (The other possible arrangements predict comparable results, so only this example will be discussed) It can be deduced from Fig. 11A that (a) 2-strand double crossing-over within the

inversion is required to produce viable adenine-independent recombinants and (b) all such recombinants are phenotypically wild type. Fig. 11B suggests that the bi and paba y diploid phenotypic classes would be found not to differ from 1:1 in mitotic recombination experiments unless, say, the occurrence of type f or type g crossovers was much more frequent than the other types.

(2) A high negative interference, characterised by localisation of the unselected crossover to the ad - paba interval and accompanied by chromatid interference and/or non-random segregation of chromatids following recombination (i.e. a high probability of crossover type f in Figs. 10A and 10B).

(3) Gene conversion or misreplication at one or other of the ad mutant sites having a high correlation with recombination in the ad - paba region.

The last explanation seems the least likely because conversion probably occurs only rarely in A. nidulans (Strickland, 1958b; Pritchard, 1960a and b -- see also discussion of conversion in section III). Moreover, it does not explain why certain combinations gave anomalous results, while other combinations involving some of the same alleles did not -- e.g. ad13 / ad15 (anomalous) and ad13 / ad9 (anomalous) vs. ad9 / ad15 (normal); ad13 / ad32 (anomalous) and ad13 / ad9 (anomalous) vs. ad9 / ad32 (normal).

On the basis of the present experiments and the allelic sequences thus far established, there is not much to choose between the first two alternatives. However, an inversion in the ad13 strains spanning the sites of ad9, ad13 and ad32 should account for all the "normal" results and the combinations ad9 / ad13 and ad13 / ad32 as well. The merit of such a proposal is that it is open to experimental verification (see next section). It is suggested that the "anomalous" results with ad15 are not due to the inviability of single and three- or four-strand double crossovers, for the ad13 - ad17 - ad15 sequence would be invalidated were either the ad13 or the ad15 strain to possess an inversion including both the ad13 and ad15 sites. This proposal can also be tested by half-tetrad analysis of the ad13 / ad15 combination (see next section).

II. The ad13 Inversion.

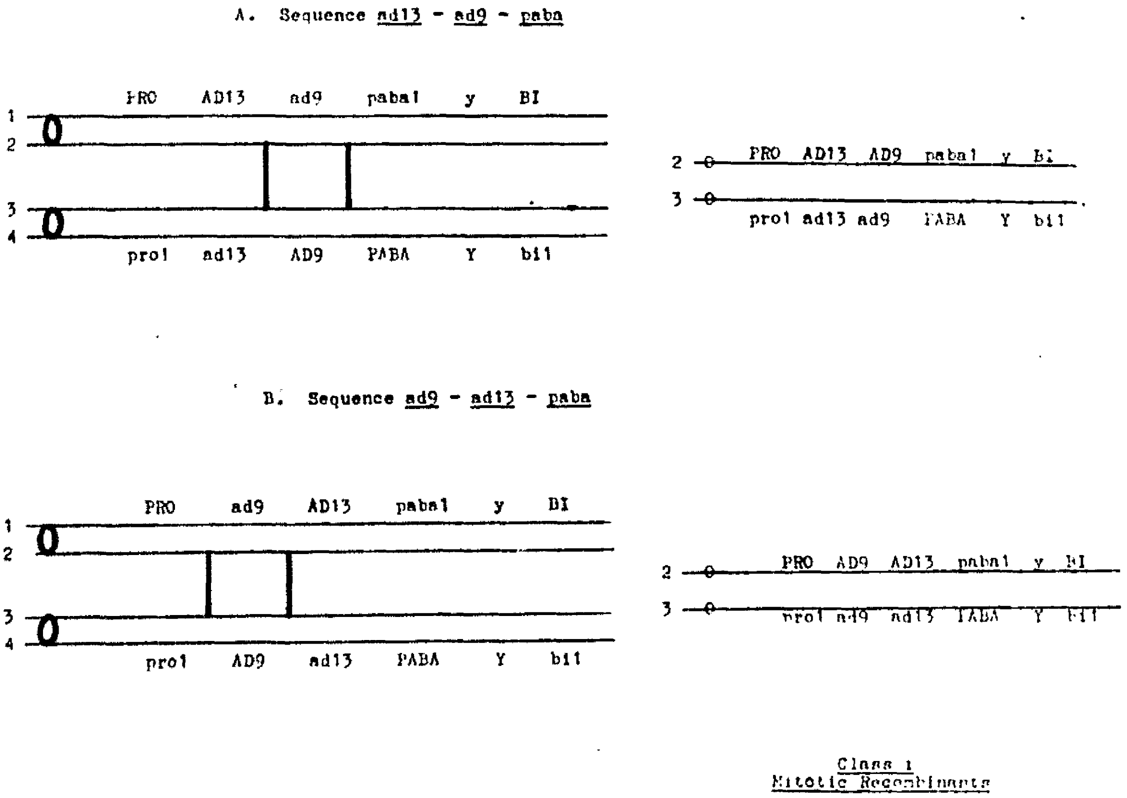
A. Introduction.

Characterisation of the component strands of a recombinant diploid is equivalent to the analysis of half-tetrads following meiosis (cf. attached-X in *Drosophila*). By this method, it is sometimes possible to recover both the double mutant strand and the doubly wild-type strand from the same diploid, following selection for recombination between the two mutants (Roper and Pritchard, 1955; Pritchard, 1955, 1960a). Haploidisation and complete genotype elucidation (i.e. half-tetrad analysis) were carried out for mitotic recombinants of some of the *trans* diploids (for experimental method, see Chapter I and Fig. 3) in an endeavour to delimit the extent of the suspected *ad13* inversion, to provide strains for the determination of the *ad13-ad9-ad32* sequence and to explain the "anomalous" combinations involving *ad15*.

B. Results.

1. Half-tetrad analysis of four allelic combinations involving ad13.

Wild-type mitotic recombinants, rather than a



Types of Mitotic Exchange Which Would Result in Class I Recombinants (Table 1), Where the Sequence of *ad9* and *ad13* Relative to *paba* is Unknown

FIG. 12

random sample of all phenotypes, were selected because diploids carrying the reciprocal products of a single crossover and all two-strand double crossovers are necessarily wild-type. These types of recombinants are crucial to the test of the suggested inversion for the *ad13* strains. The technical difficulties inherent in determining the complete genotype made it necessary to reduce the sample size as much as possible and to sacrifice the possibility of gaining direct information on chromatid segregation following mitotic recombination.

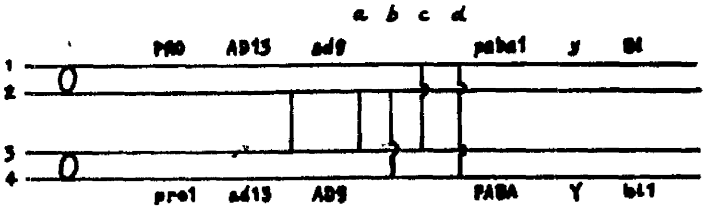
a. *ad9 / ad13.*

(1) The genotypes of sixteen wild-type mitotic recombinants from the diploid

$$\frac{\text{pro1 ad13 PABA Y bi1}}{\text{PRO ad9 paba1 y BI}} \qquad \frac{\text{W}}{\text{w3}}$$

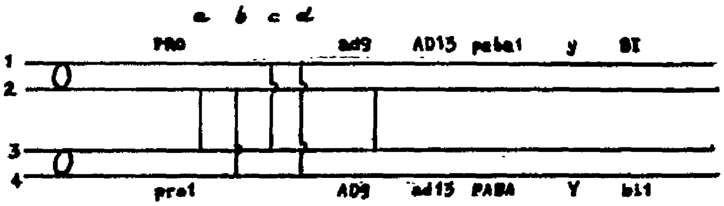
were determined. They fall into six classes with respect to genotype (Table 14), none of which could have arisen from a single crossing-over between the two *ad* alleles. One diploid (class *i*) carried the reciprocal products of a two-strand double mitotic exchange (Fig. 12). The origin of each class will now be considered in turn. (For the present, no inversion will be assumed.)

A. Sequence ad13 - ad9 - paba



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a	2 & 3	PRO AD13 AD9 paba1 y BI pro1 ad13 ad9 PABA Y bi1	i	2 & 4	PRO AD13 AD9 paba1 y BI pro1 ad13 AD9 PABA Y bi1	ii
b	2 & 3	PRO AD13 AD9 PABA Y bi1 pro1 ad13 ad9 PABA Y bi1	*	2 & 4	PRO AD13 AD9 PABA Y bi1 pro1 ad13 AD9 PABA Y bi1	*
c	2 & 3	PRO AD13 AD9 paba1 y BI pro1 ad13 ad9 paba1 y BI	*	2 & 4	PRO AD13 AD9 paba1 y BI pro1 ad13 AD9 PABA Y bi1	ii
d	2 & 3	PRO AD13 AD9 PABA Y bi1 pro1 ad13 ad9 paba1 y BI	∅	2 & 4	PRO AD13 AD9 PABA Y bi1 pro1 ad13 AD9 paba1 y BI	iv

B. Sequence ad9 - ad13 - paba



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a	2 & 3	PRO AD9 AD13 paba1 y BI pro1 ad9 ad13 PABA Y bi1	i	2 & 4	PRO AD9 AD13 paba1 y BI pro1 AD9 ad13 PABA Y bi1	ii
b	3 & 2	pro1 AD9 AD13 paba1 y BI PRO AD9 ad13 PABA Y bi1	∅	3 & 1	pro1 AD9 AD13 paba1 y BI PRO ad9 AD13 paba1 y BI	*
c	1 & 3	PRO AD9 AD13 paba1 y BI pro1 ad9 AD13 paba1 y BI	*	1 & 4	PRO AD9 AD13 paba1 y BI pro1 AD9 ad13 PABA Y bi1	ii
d	3 & 2	pro1 AD9 AD13 paba1 y BI PRO ad9 ad13 PABA Y bi1	∅	3 & 1	pro1 AD9 AD13 paba1 y BI PRO AD9 ad13 PABA Y bi1	∅

* Not phenotypically wild-type and therefore not recoverable in this experiment.
∅ Same genotype as that resulting from a single recombination between the ad mutants.

The Eight Possible Types of Adenine-independent Diploids Produced by Two Mitotic Exchanges, One between ad9 and ad13 and the Other between ad9 - paba (Sequence A or pro - ad9 (Sequence B.

FIG. 13

Class i $\left(\frac{\text{PRO}\ \ \text{AD9 AD13 } paba1\ y\ \text{BI}}{pro1\ ad9\ ad13\ \text{PABA}\ \ \text{Y}\ bi1}\right)^{*}$: - This diploid carries the reciprocal products of an exchange between the two *ad* alleles in addition to the reciprocal products of another exchange in either the interval proximal or the interval distal to the selected crossover, depending upon the linear sequence of the *ad* sites (Figs. 12 and 13) -- i.e. a two-strand double exchange would produce this genotype.

Class ii $\left(\frac{\text{PRO}\ \ \text{AD9 AD13 } paba1\ y\ \text{BI}}{pro1\ \text{AD9}\ ad13\ \text{PABA}\ \ \text{Y}\ bi1}\right)$: - The four diploids of this type could have been produced by the segregation of one crossover and one non-crossover chromatid to the same nucleus following mitotic recombination of the class *i* type. A three-strand double crossover (Fig. 13) followed by non-reciprocal segregation with respect to the recombination between *ad9* and *ad13*, would also give the same result. A less likely explanation is reversion of *ad9* to adenine independence, as back mutation was not detected in homoallelic *ad9* diploids subjected to the same experimental conditions as

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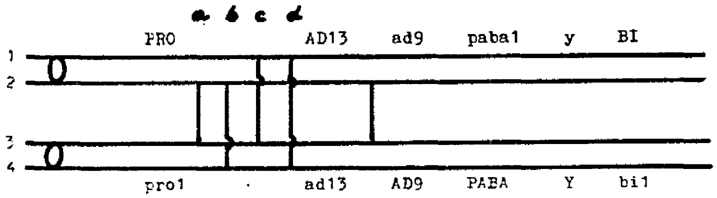
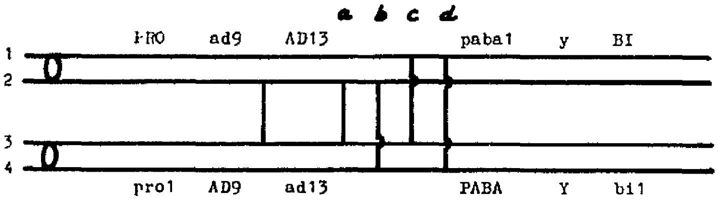
* The sequence of *ad* alleles is arbitrarily shown here to be *ad9* - *ad13*.

A. Sequence <u>ad13</u> - <u>ad9</u> - <u>paba</u>



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a	3 & 2	pro1 AD13 AD9 PABA Y bi1 PRO ad13 ad9 paba1 y BI	0	3 & 1	pro1 AD13 AD9 PABA Y bi1 PRO AD13 ad9 paba1 y BI	iii
[illegible]	4 & 2	pro1 AD13 AD9 PABA Y bi1 PRO ad13 AD9 PABA Y bi1	*	4 & 1	pro1 AD13 AD9 PABA Y bi1 PRO AD13 ad9 paba1 y BI	iii
c	2 & 3	PRO AD13 AD9 PABA Y bi1 pro1 AD13 ad9 paba1 y BI	v	2 & 4	PRO AD13 AD9 PABA Y bi1 pro1 ad13 AD9 PABA Y bi1	*
d	2 & 3	PRO AD13 AD9 PABA Y bi1 pro1 ad13 ad9 paba1 y BI	∅	2 & 4	PRO AD13 AD9 PABA Y bi1 pro1 AD13 ad9 paba1 y BI	v

B. Sequence <u>ad9</u> - <u>ad13</u> - <u>paba</u>



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a	3 & 2	pro1 AD9 AD13 PABA Y bi1 PRO ad9 ad13 paba1 y BI	0	3 & 1	pro1 AD9 AD13 PABA Y bi1 PRO ad9 AD13 paba1 y BI	iii
b	3 & 2	pro1 AD9 AD13 PABA Y bi1 PRO ad9 ad13 PABA Y bi1	*	3 & 1	pro1 AD9 AD13 PABA Y bi1 PRO ad9 AD13 paba1 y BI	iii
c	3 & 2	pro1 AD9 AD13 paba1 y BI PRO ad9 ad13 paba1 y BI	*	3 & 1	pro1 AD9 AD13 paba1 y BI PRO ad9 AD13 PABA Y bi1	∅
d	3 & 2	pro1 AD9 AD13 paba1 y BI PRO ad9 ad13 PABA Y bi1	∅	3 & 1	pro1 AD9 AD13 paba1 y BI PRO ad9 AD13 PABA Y bi1	∅

* Not phenotypically wild-type and therefore not recoverable in this experiment.
∅ Same genotype as that resulting from a single recombination between the <u>ad</u> mutants.

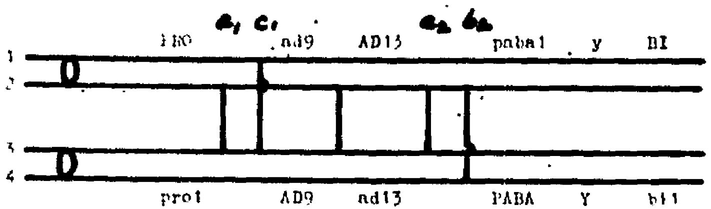
<u>The Eight Possible Types of Adenine-independent Diploids Produced by Two Mitotic Exchanges, One between ad9 and ad13 and the Other between pro - ad13 (Sequence A) or ad13 - paba (Sequence B)</u>

<u>FIG. 14</u>

the parental diploid in this analysis (see Chapter I and Table 8). Gene conversion can also probably be discounted (see discussion of previous section).

Class iii $\left(\frac{\text{pro1 AD9 AD13 PABA Y bi1}}{\text{PRO ad9 AD13 paba1 y BI}}\right)$: - The eight diploids of this type require a two- or three-strand double exchange (Fig. 14) similar to those suggested for class *ii*, excepting that (for sequence A) class *ii* is produced by a crossover in the *ad9* - *paba* region while class *iii* would result from a recombination in the *pro* - *ad13* interval. Again, reversion and gene conversion seem less likely explanations of the origin of this type of mitotic recombinant.

Classes iv $\left(\frac{\text{PRO AD9 AD13 PABA Y bi1}}{\text{pro1 AD9 ad13 paba1 y BI}}\right)$, *v* $\left(\frac{\text{PRO AD9 AD13 PABA Y bi1}}{\text{pro1 ad9 AD13 paba1 y BI}}\right)$ and *vi* $\left(\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 AD13 PABA Y bi1}}\right)$: - These three diploids are considered together because they all carry in coupling at least one recessive marker that was in repulsion in the original parental diploid, viz. *paba* and *y* in class *iv*, *pro* in class *v* and both *pro* and *bi* in class *vi*. Mutation or conversion appear to be extremely unlikely explanations for the origin of adenine independence, as both types

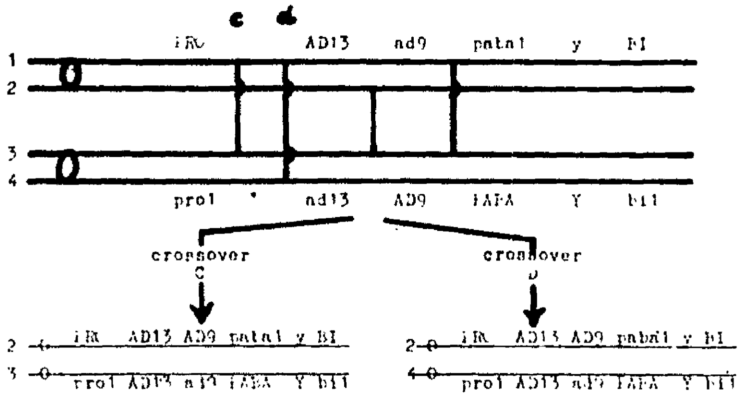


TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a_1 & b_2	2 & 4	PRO AD9 AD13 PABA Y bi1 pro1 AD9 ad13 paba1 y BI	iv
c_1 & a_2	1 & 3	PRO AD9 AD13 PABA Y bi1 pro1 ad9 AD13 paba1 y BI	v
c_1 & b_2	1 & 3	PRO AD9 AD13 PABA Y bi1 pro1 ad9 AD13 paba1 y BI	v
c_1 & b_2	1 & 4	PRO AD9 AD13 PABA Y bi1 pro1 AD9 ad13 paba1 y BI	iv

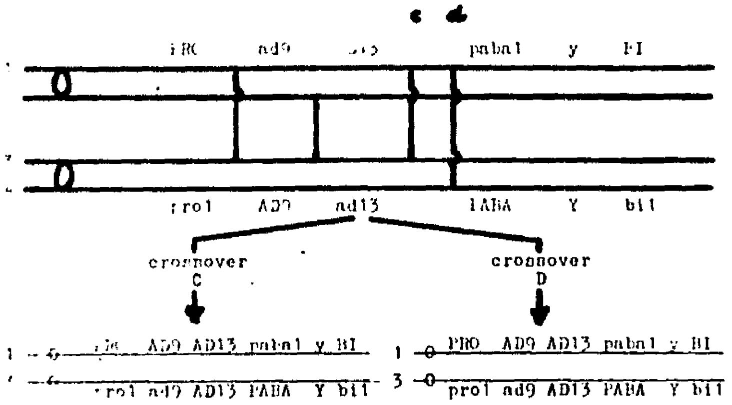
Types of Mitotic Exchanges Which Could Give Rise to Recombinant Diploids of Class iv and v (Table 14), Assuming the Linear Sequence ad9 - ad13 - paba

FIG. 15

A. Sequence ad13 - ad9 - paba



B. Sequence ad9 - ad13 - paba



Possible Mode of Origin of Class vi Diploids (Table 14)

FIG. 16

of event would require a concomitant occurrence of an unselected recombination. In the case of class vi one would have to assume either a mutation of ad13 to ad9 or coincidental reversion of ad9 and a two- or three-strand double exchange.

Class iv and v genotypes are expected from either certain types of double crossovers or triple crossovers, depending upon the ad allelic sequence. If the linear order is ad13 - ad9 - paba, class iv would result from double crossovers with the unselected exchange in the ad9 - paba1 interval (Fig. 13A) and class v from a double crossover with the unselected exchange in the pro - ad13 interval (A in Fig. 14). On the other hand, an ad9 - ad13 - paba sequence requires triple crossovers to produce these recombinant classes (Fig. 15), viz. simultaneous exchange in the pro - ad9, ad9 - ad13 and ad13 - paba intervals.

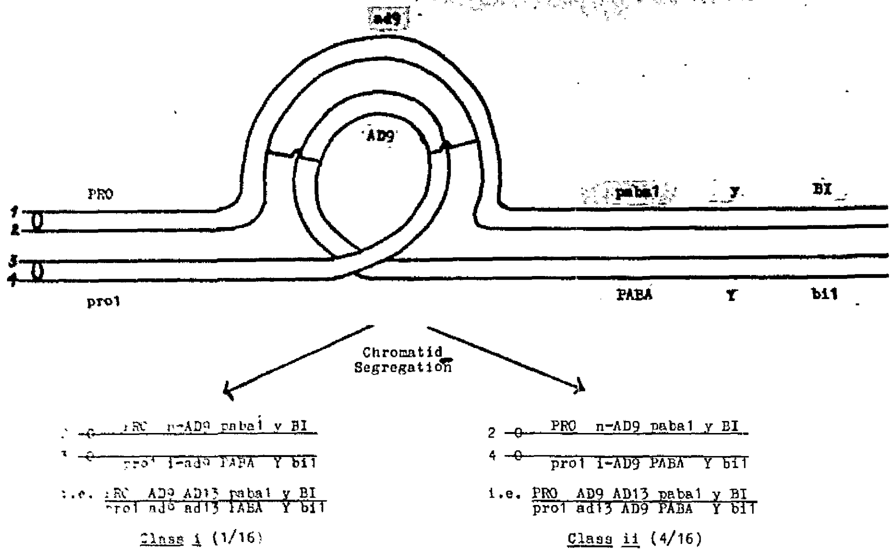
Class vi can be most simply interpreted as the consequence of a three- or four-strand triple crossover, again in the marked intervals adjacent to the selected recombination (Fig. 16).

The occurrence of these three crossover classes in a sample of sixteen diploids suggests that high negative interference, rather than a conversion phenomenon or mutation, is responsible for the observed equality of bi and paba y phenotypes in the mitotic recombination tests of the ad13 / ad9 combination.

Consideration of the following points (in addition to the ones already mentioned) would suggest that one of the chromosomes of the parental diploid contained an inversion spanning the ad9 - ad13 interval: -

1. The absence of a single-crossover class among the 16 half-tetrads analysed in this experiment,
2. The recovery of the reciprocal products of a two-strand double-crossover, viz. ad ad / + + from ad + / + ad,
3. The fact that 13 out of 16 half-tetrads could have arisen from a two-strand double crossover with the unselected recombination in an interval adjacent to the selected recombination between ad9 and ad13.

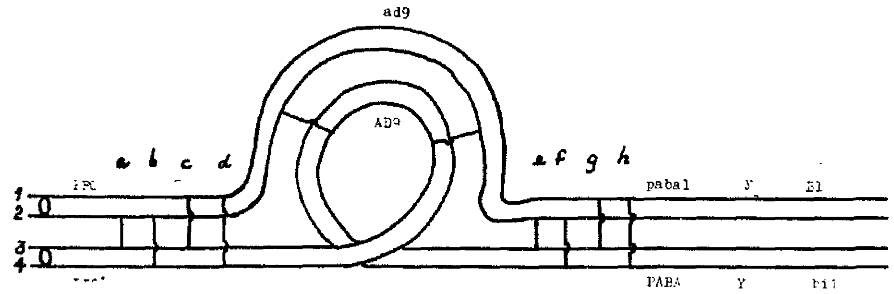
The mitotic recombination data given in Tables 12 and 13 implicates ad13, rather than ad9, as the carrier of an inversion since ad13 also gave anomalous results with ad32 and ad15.



LEGEND: i = inverted, n = non-inverted
i-AD9 = ad13, i-ad9 = ad9 ad13, n-AD9 = AD9 AD13

Origin of Adenine-independent Segregants from an Inversion Heterozygote, Where the Inversion Determines a Mutant Phenotype (See Text)

FIG. 17



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a, e	3 & 1	pro1 n-AD9 PABA Y bi1 / PRO n-ad9 paba1 y BI	iii
a, f	"	"	"
b, e	4 & 1	"	"
b, f	"	"	"
c, g	2 & 3	PRO n-AD9 paba1 y BI / pro1 n-ad9 PABA Y bi1	vi
c, h	"	"	"
d, g	2 & 4	"	"
d, h	"	"	"
d, e	2 & 4	PRO n-AD9 PABA Y bi1 / pro1 n-ad9 paba1 y BI	v
d, f	"	"	"

10/16 half-tetrads analysed

LEGEND: i = inversion, n = non-inverted
i-AD9 = ad13, i-ad9 = ad9 ad13, n-ad9 = AD9 AD13

Origin of Adenine-independent Segregants from an Inversion Heterozygote, Where the Inversion Determines a Mutant Phenotype (See Text)

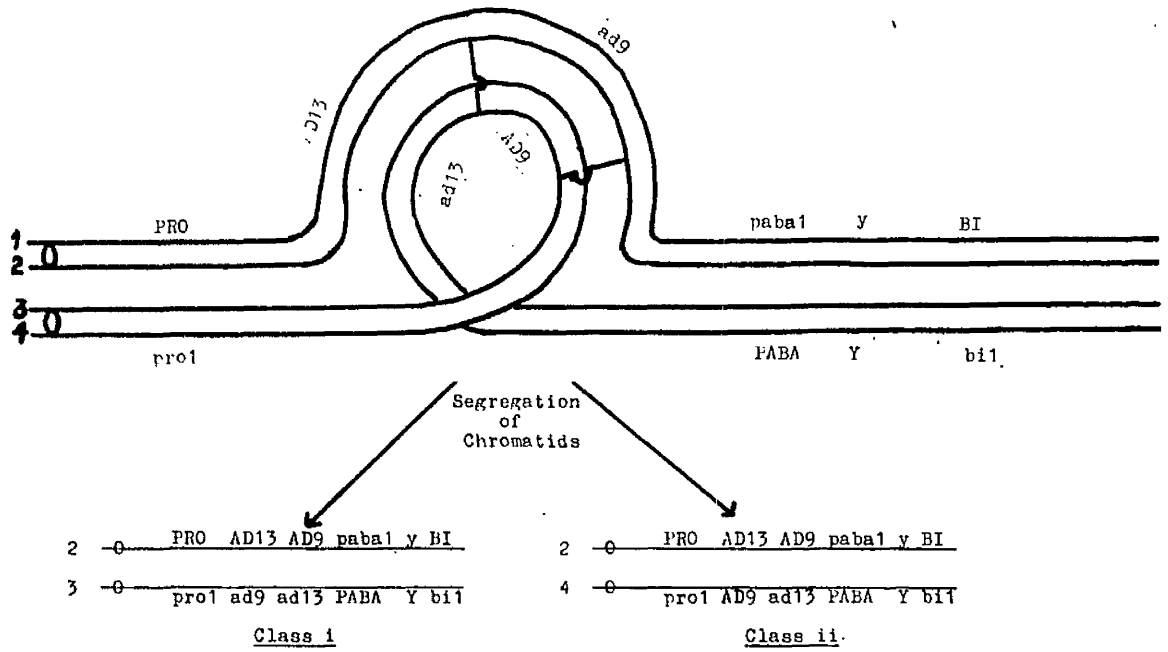
FIG. 18

The possibility that *ad13* is an inverted segment of a wild-type *ad9* cistron seems unlikely in the light of the results presented thus far: - Although classes *i* and *ii* (Table 14) would be expected to result from a two-strand double crossover within such an inversion (Fig. 17), class *iii* -- as well as classes *v* and *vi* -- would require two additional recombinations outside the inverted region (Fig. 18). The occurrence of ten such recombinants (i.e. classes *iii*, *v* and *vi*), as opposed to five double crossovers (classes *i* and *ii*) renders this scheme unlikely. It will be shown later that *ad13* can be extracted from the inversion and, therefore, that the *ad13* mutant phenotype cannot simply be due to a rearrangement in a wild-type *ad9* cistron.

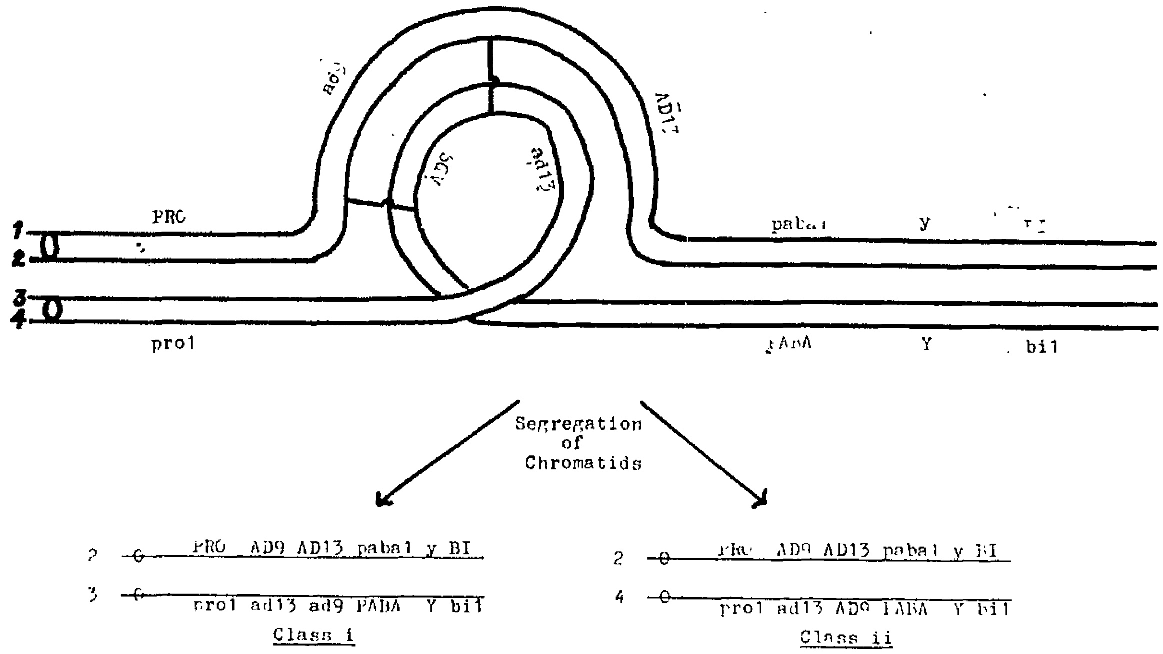
It is therefore suggested (a) that *ad13* strains carry an inversion spanning the sites of *ad13*, *ad9* and *ad32* (but not *ad17* and *ad15*, *vide infra*) and (b) *that this inversion is not in itself responsible for the mutant phenotype of ad13*.

The half-tetrad results can now be re-interpreted on this inversion hypothesis: -

A. Sequence ad13 - ad9 - paba in non-inverted strain



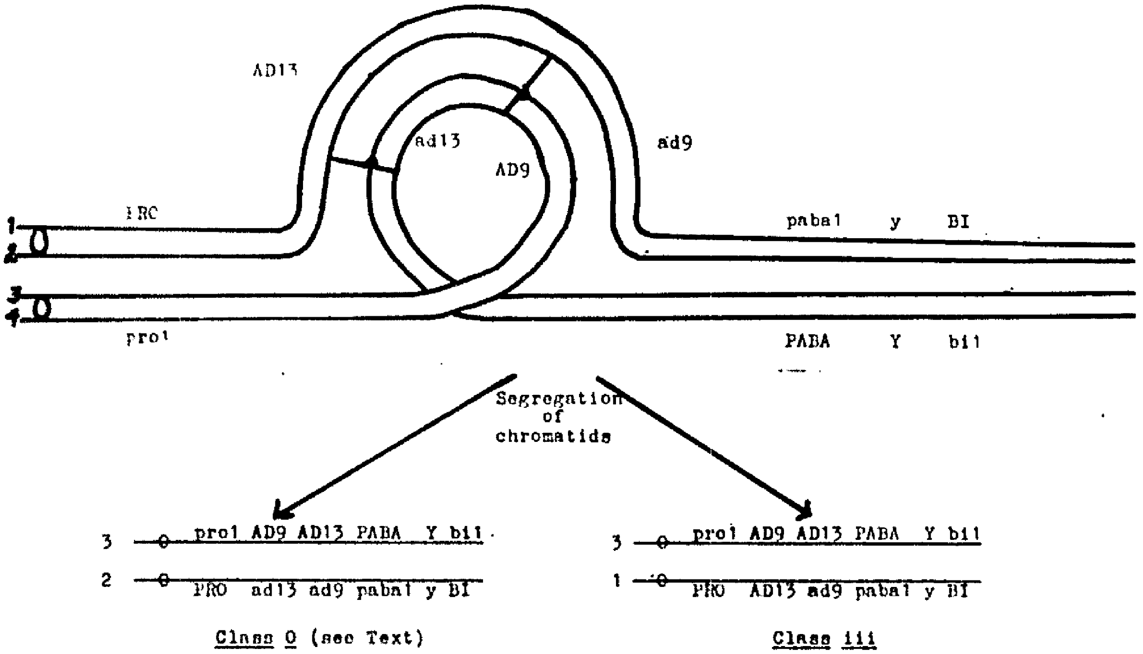
B. Sequence ad9 - ad13 - paba in non-inverted strain



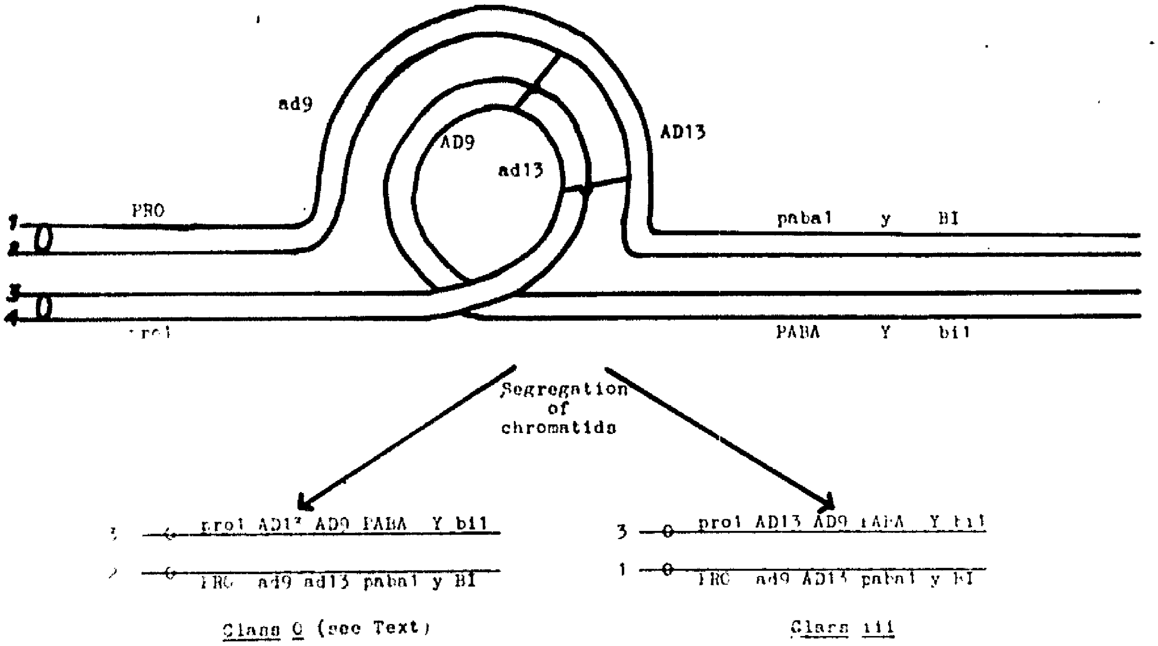
Origin of Classes i and ii from ad9 paba1 y; w3 / pro1 ad13 bi1, Assuming that the ad13 Strain Carries an Inversion (See Text)

FIG. 19

A. Sequence ad13 - ad9 - paba in non-inverted strain



B. Sequence ad9 - ad13 - paba in non-inverted strain



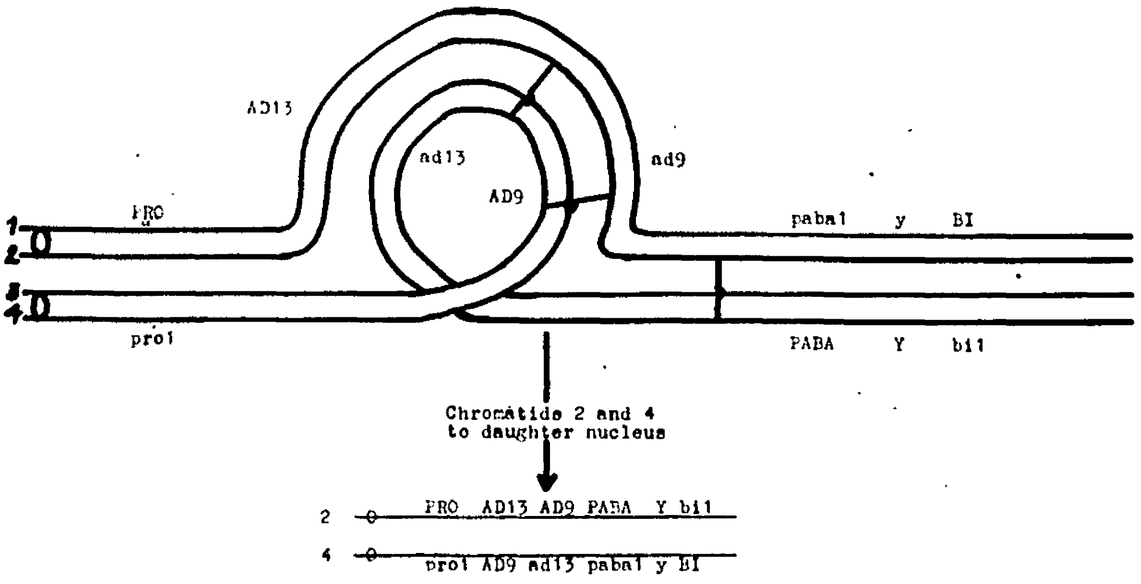
Origin of Classes "0" and iii from ad9 paba1 y; w3 / pro1 ad13 bi1, Assuming that the ad13 Strain Carries an Inversion (See Text

FIG. 20

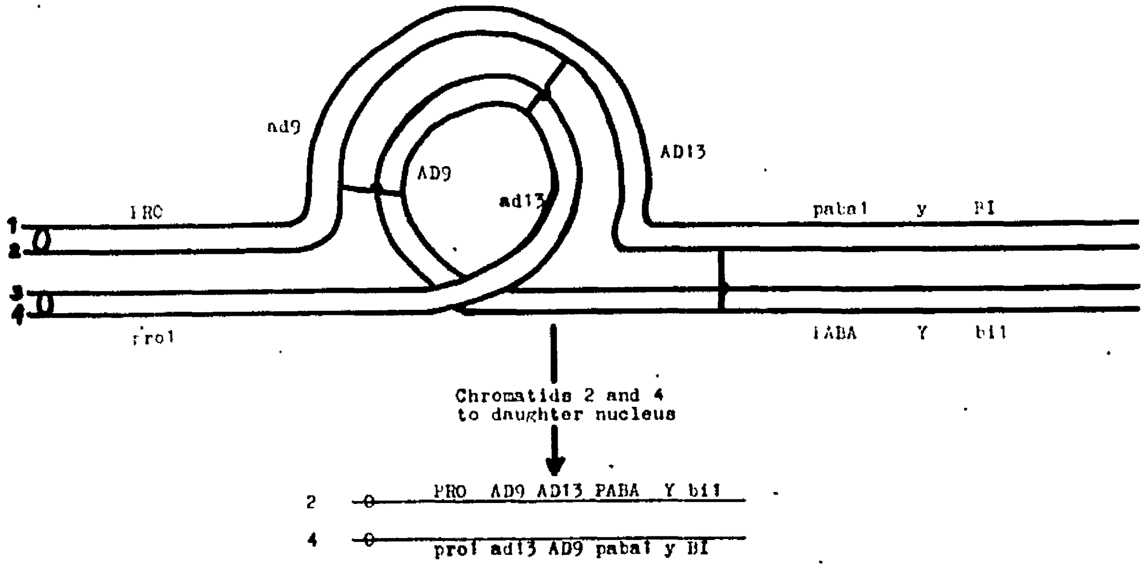
Class	No. obtained	Crossover types
i	1	2-strand double within inversion
ii	4	"
iii	8	"
iv	1	2-strand double within inversion and a 3rd crossover in *inversion* - *paba* interval
v	1	2-strand double within inversion and a 3rd crossover in *pro* - *inversion* interval
vi	1	2-strand double within inversion and 3rd & 4th crossovers in *pro* - *inversion* and *inversion* - *paba* intervals

It is predicted that class *i* carries the *ad9 ad13* strand in the inverted (symbolised by *i-*) sequence (Fig. 19) -- regardless of the allelic order -- as the result of a two-strand double recombination within the inversion. No diploids supposedly carrying the non-inverted (symbolised by *n-*) sequence of *ad9 ad13* (expected as a consequence of the crossing-over shown in Class *0* $\left\{ \text{i.e. } \frac{\text{pro1 AD9 AD13 PABA Y bi1}}{\text{PRO ad13 ad9 paba1 y BI}} \right\}$ of Figs. 14 and 20) were recovered in this experiment. Classes *ii* and *iii* would be expected to result from the recombina-

A. Sequence *ad13* - *ad9* - *paba* in non-inverted strain



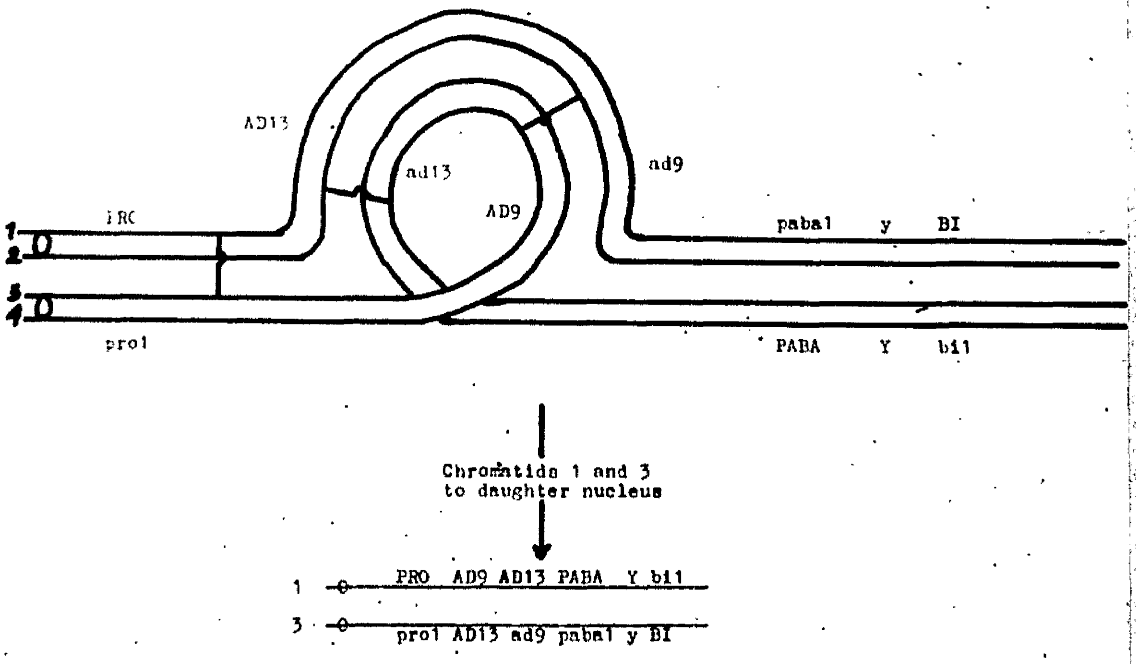
B. Sequence *ad9* - *ad13* - *paba* in non-inverted strain



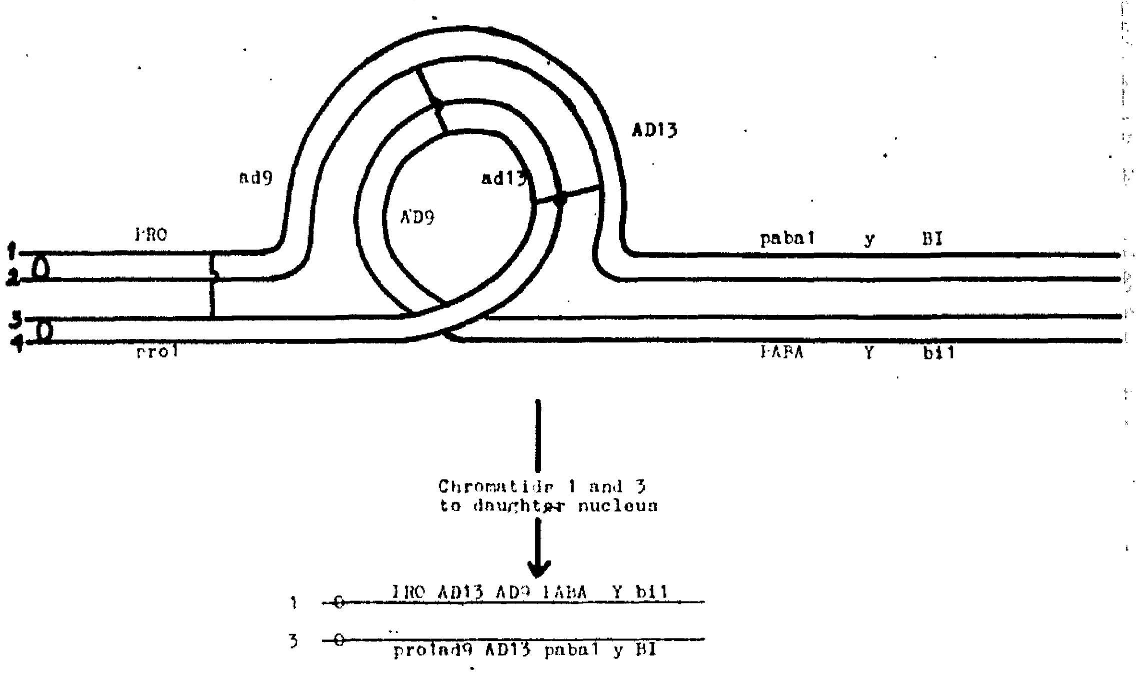
Origin of Class iv from ad9 paba1 y; w3 / pro1 ad13 bi1,
Assuming that the ad13 Strain Carries an Inversion (See Text)

FIG. 21

A. Sequence ad13 - ad9 - paba in non-inverted strain



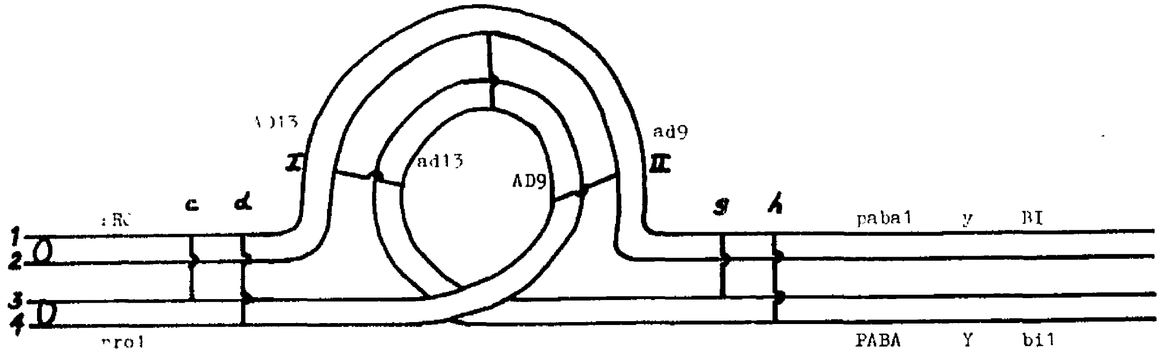
B. Sequence ad9 - ad13 - paba in non-inverted strain



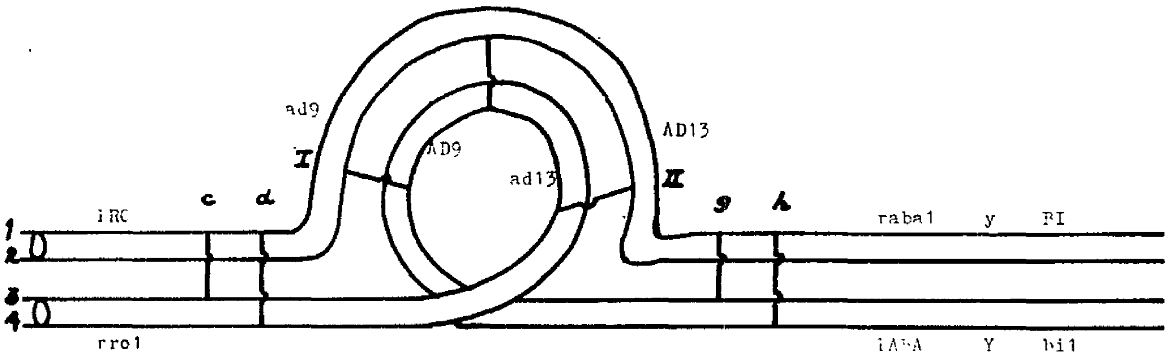
Origin of Class v from ad9 pabal y; w3 / pro1 ad13 bi1,
Assuming that the ad13 Strain Carries an Inversion (See Text)

FIG. 22

A. Sequence ad13 - ad9 - paba in non-inverted strain



B. Sequence ad9 - ad13 - paba in non-inverted strain



A Order			B Order		
TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE OF RECOMBINANT	TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE OF RECOMBINANT
I, c, g	1 & 3	PRO AD9 AD13 paba1 y BI / pro1 AD13 ad9 PABA Y bi	II, c, g	1 & 3	PRO AD13 AD9 paba1 y BI / pro1 ad9 AD13 PABA Y bi1
II, c, g	2 & 3	PRO AD13 AD9 paba1 y BI / pro1 AD13 ad9 PABA Y bi	I, c, g	2 & 3	PRO AD9 AD13 paba1 y BI / pro1 ad9 AD13 PABA Y bi1
II, c, h	"	"	I, c, h	"	"
II, d, g	2 & 4	"	I, d, g	2 & 4	"
II, d, h	"	"	I, d, h	"	"

Origin of Class vi from ad9 paba1 y; w3 / pro1 ad13 bi1, Assuming that the ad13 Strain Carries an Inversion (See Text)

FIG. 23

tion events leading to classes *i* and *O*, respectively (Figs. 19 and 20). Class *iv* (Fig. 21) and class *v* (Fig. 22) require an additional recombination in either the *pro* - *inversion* or *inversion* - *paba* intervals. Class *vi* (Fig. 23) would be the result of crossing-over in both the *pro* - *inversion* and *inversion* - *paba* intervals concomitant with a two-strand double mitotic recombination within the inversion.

(2) Thirty wild-type mitotic recombinants from the diploid

$$\frac{\text{pro1 ad9 PABA Y bi1}}{\text{PRO ad13 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{w3 acr2}}$$

(reciprocal of 1, p. 74) were haploidised (Table 15) in an endeavour to obtain a haploid of the constitution

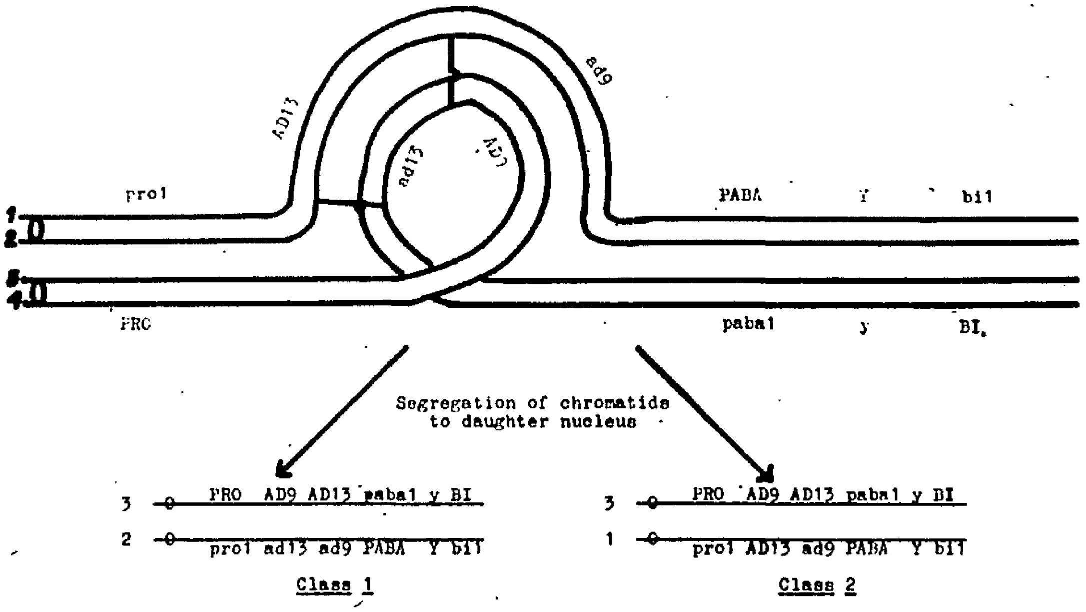
pro1 n-ad13 ad9 PABA Y bi1

for use in the further elucidation of the *ad13* inversion.

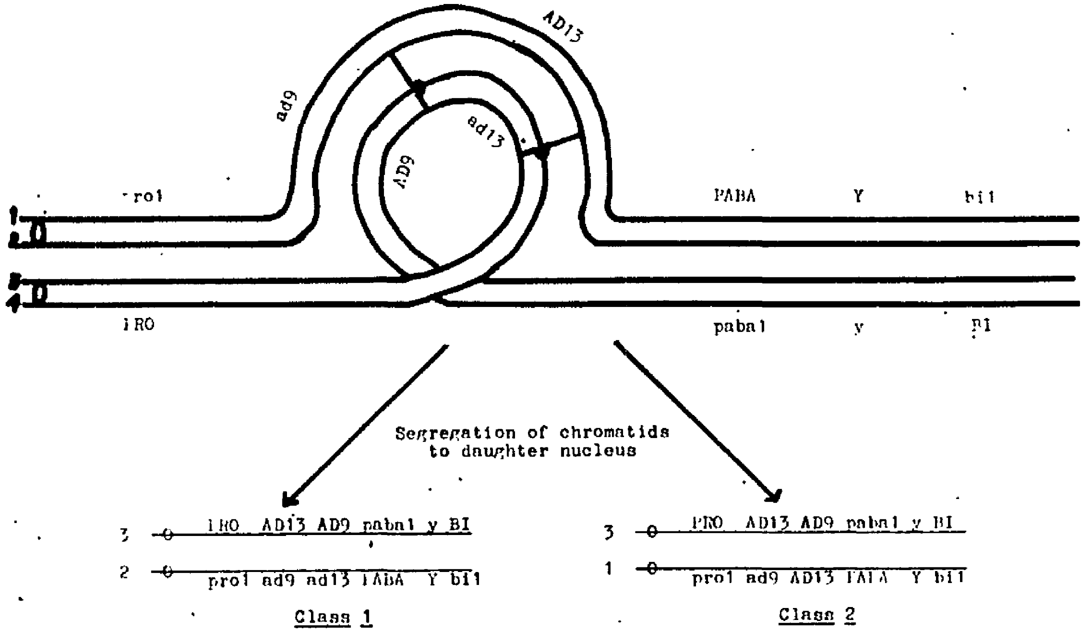
The *ad* genotypes of only the *pro1 ad bi1* / *paba1 y* group were determined (Table 16), since on the inversion hypothesis *n-ad13 ad9* / *AD9 AD13* should have that arrangement of outside markers (cf. Class O of diploid 1, Figs. 14 and 20).

Classes 1 $\left(\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 ad13 PABA Y bi1}}\right)$ and *2* $\left(\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 AD13 PABA Y bi1}}\right)$: - The genotypes of these

A. Sequence _ad13_ - _ad9_ - _paba_ in non-inverted strain



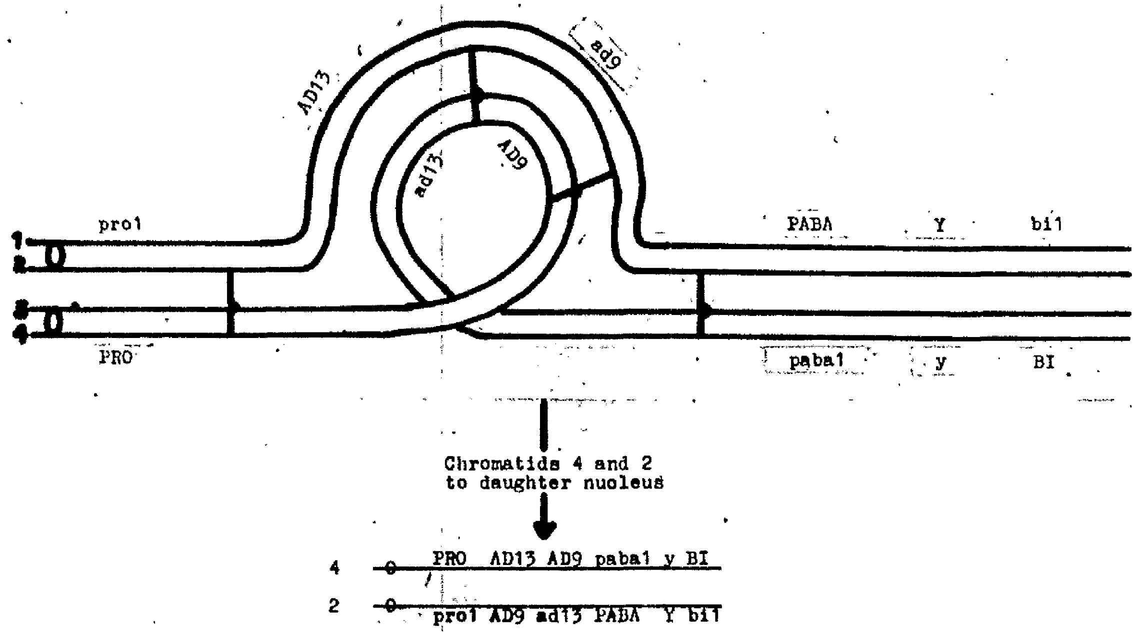
B. Sequence _ad9_ - _ad13_ - _paba_ in non-inverted strain



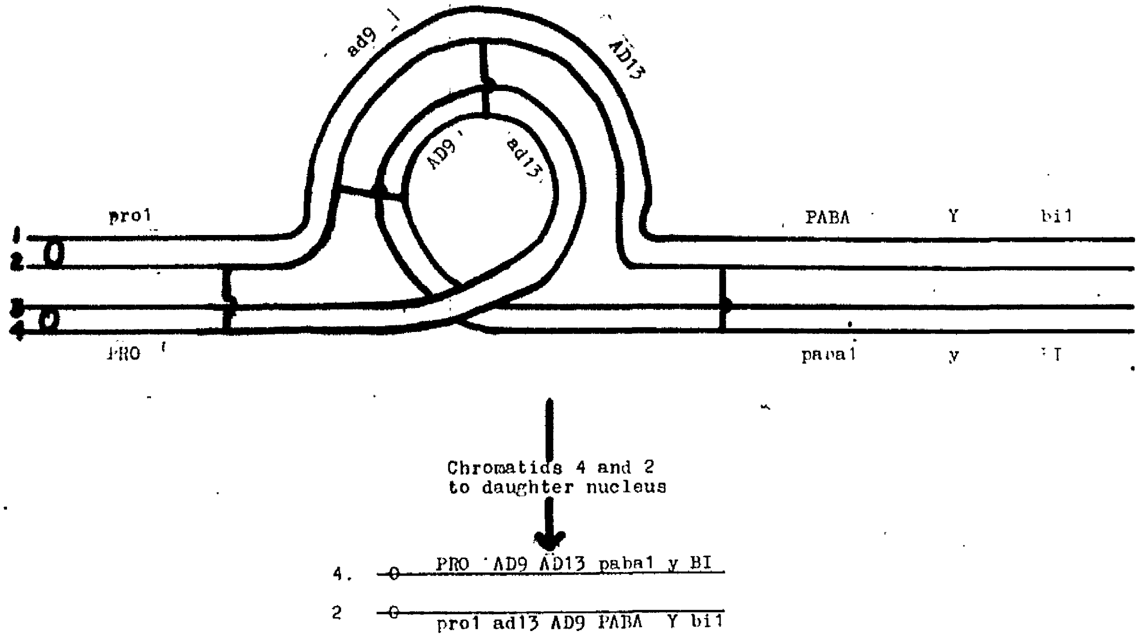
Origin of Classes 1 and 2 from pro1 ad9 bi1 / ad13 paba1 y; acr2 w3, Assuming the _ad13_ Strain Carries an Inversion (See Text)

FIG. 24

A. Sequence ad13 - ad9 - paba in non-inverted strain



B. Sequence ad9 - ad13 - paba in non-inverted strain



Origin of Class 3 from pro1 ad9 bi1 / ad13 paba1 y; acr2 w3,
Assuming the ad13 Strain Carries an Inversion (See Text)

FIG. 25

diploids would be expected as the result of a double crossover within the inversion, followed by segregation of both crossover chromatids to the same daughter nucleus in the case of class *1* (Fig. 24, cf. Fig. 20, class *0*) or of the *AD9 AD13* recombinant strand with a non-crossover strand to the same daughter nucleus in the case of class *2* (Fig. 24, cf. Fig. 20, class *iii*). While making no assumptions with regard to either the normal or inverted order of *ad* sites it is predicted that class *1* has *ad13-ad9* in the non-inverted order and that both classes have the *AD9 AD13* sites in the inverted sequence.

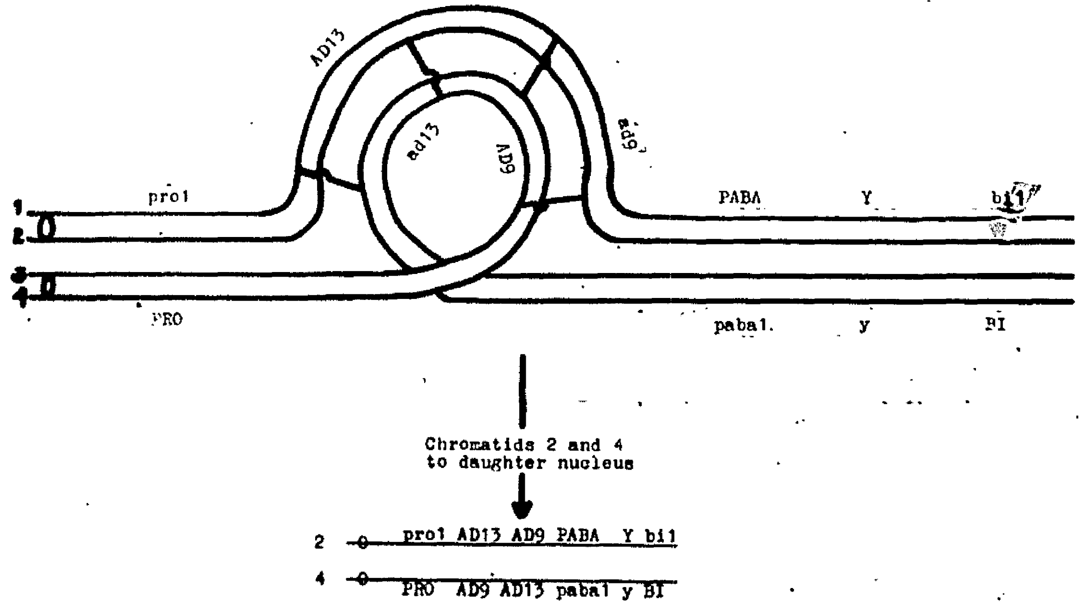
Class 3 (Table 16) $\left(\dfrac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 AD9 ad13 PABA Y bi1}}\right)$: -

Simultaneous crossing-over in the *pro* - *inversion* and *inversion* - *paba* intervals, coincident with a double recombination within the inversion, must be invoked to explain the origin of this mitotic recombinant (Fig. 25). Class *3* is similar to class *vi* of the reciprocal parental diploid (Table 14) in that the *ad* mutant originally in coupling (in the parental diploid) with the markers

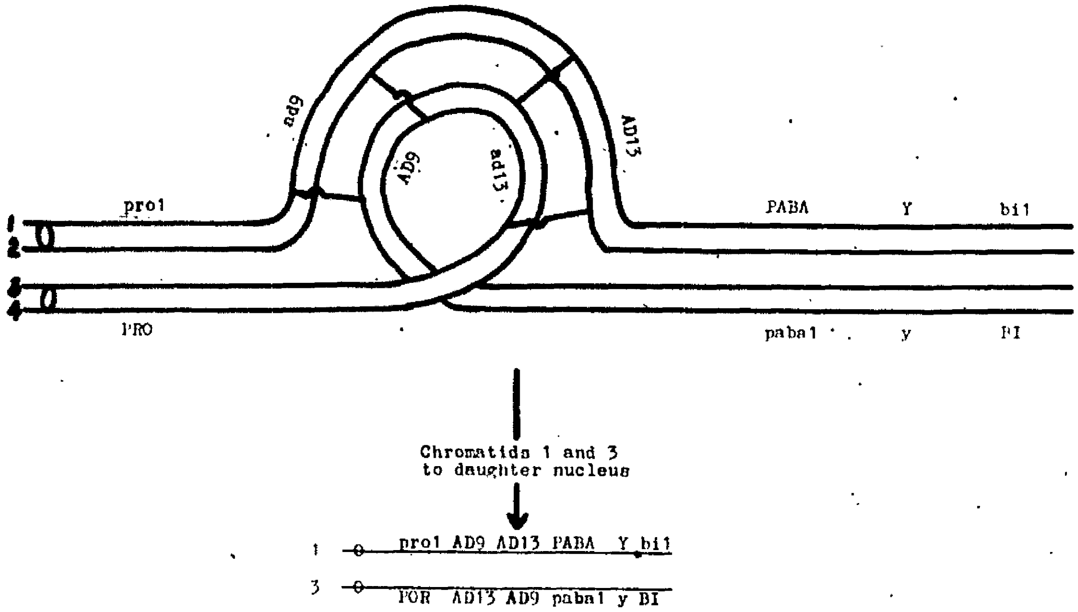
PRO paba1 y BI

is recovered (in the recombinant diploid) in repulsion of these markers.

A. Sequence ad13 - ad9 - paba in non-inverted strain



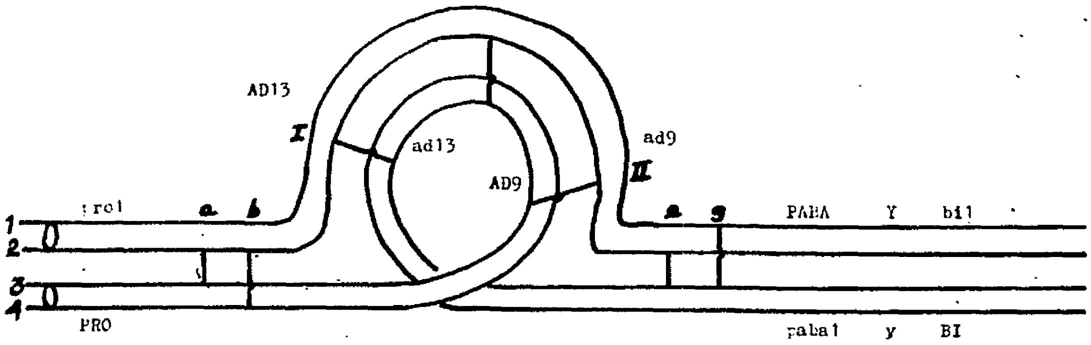
B. Sequence ad9 - ad13 - paba in non-inverted strain



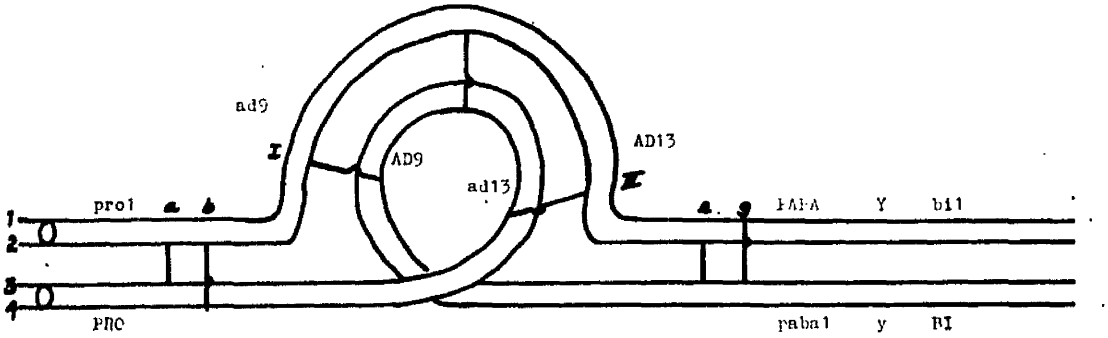
Possible Recombinational Origin of Class F (Table 15) from pro1 ad9 bi1 / ad13 paba1 y; acr1 w^3, Assuming the ad13 Strain Carries an Inversion (See Text)

FIG. 26

A. Sequence ad13 - ad9 - paba in non-inverted strain



B. Sequence ad9 - ad13 - paba in non-inverted strain



A Order			B Order		
TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE OF RECOMBINANT	TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE OF RECOMBINANT
I, a	3 & 2	PRO AD9 AD13 PABA Y bi1 / pro1 ad13 ad9 paba1 y BI	a, I	3 & 2	PRO AD9 AD13 PABA Y bi1 / pro1 ad13 ad9 paba1 y BI
I, b	3 & 1	PRO AD9 AD13 PABA Y bi1 / pro1 AD13 ad9 paba1 y BI	b, I	4 & 2	PRO AD9 AD13 PABA Y bi1 / pro1 ad13 AD9 paba1 y BI
a, II	3 & 2	PRO AD13 AD9 PABA Y bi1 / pro1 ad9 ad13 paba1 y BI	II, a	3 & 2	PRO AD13 AD9 PABA Y bi1 / pro1 ad9 ad13 paba1 y BI
b, II	4 & 2	PRO AD13 AD9 PABA Y bi1 / pro1 AD9 ad13 paba1 y BI	II, b	3 & 1	PRO AD13 AD9 PABA Y bi1 / pro1 ad9 AD13 paba1 y BI

Possible Origin of Group E Recombinants (Table 15) from pro1 ad9 bi1 / ad13 paba1 y; acr1 w3, Assuming the ad13 Strain Carries an Inversion (See Text)

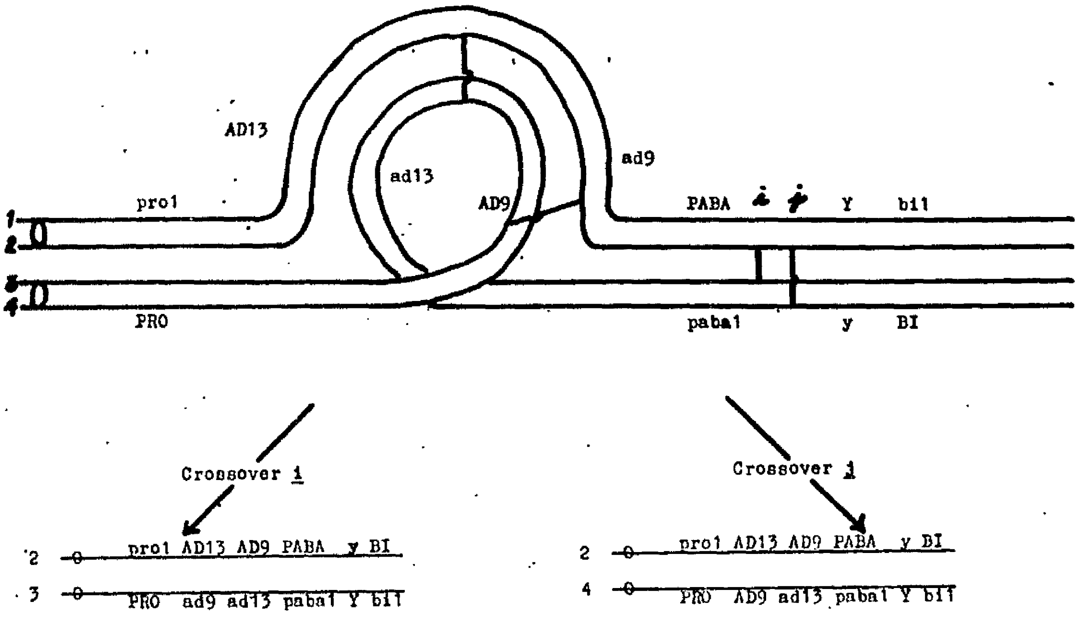
FIG. 27

Group F (Table 15) $\left(\frac{\text{PRO AD paba1 y BI}}{\text{pro1 AD PABA Y bi1}}\right)$: - This diploid has no ad mutant at all. Either simultaneous reversion of both ad9 and ad13, or either ad9 or ad13 coincident with a double crossover within the inversion, are required to explain a diploid of this genotype unless one accepts the possibility that four mitotic exchanges occurred within the inversion, two of which must have been between the ad alleles (Fig. 26).

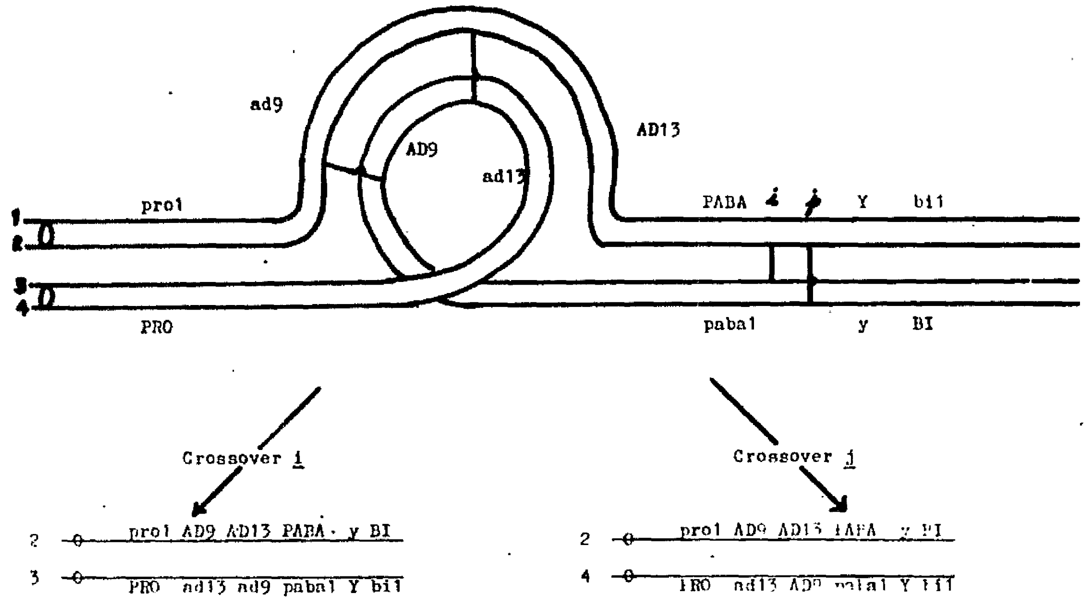
Groups B $\left(\frac{\text{pro1 AD PABA Y bi1}}{\text{PRO ad paba1 y BI}}\right)$, C $\left(\frac{\text{pro1 AD paba1 y BI}}{\text{PRO ad PABA Y bi1}}\right)$, D $\left(\frac{\text{pro1 AD PABA y BI}}{\text{PRO ad paba1 Y bi1}}\right)$ and E $\left(\frac{\text{PRO AD PABA Y bi1}}{\text{pro1 ad paba1 y BI}}\right)$ (Table 15) : - Since the ad genotypes of these groups were not determined it is possible only to suggest that (1) group B corresponds to classes i, ii and vi of the reciprocal diploid (cf. Fig. 19), (2) group C could represent triple crossovers of the class iv and v types (cf. Figs. 21 and 22), (3) group E probably results from the concomitant occurrence of three mitotic recombinations (Fig. 27) and (4) group D corresponds to the triple crossover type shown in Fig. 28.

It should be noted that group D requires a recom-

A. Sequence ad13 - ad9 - paba in non-inverted strain



B. Sequence ad9 - ad13 - paba in non-inverted strain



Probable Origin of Group D Recombinants (Table 15) from pro1 ad9 bi1 / ad13 paba1 y; [illegible] w3, Assuming the ad13 Strain Carries an Inversion (See Text)

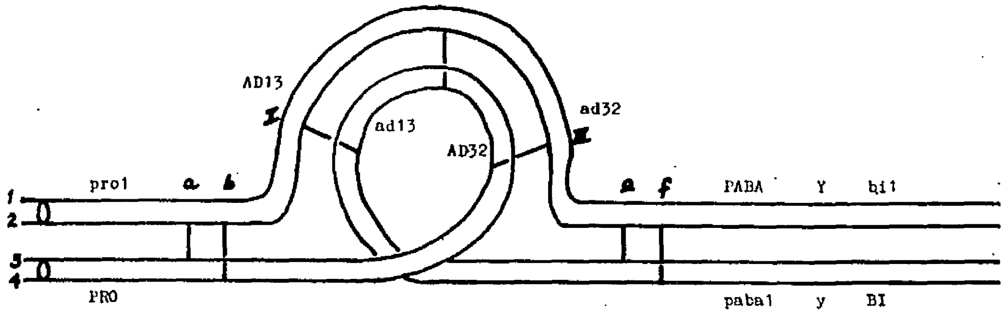
FIG. 28

bination in the paba - y interval and is the only recombinant thus far encountered that arose from a crossing-over in an interval not adjacent to the inversion.

The foregoing interpretation of the half-tetrad analysis of diploid (2) can be summarised as follows: -

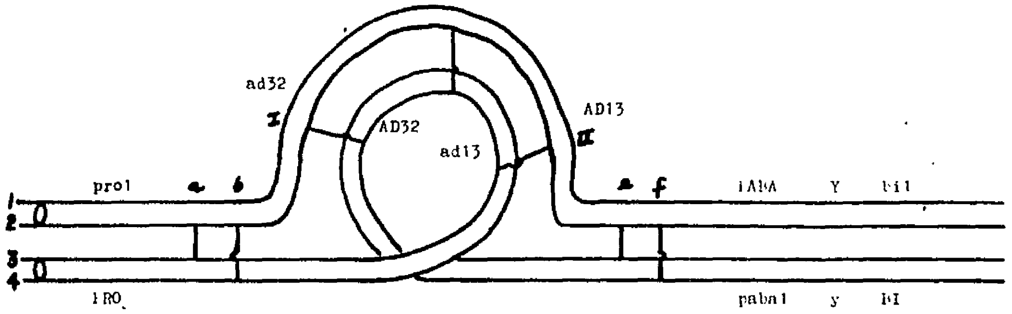
Class or group	No. obtained	Crossover types
1	1	2-strand double within inversion
2	10	"
B	10	" or a quadruple:- 2-strand double within inversion and 3rd & 4th crossovers in pro - inversion and inversion - paba intervals
C	4	Triple:- 2-strand double within inversion and a 3rd crossover in either pro - inversion or inversion - paba intervals
D	1	Triple:- 2-strand double within inversion and a 3rd crossover in paba - y interval
E	2	Triple:- 2-strand double within inversion and a 3rd crossover in either pro - inversion or inversion - paba intervals
3	1	Quadruple:- 2-strand double within inversion and 3rd & 4th crossovers in pro - inversion and inversion - paba intervals
F	1	Quadruple:- 2 2-strand doubles within inversion with each double having a crossover between the ad sites.

A. Sequence ad13 - ad32 - paba in non-inverted strain



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a, I	2 & 3	pro1 AD32 AD13 paba1 y BI PRO ad13 ad32 PABA Y bi1	v
II, e	2 & 3	pro1 AD13 AD32 paba1 y BI PRO ad32 ad13 PABA Y bi1	v
II, f	2 & 4	pro1 AD13 AD32 paba1 y BI PRO AD32 ad13 PABA Y bi1	vi
II, b	4 & 2	PRO AD13 AD32 PABA Y bi1 pro1 AD32 ad13 paba1 y BI	vii

B. Sequence ad32 - ad13 - paba in non-inverted strain



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a, II	2 & 3	pro1 AD13 AD32 paba1 y BI PRO ad32 ad13 PABA Y bi1	v
I, e	2 & 3	pro1 AD32 AD13 paba1 y BI PRO ad13 ad32 PABA Y bi1	v
I, f	2 & 4	pro1 AD32 AD13 paba1 y BI PRO ad13 AD32 PABA Y bi1	vi
I, I	4 & 2	PRO AD32 AD13 PABA Y bi1 pro1 ad13 AD32 paba1 y BI	vii

Origin of Class v, vi and vii Mitotic Recombinants from pro1 ad32 bi1 / ad13 paba1 y; acr2 w3, Assuming that the ad13 Strain Carries an Inversion (See Text)

FIG. 29

b. *ad13 / ad32*

(1) A half-tetrad analysis was made of thirty-three wild-type recombinants isolated from the diploid

$$\frac{\text{pro1 ad32 PABA Y bi1}}{\text{PRO ad13 paba1 y BI}}\ \frac{\text{W ACR}}{\text{w3 acr2}}\ .$$

Table 17 lists the ten different genotypes discerned and the number of recombinants found in each class. Six diploids possessed an *ad13 ad32* strand, viz.

$$\text{class } \underline{\text{i}}\ \left(\frac{\text{pro1 AD13 AD32 PABA Y bi1}}{\text{PRO ad13 ad32 paba1 y BI}}\right)^{*}\ ,$$

$$\text{class } \underline{\text{iii}}\ \left(\frac{\text{PRO AD13 AD32 paba1 y BI}}{\text{pro1 ad13 ad32 PABA Y bi1}}\right)\ \text{and}$$

$$\text{class } \underline{\text{v}}\ \left(\frac{\text{pro1 AD13 AD32 paba1 y BI}}{\text{PRO ad13 ad32 PABA Y bi1}}\right)\ .$$

The class *v* recombinants would be expected as the result of a single mitotic recombination between *ad13* and *ad32* in the absence of an inversion of the type proposed for *ad13* strains and would indicate the allelic sequence *ad13* - *ad32* - *paba*. However, it is suggested that this class represents certain types of triple crossovers (Fig. 29).

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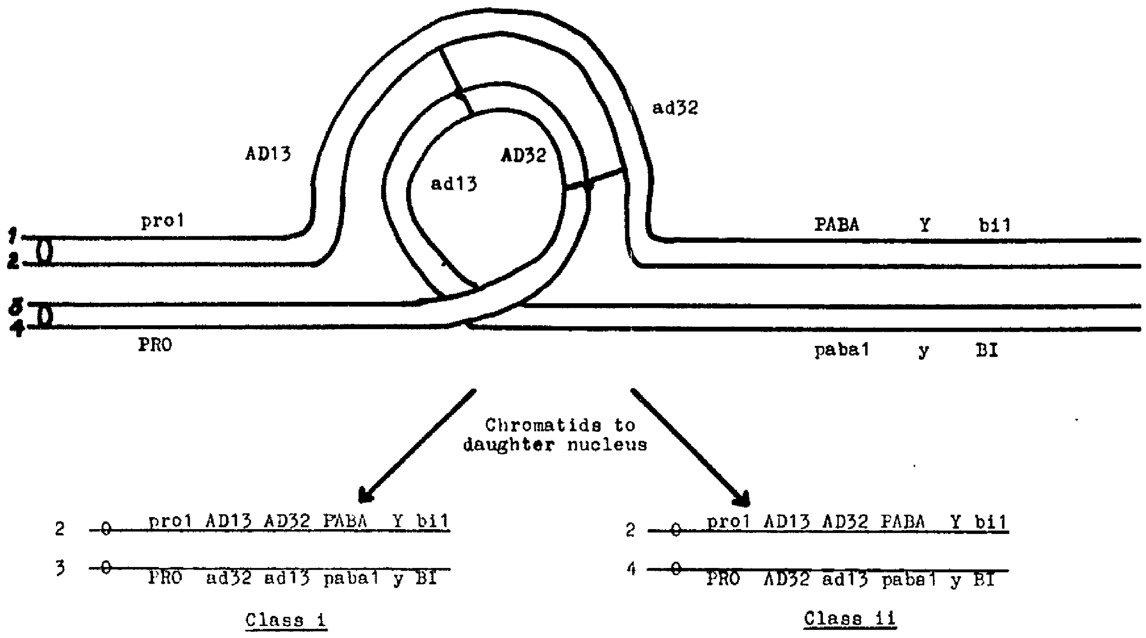
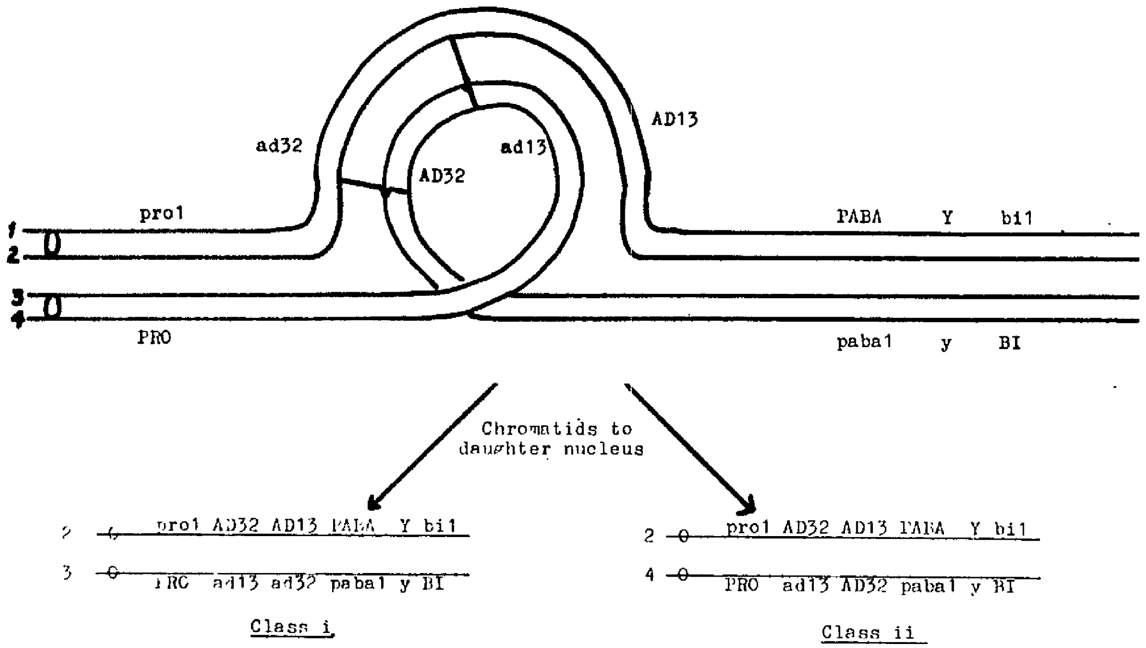
* The sequence of *ad* alleles is arbitrarily shown here to be *ad13* - *ad32*.

A. Sequence ad 13 - ad32 - paba in non-inverted strain



B. Sequence ad32 - ad13 - paba in non-inverted strain



Origin of Class i and ii Mitotic Recombinants from
pro1 ad32 bi1 / ad13 paba1 y; acr2 w3, Assuming that the ad13 Strain Carries an Inversion
(See Text)

FIG. 30

The possible origin of each genotype will be considered in turn on the basis of an *ad13* inversion spanning the sites of *ad13* and *ad32*.

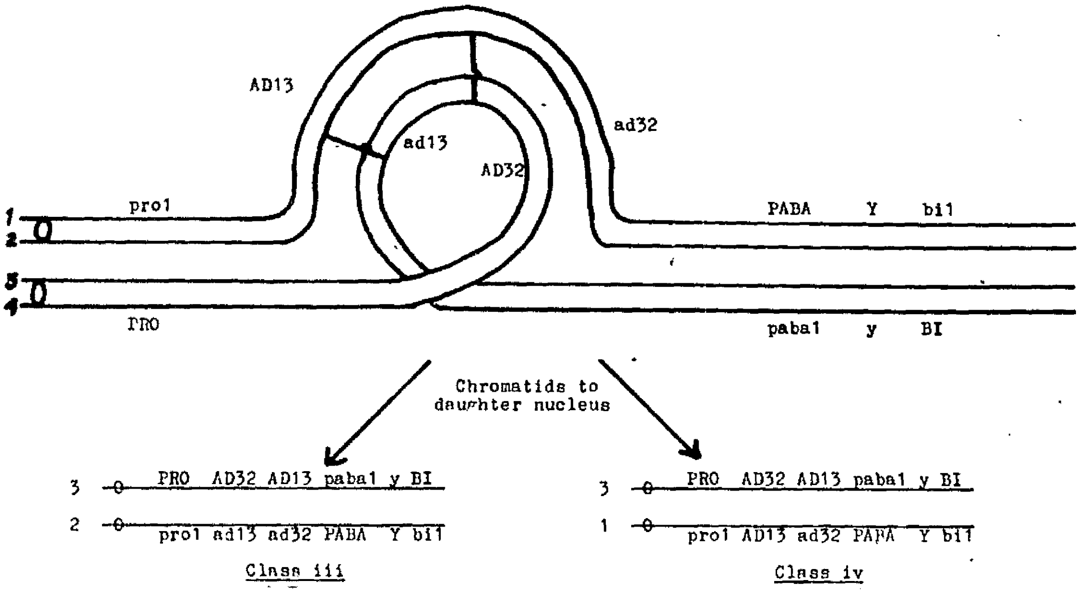
Class i $\left(\frac{\text{pro1 AD13 AD32 PABA Y bi1}}{\text{PRO ad13 ad32 paba1 y BI}}\right)$: - This mitotic recombinant would be the result of a two-strand double crossover within the inversion, followed by segregation of the two crossover chromatids into the same daughter nucleus, giving rise to a double *ad* mutant strand in the inverted sequence (Fig. 30).

Class ii $\left(\frac{\text{pro1 AD13 AD32 PABA Y bi1}}{\text{PRO ad13 AD32 paba1 y BI}}\right)$: - The segregation of a recombinant and a non-recombinant chromatid following the same type of crossing-over as for class *i* would produce the 12 diploids of this genotype (Fig. 30).

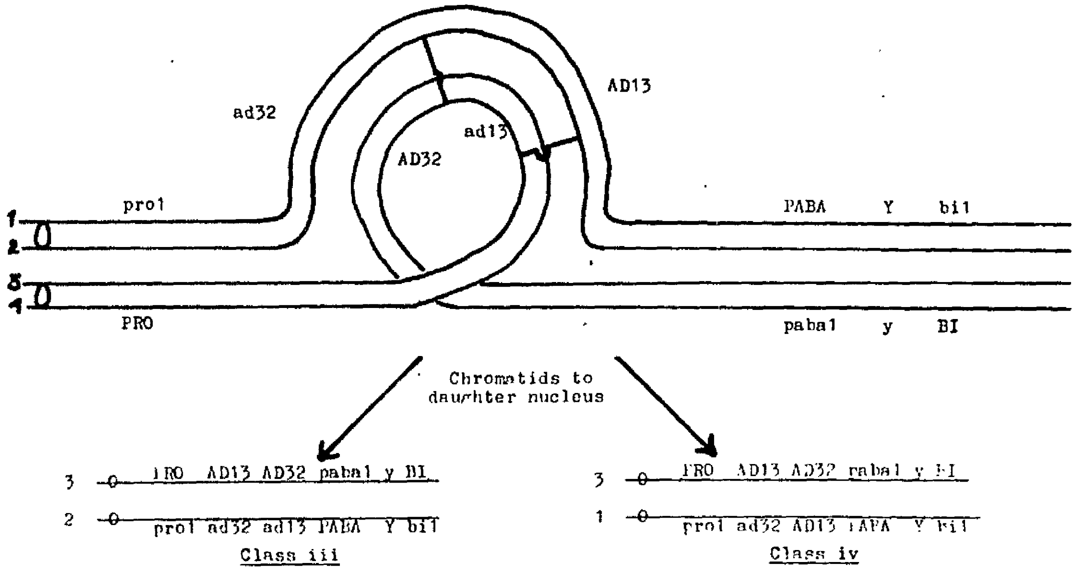
Class iii $\left(\frac{\text{PRO AD13 AD32 paba1 y BI}}{\text{pro1 ad13 ad32 PABA Y bi1}}\right)$: - This diploid carries both crossover strands of a two-strand double mitotic recombination (Fig. 31). According to the inversion hypothesis, the *ad* sites should be in the non-inverted sequence in the *ad13 ad32* strand and in the inverted order in the *AD32 AD13* strand.

Class iv $\left(\frac{\text{PRO AD13 AD32 paba1 y BI}}{\text{pro1 AD13 ad32 PABA Y bi1}}\right)$: - These

A. Sequence ad13 - ad32 - paba in non-inverted strain



B. Sequence ad32 - ad13 - paba in non-inverted strain



Origin of Class iii and iv Mitotic Recombinants from
pro1 ad32 bi1 / ad13 paba1 y; acr2 w3, Assuming that the ad13 Strain Carries an Inversion.
(See Text)

FIG. 31

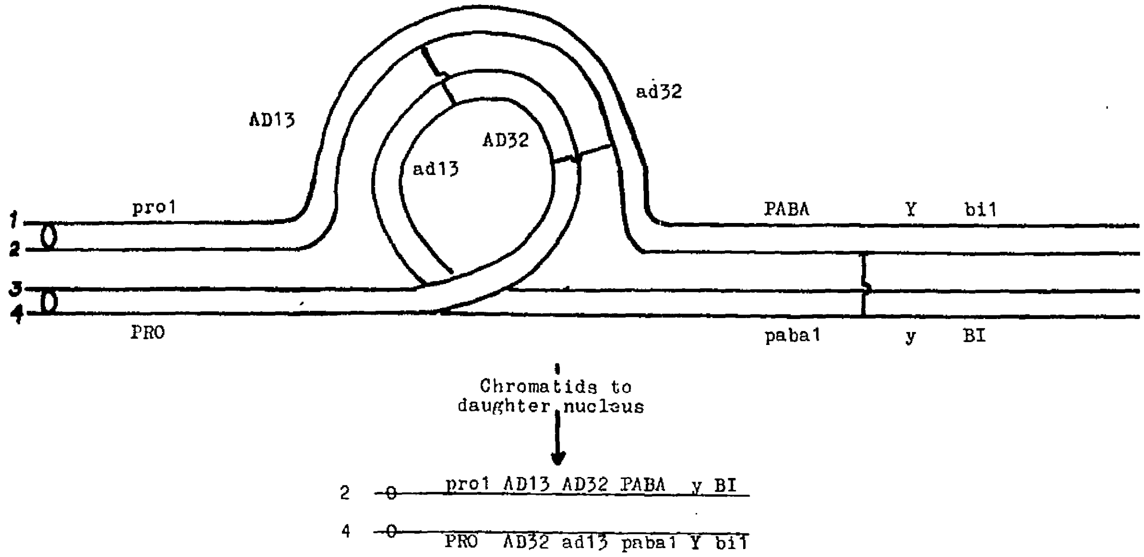
10 recombinants carry one crossover and one non-crossover strand from the same type of mitotic recombination postulated for class *iii* (Fig. 31).

Classes v $\frac{(\text{pro1 AD13 AD32 paba1 y BI})}{(\text{PRO ad13 ad32 PABA Y bi1})}$,

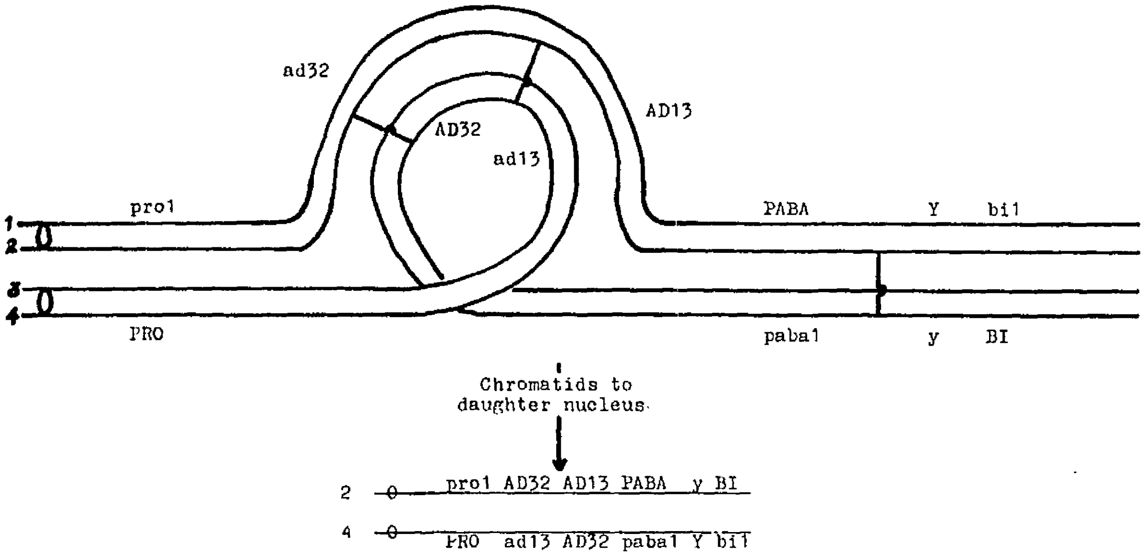
vi $\frac{(\text{pro1 AD13 AD32 paba1 y BI})}{(\text{PRO ad13 AD32 PABA Y bi1})}$ and *vii*

$\frac{(\text{PRO AD13 AD32 PABA Y bi1})}{(\text{pro1 ad13 AD32 paba1 y BI})}$: - The probable origin of these diploids is given in Fig. 29. The four class *v* diploids could arise from a three-strand triple crossover, with the recombination outside the inversion occurring in either the *pro* - *inversion* or *inversion* - *paba* intervals. The class *vi* recombinant would be expected as the result of one type of three-strand triple crossover with the third recombination in the *inversion* - *paba* interval. For the class *vii* half-tetrad, the crossover outside the inversion would be in the *pro* - *inversion* region. It is expected that class *v* would occur as frequently as classes *vi* + *vii*, assuming no chromatid interference and random chromatid segregation. In fact, four class *v* diploids were recovered and two class *vi* + *vii* (the difference is not significant). This argues against class *v* representing single crossovers (see p.85), since in that case one would expect to find many fewer triple

A. Sequence ad13 - ad32 - paba in non-inverted strain



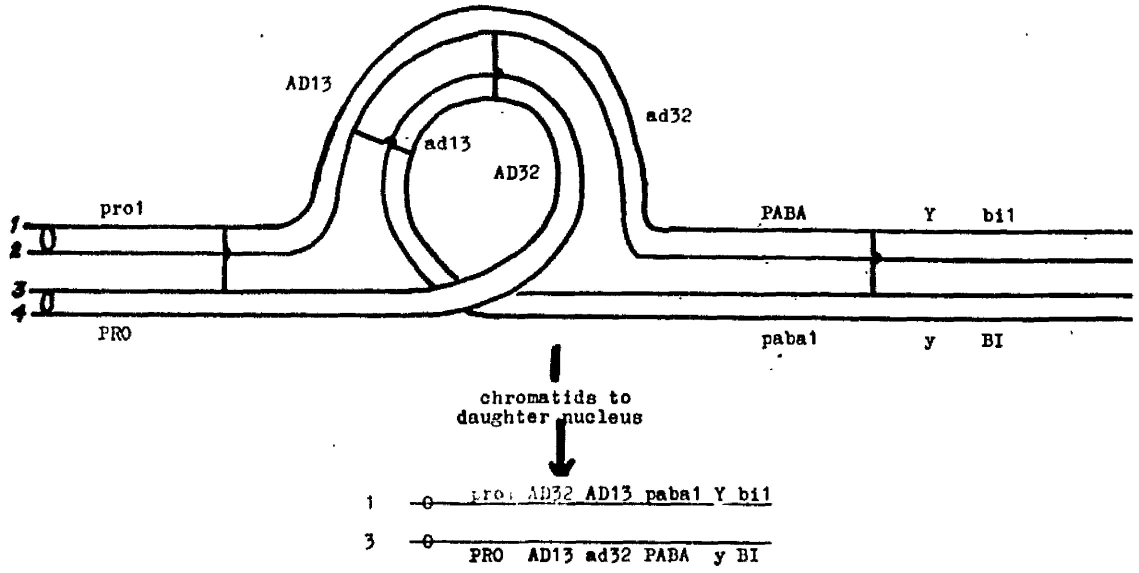
B. Sequence ad32 - ad13 - paba in non-inverted strain



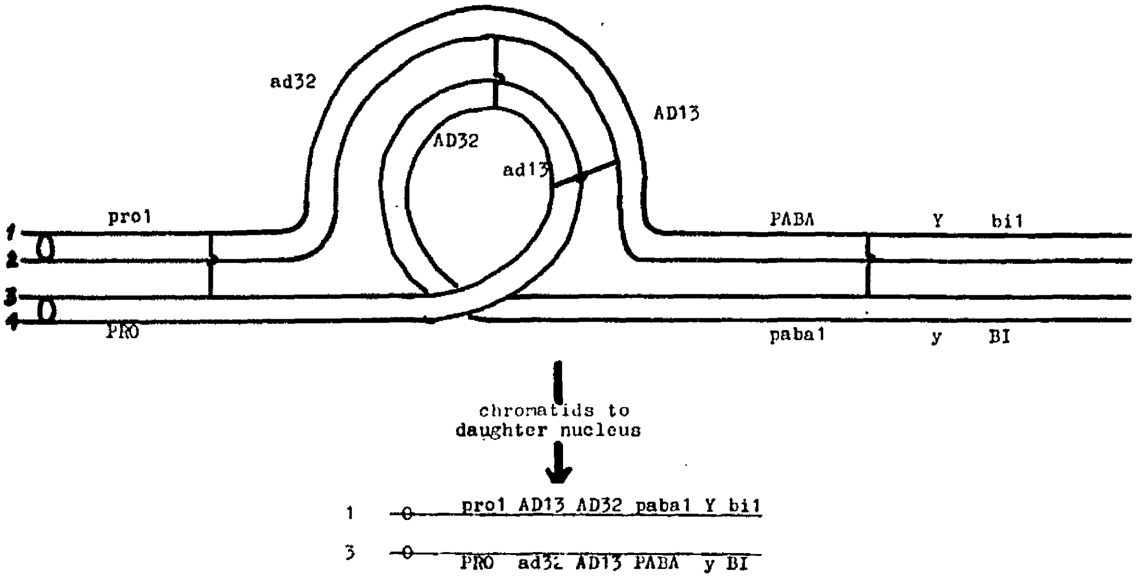
Origin of Class viii Mitotic Recombinant from
pro1 ad32 bi1 / ad13 paba1 y; acr2 w3, Assuming that the ad13 Strain Carries an Inversion
(See Text)

FIG. 32

A. Sequence ad13 - ad32 - paba in non-inverted strain



B. Sequence ad32 - ad13 - paba in non-inverted strain



Origin of the Class ix Diploid from pro1 ad32 bi1 / ad13 paba1 y; acr2 w3,
Assuming the ad13 Strain Carries an Inversion (See Text)

FIG. 33

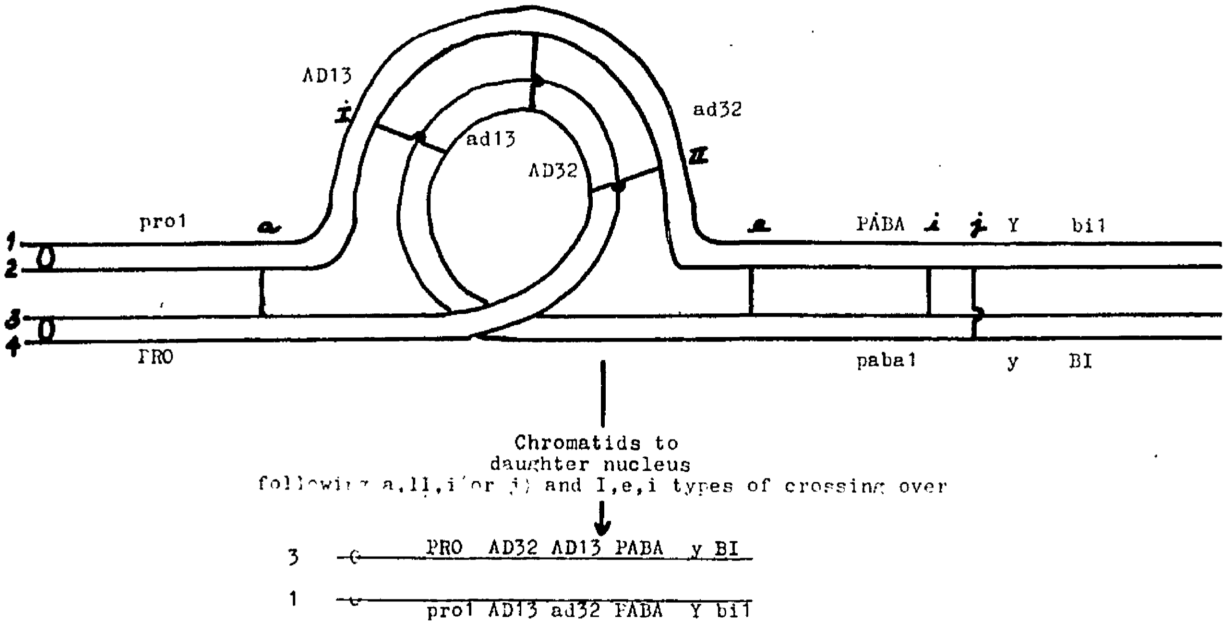
recombinants (i.e. classes *vi* and *vii*) than singles.

Classes viii $\left(\frac{\text{pro1 AD13 AD32 PABA y BI}}{\text{PRO ad13 AD32 paba1 Y bi1}}\right)$ *and ix* $\left(\frac{\text{pro1 AD13 AD32 paba1 Y bi1}}{\text{PRO AD13 ad32 PABA y BI}}\right)$: - Both these mitotic recombinants require a crossover in the *paba* - *y* interval. Class *viii* would be the consequence of a triple recombination of the type shown in Fig. 32, and class *ix* would arise following four mitotic recombinations (Fig. 33).

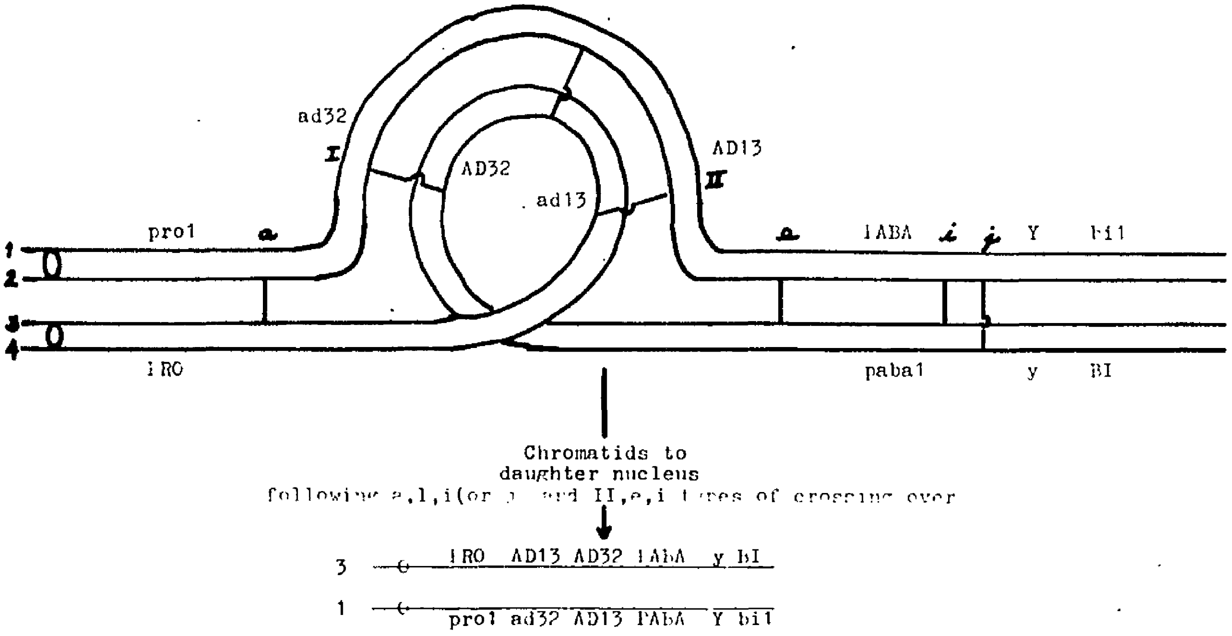
Class x $\left(\frac{\text{PRO AD13 AD32 PABA y BI}}{\text{pro1 AD13 ad32 PABA Y bi1}}\right)$: - The extraordinary aspect of this genotype is its homozygosis for *PABA*. Reversion of *paba1* accompanied by a class *iv* type recombination and segregation (Fig. 31) would produce this diploid, although such a coincidence of an *un*selected reversion with a selected double mitotic recombination seems highly unlikely. Alternatively, recombination within the inversion, in the *pro* - *inversion* (or *inversion* - *paba*) and *paba* - *y* intervals must be invoked to explain the origin of this class (Fig. 34).

The recombinant diploids recovered in this experiment are classified according to number of crossovers in Table 18. It should be noted that, for double

A. Sequence <u>ad13</u> - <u>ad32</u> - <u>paba</u> in non-inverted strain



B. Sequence <u>ad32</u> - <u>ad13</u> - <u>paba</u> in non-inverted strain



Origin of the Class x Diploid from pro1 ad13 bi1 / ad32 paba1 ; [illegible] Assuming the ad13 Strain Carried an Inversion (See Text)

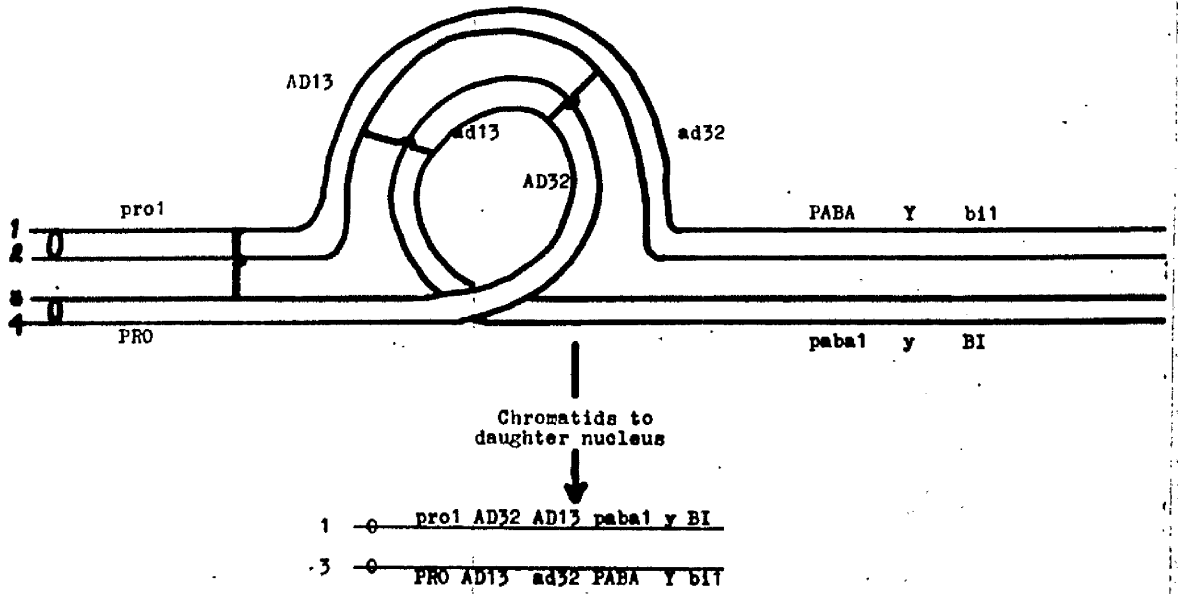
FIG. 34

crossover types (classes i - iv, Table 17), the ratio of reciprocal to non-reciprocal segregation of chromatids is 2:22, which shows a significant excess of non-reciprocal segregation from two-strand double crossovers ($P < 0.01$ for reciprocal=non-reciprocal segregation).

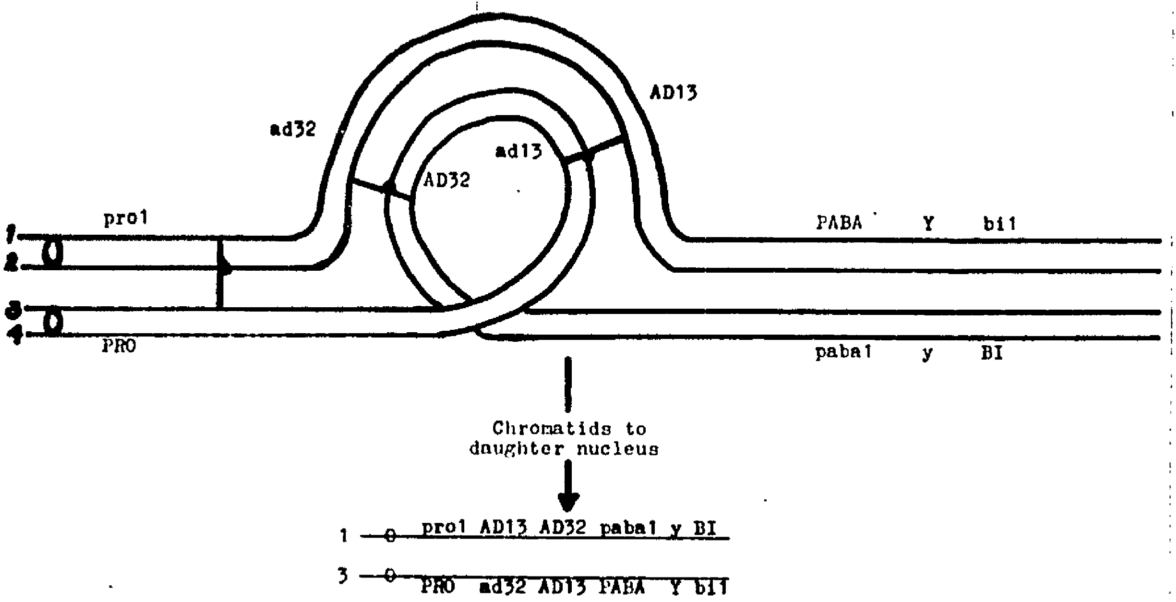
The foregoing discussion of the ad13 / ad32 half-tetrad analysis can be summarised as follows: -

Class	No. obtained	Crossover types
i	1	2-strand double within inversion
ii	12	"
iii	1	"
iv	10	"
v	4	Triple:- 2-strand double within inversion and a 3rd crossover in either pro - inversion or inversion - paba intervals
vi	1	Triple:- 2-strand double within inversion and a 3rd crossover in inversion - paba interval
vii	1	Triple:- 2-strand double within inversion and a 3rd crossover in pro - inversion interval
viii	1	Triple:- 2-strand double within inversion and a 3rd crossover in paba - y interval
ix	1	Quadruple:- 2-strand double within inversion, 3rd & 4th crossovers in pro - inversion and paba - y intervals
x	1	Quadruple:- 2-strand double within inversion, 3rd & 4th crossovers in pro - inversion and paba - y intervals (or inversion - paba & paba - y intervals)

A. Sequence *ad13* - *ad32* - *paba* in non-inverted strain



B. Sequence *ad32* - *ad13* - *paba* in non-inverted strain

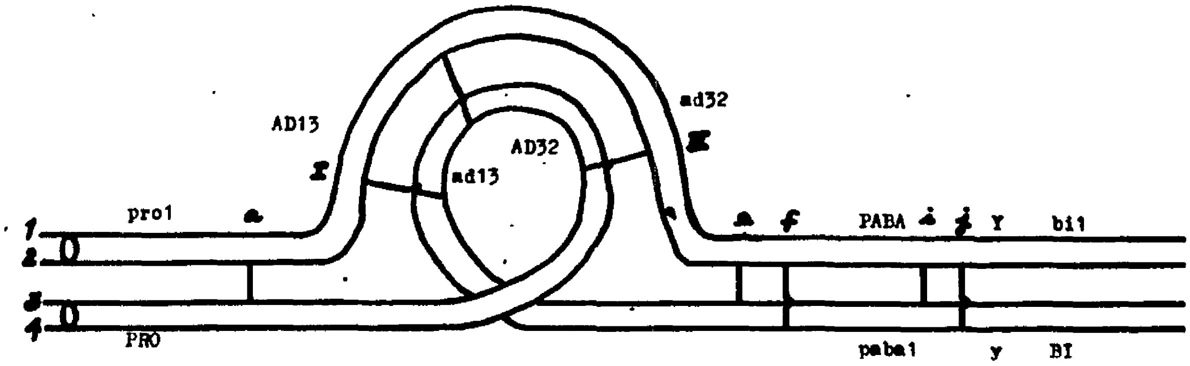


cf. Fig. 29 for recombinational origin of the other possible *ad* genotypes, *viz*. *ad13* *ad32* and *ad13* *AD32* in *ad* strand.

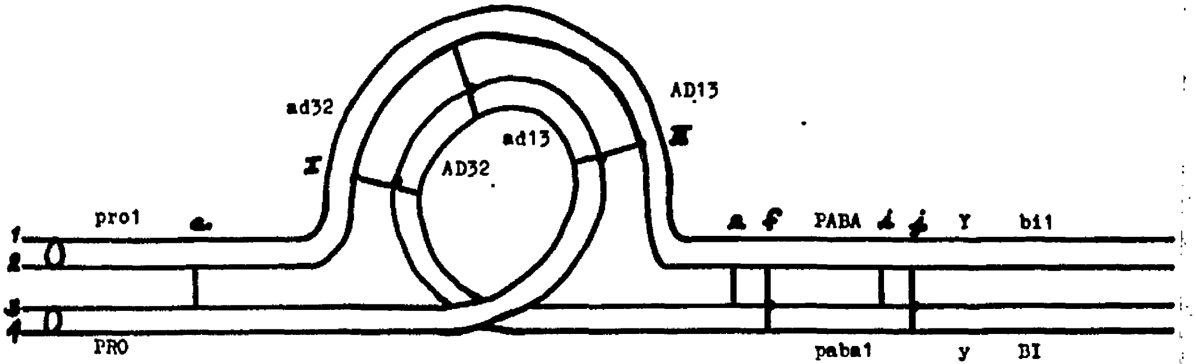
Possible Origin of Class C Recombinants from pro1 ad32 bi1 / ad13 paba1 y; acr2 w3 (See Table 19)

FIG. 35

A. Sequence ad13 - ad32 - paba in non-inverted strain



B. Sequence ad32 - ad13 - paba in non-inverted strain



A Order			B Order		
TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE
a, I, i	2 & 3	pro1 AD32 AD13 paba1 Y bi1 PRO ad13 ad32 PABA y BI	a,II,i	2 & 3	pro1 AD13 AD32 paba1 Y bi1 PRO ad32 ad13 PABA y BI
II, e,i	"	pro1 AD13 AD32 paba1 Y bi1 PRO ad32 ad13 PABA y BI	I, e,i	"	pro1 AD32 AD13 paba1 Y bi1 PRO ad13 ad32 PABA y BI
II, f,j	2 & 4	pro1 AD13 AD32 paba1 Y bi1 PRO AD32 ad13 PABA y BI	I, f,j	2 & 4	pro1 AD32 AD13 paba1 Y bi1 PRO ad13 AD32 PABA y BI

cf. Fig. 33 for recombinational origin of other possible ad genotypes, viz. those having an ad32 strand.

Possible Origin of Class D Recombinants from pro1 ad32 bi1 / ad13 paba1 y; acr2 w3
(See Table 19)

FIG. 36

(2) The results of haploidisation of a further seventeen wild-type recombinants from the *ad13* / *ad32* diploid (p. 85) are listed in Table 19. Only the type *A* diploids had their *ad* genotype determined* and all proved to belong to class *ii* (i.e. $\frac{\text{pro1 AD13 AD32 PABA Y bi1}}{\text{PRO ad13 AD32 paba1 y BI}}$, cf. Table 17 and Fig. 30).

The type *B* recombinants (i.e. $\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad PABA Y bi1}}$, Table 19) are probably class *iii* and *iv* genotypes (cf. Table 17 and Fig. 31), while the type *C* diploids (i.e. $\frac{\text{pro1 AD paba1 y BI}}{\text{PRO ad PABA Y bi1}}$, Table 19) would result from any one of several possible triple mitotic crossovers, depending upon the *ad* genotype (Figs. 29 and 35). Type *D* (i.e. $\frac{\text{pro1 AD paba1 Y bi1}}{\text{PRO ad PABA y BI}}$, Table 19) is comparable to class *ix* (Table 17 and Fig. 33) in that it requires four mitotic recombinations with one crossover taking place in the *paba* - *y* interval. Fig. 36 shows other possible types of

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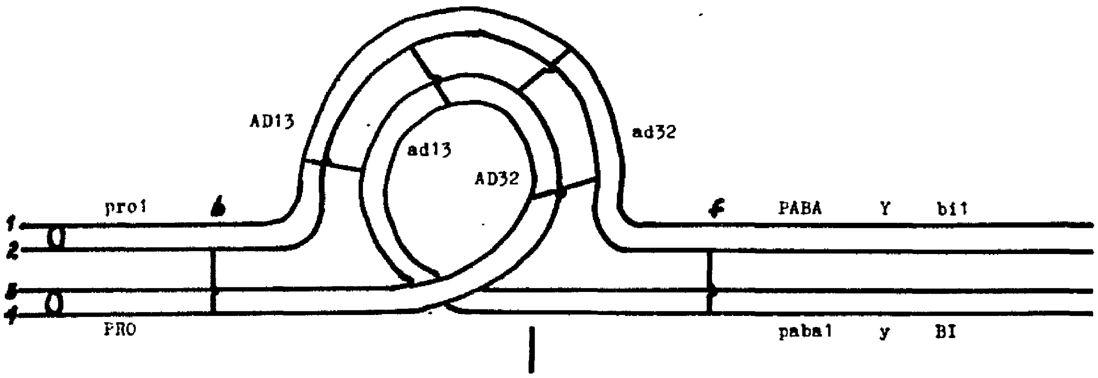
* As a class *i* diploid (Fig. 30) was not found among the first 32 recombinants analysed and as a haploid with *ad13 ad32* in inverted sequence was required for further experiments, the genotypes of type *A* diploids of a further 18 recombinants were determined. However, the 33rd recombinant (in order of isolation in the mitotic recombination analysis) proved to be a class *i* diploid and was therefore included in part (1), p.86.

A. Sequence ad13 - ad32 - paba in non-inverted strain

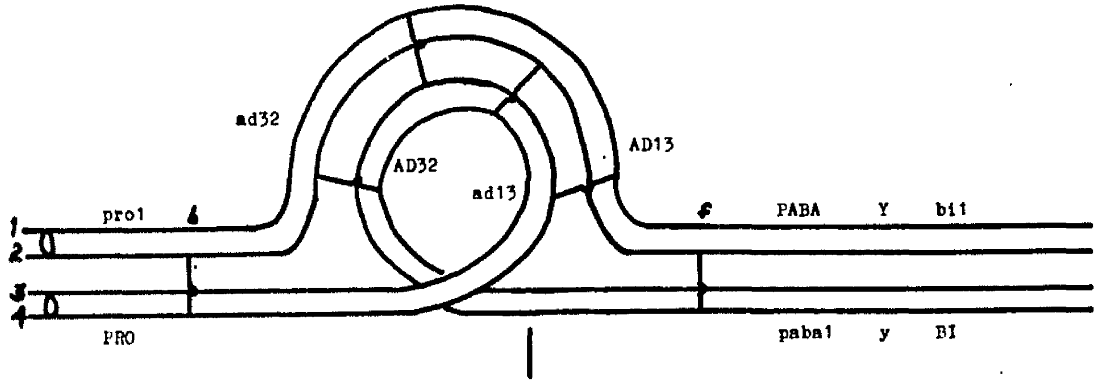


Chromatids 2 & 4 to daughter nucleus
following either the b type or f type of crossing over

2 prb1 AD13 AD32 paba1 y BI

4 PRO AD32 AD13 PABA Y bi1

B. Sequence ad32 - ad13 - paba in non-inverted strain



Chromatids 2 & 4 to daughter nucleus
following either the b type or f type of crossing over

2 pro1 AD32 AD13 paba1 y BI

4 PRO AD13 AD32 PABA Y bi1

Possible Origin of the Type E Recombinant from pro1 ad32 bi1 / ad13 paba1 y; [illegible]
(See Table 19)

FIG. 37

quadruple recombinations that could give rise to type *D*, where the *ad* genotype is either *ad13 ad32* or *ad13*.

One of the recombinants listed in Table 19 carried no *ad* allele (Type *E*, i.e. $\left(\frac{pro1\ AD\ paba1\ y\ BI}{PRO\ AD\ PABA\ Y\ bi1}\right)$. This diploid is reminiscent of the one found in the analysis of recombinants from *pro1 ad9 bi1* / *ad13 paba1 y; acr2 w3* (Table 15, Group *F*; see also p. 83). However, the homozygous *AD* recombinant from the present analysis requires, in addition to four crossovers within the inversion, a recombination in either the *pro* - *inversion* or *inversion* - *paba* intervals (Fig. 37). The alternative of simultaneous reversion and recombination seems highly unlikely.

The interpretation of the part (2) of the *ad13* / *ad32* half-tetrad analysis is summarised below: -

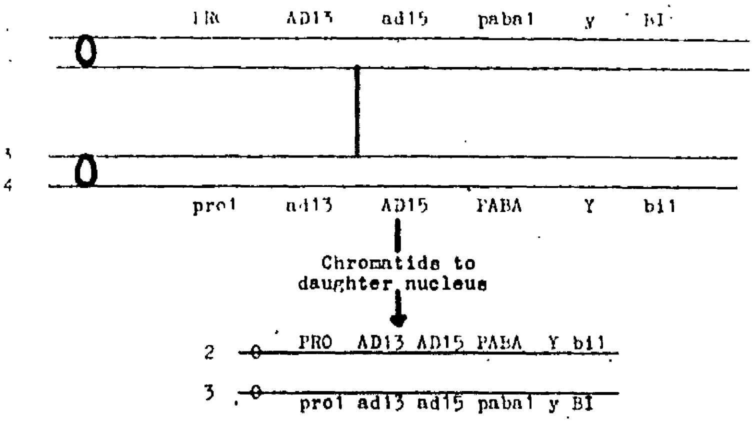
Class	No. obtained	Crossover types
A	5	2-strand double within inversion
B	4	"
C	6	Triple:- 2-strand double within inversion and a 3rd crossover in *pro* - *inversion* or *inversion* - *paba* intervals
D	1	Quadruple:- 2-strand double within inversion, a 3rd crossover in *paba* - *y* interval & a 4th in either *pro* - *inversion* or *inversion* - *paba* intervals

Class	No. obtained	Crossover types
E	1	Quintuple:- 2 2-strand doubles within inversion with each double having a crossover between the *ad* sites, and a 5th crossover in either the *pro* - *inversion* or *inversion* - *paba* intervals.

c. *ad13 / ad15*

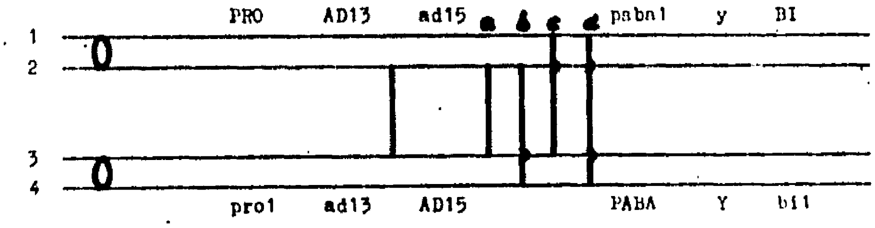
It was expected, on the basis of the *ad13* / *ad17* mitotic analysis and the fact that *ad15* has been shown by Calef (1957) to be distal of *ad17*, that the *ad13* inversion terminated proximally to the site of *ad17*. Other explanations were therefore sought for the results from mitotic recombination analyses of the *ad13* / *ad15* combination. The possibility that either *ad13* or *ad15* strains had inversions covering the sites of both *ad13* and *ad15* would be excluded were half-tetrad analysis to reveal the occurrence of a single recombination between to two *ad* alleles, without any additional recombination in the adjacent intervals.

Accordingly, fifteen wild-type mitotic recombinants from $\frac{\text{pro1 ad13 AD15 PABA Y bi1}}{\text{PRO AD13 ad15 paba1 y BI}}\ \ \frac{\text{W ACR}}{\text{w3 acr2}}$ were genotyped. The results, listed in Table 20, indicate that four



Recombination Which Would Produce Class i Mitotic Recombinants from ad15 paba1 y; acr2 w3 / pro1 ad13 bi1

FIG. 38



TYPE OF CROSSOVER	SEGREGATION OF CHROMATIDS 2 & 3 TO DAUGHTER NUCLEUS	CLASS	SEGREGATION OF CHROMATIDS 2 & 4 TO DAUGHTER NUCLEUS	CLASS
a	PRO AD13 AD15 paba1 y BI pro1 ad13 ad15 PABA Y bi1		PRO AD13 AD15 paba1 y BI pro1 ad13 AD15 PABA Y bi1	iv
b	PRO AD13 AD15 PABA Y bi1 pro1 ad13 ad15 PABA Y bi1	*	PRO AD13 AD15 PABA Y bi1 pro1 ad13 AD15 paba1 y BI	ii
c	PRO AD13 AD15 paba1 y BI pro1 ad13 ad15 paba1 y BI	*	PRO AD13 AD15 paba1 y BI pro1 ad13 AD15 PABA Y bi1	iv
d	PRO AD13 AD15 PABA Y bi1 pro1 ad13 ad15 paba1 y BI	Ø	PRO AD13 AD15 PABA Y bi1 pro1 ad13 AD15 paba1 y BI	ii

* Not recoverable in this experiment, as only wild-type diploids were analysed.
Ø Same as single crossover

Types of Recombinants Expected Following Double Recombination in the ad13 - ad15 and ad15 - paba1 intervals (See Text

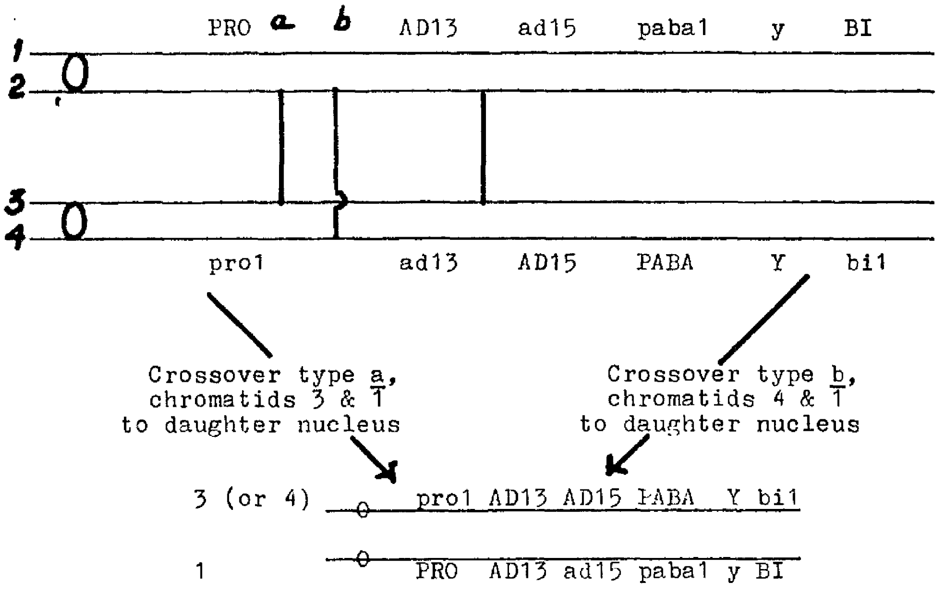
FIG. 39

single recombinants and eleven double recombinants were detected. The origin and significance of each of the four genotypes recovered will be considered in turn.

Class i $\left(\frac{\text{PRO AD13 AD15 PABA Y bi1}}{\text{pro1 ad13 ad15 paba1 y BI}}\right)$: - These four diploids are the expected result of a single recombination between *ad13* and *ad15* (Fig. 38). It should be noted that the allelic sequence, *ad13* - *ad15* - *paba*, suggested by the mitotic recombination experiments (see p. 67), is fully corroborated by the finding of this class. Discounted is the possibility that either *ad13* or *ad15* strains have an inversion spanning the *ad13* - *ad15* interval.

Class ii $\left(\frac{\text{PRO AD13 AD15 PABA Y bi1}}{\text{pro1 ad13 AD15 paba1 y BI}}\right)$: - These two diploids are of especial interest as both strands are recombinant with respect to the markers outside the *ad* cistron. Such a genotype requires a three- or four-strand double mitotic recombination of the types shown in Fig. 39 and again indicates the allelic sequence *ad13* - *ad15*.

Class iii $\left(\frac{\text{pro1 AD13 AD15 PABA Y bi1}}{\text{PRO AD13 ad15 paba1 y BI}}\right)$: - A



Origin of Class iii Recombinants from pro1 ad13 bi1 / ad15 paba1 y; acr2 w3

FIG. 40

two- or three-strand double crossover in the *pro* - *ad13* and *ad13* - *ad15* intervals, followed by segregation of one crossover and one non-crossover chromatid into the daughter nucleus, would give rise to the three mitotic recombinants of this genotype (Fig. 40). Reversion and gene conversion are thought to be less likely explanations of the origin of these and the class *iv* diploids for the reasons already advanced in connection with the half-tetrad analysis of *ad9* / *ad13*.

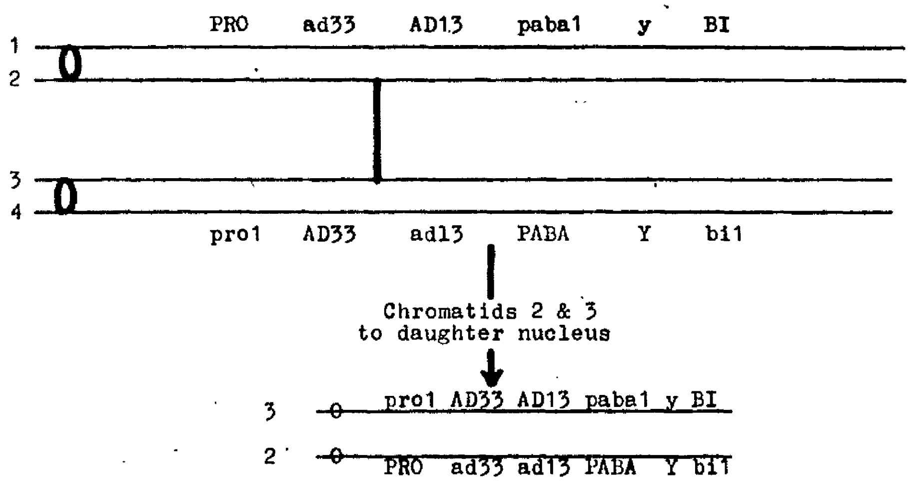
Class iv $\left(\frac{\text{PRO AD13 AD15 paba1 y BI}}{\text{pro1 ad13 AD15 PABA Y bi1}}\right)$: - The six mitotic recombinants of this genotype would be the consequence of a two- or three-strand double crossover in the *ad13* - *ad15* and *ad15* - *paba* intervals, followed by recovery of one crossover and one non-crossover strand (Fig. 39). This class would be expected to arise as frequently as class *ii* (Fig. 39), assuming no chromatid interference. However, class *iv* is in excess (though not significantly so), which might suggest chromatid interference.

It is notable that of the eleven double recombinants recovered in this experiment all had arisen as a consequence of an unselected recombination in an interval adjacent to the region of selection, viz. *pro* - *ad13* and *ad15* - *paba*. Moreover, eight are recombinant in the *ad15* - *paba* interval. The high frequency of recombination in the latter interval, correlated with the predominance of the class *iv* recombinants over the class *ii* type, might be implicated as a cause of the "anomalous" results obtained in the *ad13* / *ad15* mitotic recombination experiments (cf. Fig. 10 and Table 13).

The *ad13* / *ad15* half-tetrad analysis can be summarised as follows: -

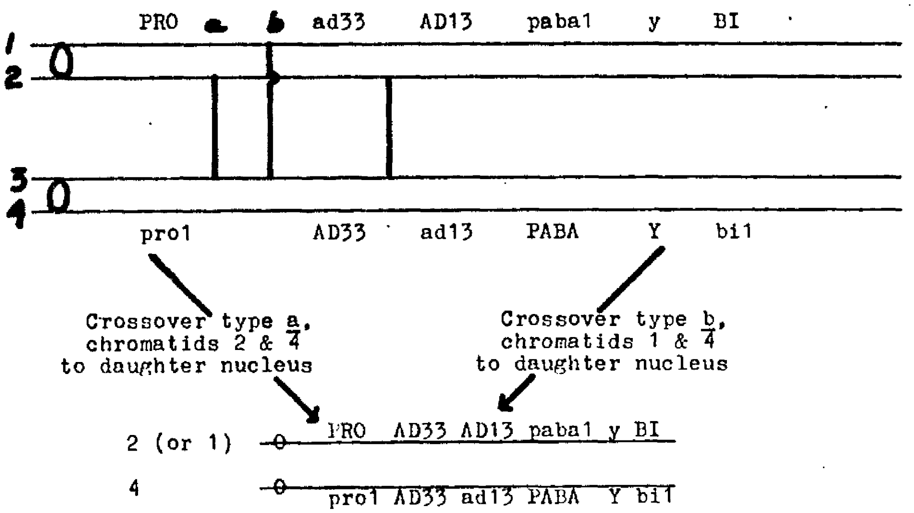
Class	No. obtained	Crossover type
i	4	single crossover between *ad* sites
ii	2	3- or 4-strand double crossover, with unselected crossover in *ad15* - *paba* interval
iii	3	2- or 3-strand double crossover, with unselected crossover in *pro* - *ad13* interval
iv	6	2- or 3-strand double crossover, with unselected crossover in *ad15* - *paba* interval

d. *ad33 / ad13*



Origin of the Class i Recombinant from ad33 paba1 y; acr2 w3 / pro1 ad13 bi1

FIG. 41



Origin of Class ii Recombinants from ad33 paba1 y; acr2 w3 / pro1 ad13 bi1

FIG. 42

This combination was investigated to determine whether the *ad13* inversion terminates distally to the *ad33* site. A sample of sixteen wild-type recombinants from the diploid

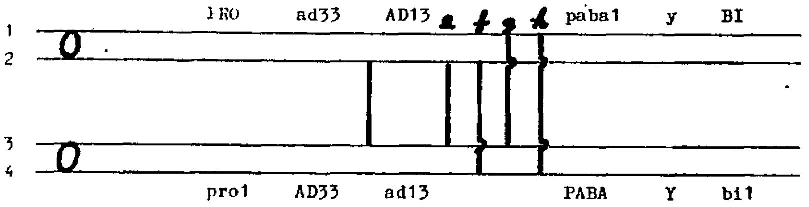
$$\frac{\text{PRO ad33 AD13 paba1 y BI}}{\text{pro1 AD33 ad13 PABA Y bi1}}\quad\frac{\text{w3 acr2}}{\text{W ACR}}$$

was analysed. The recombinants fall into seven genotypic classes (Table 21).

Class i $\left(\frac{\text{pro1 AD33 AD13 paba1 y BI}}{\text{PRO ad33 ad13 PABA Y bi1}}\right)$: - This diploid carries the reciprocal products of a single recombination between *ad33* and *ad13* (Fig. 41), indicating that the sequence determined in the mitotic recombination experiments (see p. 67) is correct and also that a single crossing-over leads to a viable recombinant. Thus, it has been shown that the *ad33* site is not included within the *ad13* inversion.

Class ii $\left(\frac{\text{PRO AD33 AD13 paba1 y BI}}{\text{pro1 AD33 ad13 PABA Y bi1}}\right)$: - These three recombinants would be expected as the consequence of a double crossing-over in the *pro* - *ad33* and *ad33* - *ad13* intervals (Fig. 42) or, less likely, as the result of mutation or conversion of *ad33* to *AD*.

Class iii $\left(\frac{\text{pro1 AD33 AD13 PABA Y bi1}}{\text{PRO ad33 AD13 paba1 y BI}}\right)$ and

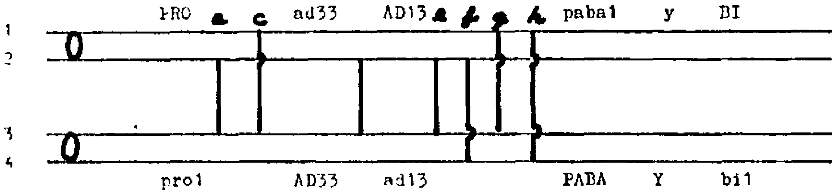


TYPE OF CROSSOVER	CHROMATIDS 3 AND 2 TO DAUGHTER NUCLEUS	CLASS	CHROMATIDS 3 AND 1 TO DAUGHTER NUCLEUS	CLASS
e	pro1 AD33 AD13 PABA Y bi1 PRO ad33 ad13 paba1 y BI		pro1 AD33 AD13 PABA Y bi1 PRO ad33 AD13 paba1 y BI	iii
f	pro1 AD33 AD13 PABA Y bi1 PRO ad33 ad13 PABA Y bi1	*	pro1 AD33 AD13 PABA Y bi1 PRO ad33 AD13 paba1 y BI	iii
g	pro1 AD33 AD13 paba1 y BI PRO ad33 ad13 paba1 y BI	*	pro1 AD33 AD13 paba1 y BI PRO ad33 AD13 PABA Y bi1	iv
h	pro1 AD33 AD13 paba1 y BI PRO ad33 ad13 PABA Y bi1	∅	pro1 AD33 AD13 paba1 y BI PRO ad33 AD13 PABA Y bi1	iv

* not phenotypically wild-type and therefore not recoverable in this experiment
∅ same as single cross-over type

Types of Recombination Which Could Result in Class iii and iv Recombinants from ad33 paba1 y; acr2 w3 / pro1/ad13 bi1

FIG. 43



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
a, f	2 & 4	PRO AD33 AD13 PABA Y bi1 pro1 AD33 ad13 paba1 y BI	vii
c, e	1 & 3	PRO AD33 AD13 PABA Y bi1 pro1 ad33 AD13 paba1 y BI	vi
c, f	1 & 4	PRO AD33 AD13 PABA Y bi1 pro1 AD33 ad13 paba1 y BI	vii
c, f	1 & 3	PRO AD33 AD13 PABA Y bi1 pro1 ad33 AD13 paba1 y BI	vi
c, g	1 & 3	PRO AD33 AD13 paba1 y BI pro1 ad33 AD13 PABA Y bi1	v
c, h	1 & 3	PRO AD33 AD13 paba1 y BI pro1 ad33 AD13 PABA Y bi1	v

Types of Recombination Which Could Give Rise to Class v, vi and vii Recombinants from ad33 paba1 y; acr2 w3 / pro1 ad13 bi1

FIG. 44

iv $\left(\frac{\text{pro1 AD33 AD13 paba1 y BI}}{\text{PRO ad33 AD13 PABA Y bi1}}\right)$: - Eight of the diploids analysed could have arisen from a double recombination in the *ad33* - *ad13* and *ad13* - *paba* intervals (Fig. 43). The class *iii* genotype could also result from reversion of *ad13*, although a recombinational origin is more probable. The class *iv* diploid is of interest as both strands are recombinant for outside markers; it also corroborates the *ad33* - *ad13* - *paba* sequence.

Classes v $\left(\frac{\text{PRO AD33 AD13 paba1 y BI}}{\text{pro1 ad33 AD13 PABA Y bi1}}\right)$, *vi* $\left(\frac{\text{PRO AD33 AD13 PABA Y bi1}}{\text{pro1 ad33 AD13 paba1 y BI}}\right)$ and *vii* $\left(\frac{\text{PRO AD33 AD13 PABA Y bi1}}{\text{pro1 AD33 ad13 paba1 y BI}}\right)$: - These three genotypes would be the result of certain types of triple crossing-over, with recombination in the *pro* - *ad33*, *ad33* - *ad15* and *ad15* - *paba* intervals (Fig. 44). In the class *vi* and *vii* diploids both strands are recombinant for outside markers, while the class *v* diploids have in coupling with *ad33* all the markers that were in repulsion of *ad33* in the parental diploid. Thus a non-recombinational origin of adenine-independence in these diploids is exceedingly unlikely.

Of the sixteen recombinants analysed in this experiment, seven could have resulted from an unselected recombination in the *pro* - *ad33* interval and twelve from an unselected recombination in the *ad13* - *paba* interval. (In both cases these recombinations are, of course, additional to the selected recombination between *ad33* and *ad13*.) This apparent polarisation of recombination in favour of the *ad* - *paba* interval is similar to that found in the *ad13* / *ad15* experiment (see p. 95).

The foregoing interpretation of the *ad33* / *ad13* half-tetrad analysis can be summarised as follows: -

Class	No. obtained	Crossover types
i	1	Single crossover between *ad* sites
ii	3	2- or 3-strand double with unselected crossover in *pro* - *ad33* interval
iii	7	2- or 3-strand double with unselected crossover in *ad13* - *paba* interval
iv	1	3- or 4-strand double with unselected crossover in *ad13* - *paba* interval
v	2	Triple:- unselected crossovers in *pro* - *ad33* and *ad13* - *paba* intervals
vi	1	"
vii	1	"

2. Non-inverted order of the sites ad13, ad9 and ad32.

a. Meiotic analysis

Four crosses relevant to the ordering of *ad13*, *ad9* and *ad32* were investigated: - *ad9* // *ad32* , *ad9* // *n-ad13 ad32** , *ad32* // *n-ad13 ad9* and *i-ad13** // *ad9* . Techniques employed in recombinant selection and in estimating recombination fractions were described in Chapter I (see also Fig. 5).

ad9 // ad32 . Data from three replicate experiments are given in Tables 22 and 23. The two *ad* alleles recombine with a frequency of *ca*. 4×10^{-6}. Classification of the adenine-independent recombinants with respect to unselected markers (Table 23) clearly indicates that the *ad9* - *ad32* - *paba1* sequence derived from the mitotic recombination data in Table 12 (see also p. 67) is correct.

ad32 // n-ad13 ad9 . The *n-ad13 ad9* parent in this cross was obtained during the half-tetrad analysis of *pro1 ad9 bi1* / *ad13 paba1 y; acr2 w3* (see class *1*, Table 15). It

---

* "n-" denotes non-inverted sequence, while "i-" indicates the inverted sequence.

was inferred, from the distribution of outside markers in the recombinant diploid from which it was derived, to have the non-inverted sequence of the *ad13*, *ad9* and *ad32* sites.

The classification of recombinants presented in Table 24 places *n-ad13* proximally to *ad32*. Heterogeneity tests on the three values for the *ad9* - *ad32* recombination fraction estimate indicate that the value obtained in the *n-ad13 ad9* // *ad32* experiment is homogeneous with one of the three replicates of the previous cross (i.e. with *iii*, $X_1^2 = 0.70$, $P > 0.30$). The weighted and pooled data from *n-ad13 ad9* // *ad32* and (*iii*) *ad9* // *ad32* gives a recombination fraction estimate of $9.3 \times 10^{-6} \pm 9.5 \times 10^{-7}$.

ad9 // n-ad13 ad32 . The *n-ad13 ad32* strain used in this cross was recovered from the class *i* diploid recombinant of the *pro1 ad32 bi1* / *ad13 paba1 y; acr2 w3* half-tetrad analysis (Table 17). Again, it was assumed to have the non-inverted order of the *ad13*, *ad9* and *ad32* sites.

Should the sequence be *ad13 ad9 ad32* in non-inverted strains, selection for adenine-independent recombinants would require two crossovers. On the other hand, a single recombination between *ad13* and *ad32* would give rise to an adenine-independent colony if the order were ad9 ad13 ad32. The results given in Table 25 would suggest that the first sequence is the more likely since the recombination fraction appears to be less than 1×10^{-6}.

The recombinant phenotypes are not those expected with either sequence. It is possible that reversion of ad9 is responsible for at least the pro w colony in plating 7.

The next series of crosses shows that the ad13 - ad9 interval must be larger than the ad9 - ad32 interval, hence indicating that ad13 - ad9 - ad32 - paba is indeed the non-inverted order of these alleles.

ad9 // i-ad13 . Three estimates of the frequency of the recombinations producing adenine-independent progeny were obtained (Table 26). It must be remembered that these are not estimates of the recombination fraction for the ad13 - ad9 interval because the presence of the inversion in the i-ad13 parent makes necessary an additional crossing-over within the inversion for the production of AD progeny. Although the three estimates are heterogeneous (Table 26), they indicate that the recombination events leading to adenine independence occur at least as frequently in these crosses as in the _ad9_ // _ad32_ and _n-ad13 ad9_ // _ad32_ experiments: -

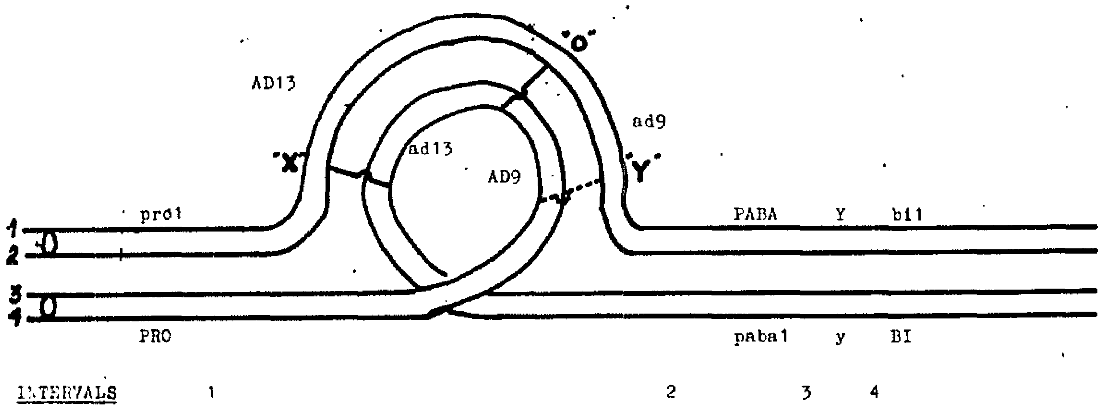
_ad9_ // _ad32_ (pooled estimate, Table 22) = $4.5 \times 10^{-6} \pm 3.6 \times 10^{-7}$ _vs._ _ad9_ // _i-ad13_ (estimate _i_, Table 26) = $5.1 \times 10^{-5} \pm 6.9 \times 10^{-6}$. Test for heterogeneity gives $X_1^2 = 45.29$, $P \ll 0.01$.

_ad9_ // _ad32_ (pooled estimate, Table 22) = $4.5 \times 10^{-6} \pm 3.6 \times 10^{-7}$ _vs._ _ad9_ // _i-ad13_ (estimate _iii_, Table 26) = $1.4 \times 10^{-5} \pm 5.0 \times 10^{-6}$. Test for heterogeneity gives $X_1^2 = 3.59$, $P > 0.05$.

_n-ad13 ad9_ // _ad32_ (Table 24) = $1.0 \times 10^{-5} \pm 1.3 \times 10^{-6}$ _vs._ _ad9_ // _i-ad13_ (estimate _i_, Table 26) = $5.1 \times 10^{-5} \pm 6.9 \times 10^{-6}$. Test for heterogeneity gives $X_1^2 = 34.10$, $P \ll 0.01$.

_n-ad13 ad9_ // _ad32_ (Table 24) = $1.0 \times 10^{-5} \pm 1.3 \times 10^{-6}$ _vs._ _ad9_ // _i-ad13_ (estimate _iii_, Table 26) = $1.4 \times 10^{-5} \pm 5.0 \times 10^{-6}$. Test for heterogeneity gives $X_1^2 = 0.60$, $P > 0.30$.

When one considers that the _i-ad13_ strains carry the inversion and hence _two_ recombinations are required within the inversion, it is readily apparent that the



LEGEND: "O" is the selected crossover
"X" is the second crossover within the inversion, between ad13 and the proximal break-point (in non-inverted sequence)
"Y" is the second crossover within the inversion, between ad9 and the distal break-point (in the non-inverted sequence)

The solid line for crossover type "X" and the dashed line for crossover type "Y" are used to emphasise that the "X" type seems to be the more frequent.

Interpretation of Recombination in ad9 x i-ad13 crosses (See Table 27)

FIG. 45

ad13 site must be located proximally to ad9 in the non-inverted sequence.

Classification of recombinants from four platings (two for each cross) show that there is also great heterogeneity in the types of recombinants recovered (Table 27). Assuming for a moment that one has no knowledge of either the ad13 inversion or of the ad allelic sequence, only plating iii (Table 27) would suggest an allelic order. All the results are compatible, however, with the interpretation that the ad13 strain has the ad13 - ad9 - ad32 region of the cistron inverted.

It appears that the unselected recombination within the inversion most often occurs proximally to the ad13 site ("X" in Fig. 45), suggesting that the distance between ad13 and the proximal break point of the inversion is much longer than that from ad32 to the distal break point. Taking into account the recombination fraction estimations of the several crosses described in this section, it would seem that the distance from ad9 to the distal break point of the inversion is a small proportion of the total inverted length.

b. Mitotic recombination analysis

A comparison was made of the mitotic recombination behaviour of three diploids heterozygous for *ad13*, *ad9* and *ad32* in different arrangements -- viz.

(1) $\frac{\text{pro1 n-ad13 ad9 AD32 PABA Y bi1}}{\text{PRO n-AD13 AD9 ad32 paba1 y BI}}\;\frac{\text{w3 acr2}}{\text{W ACR}}$

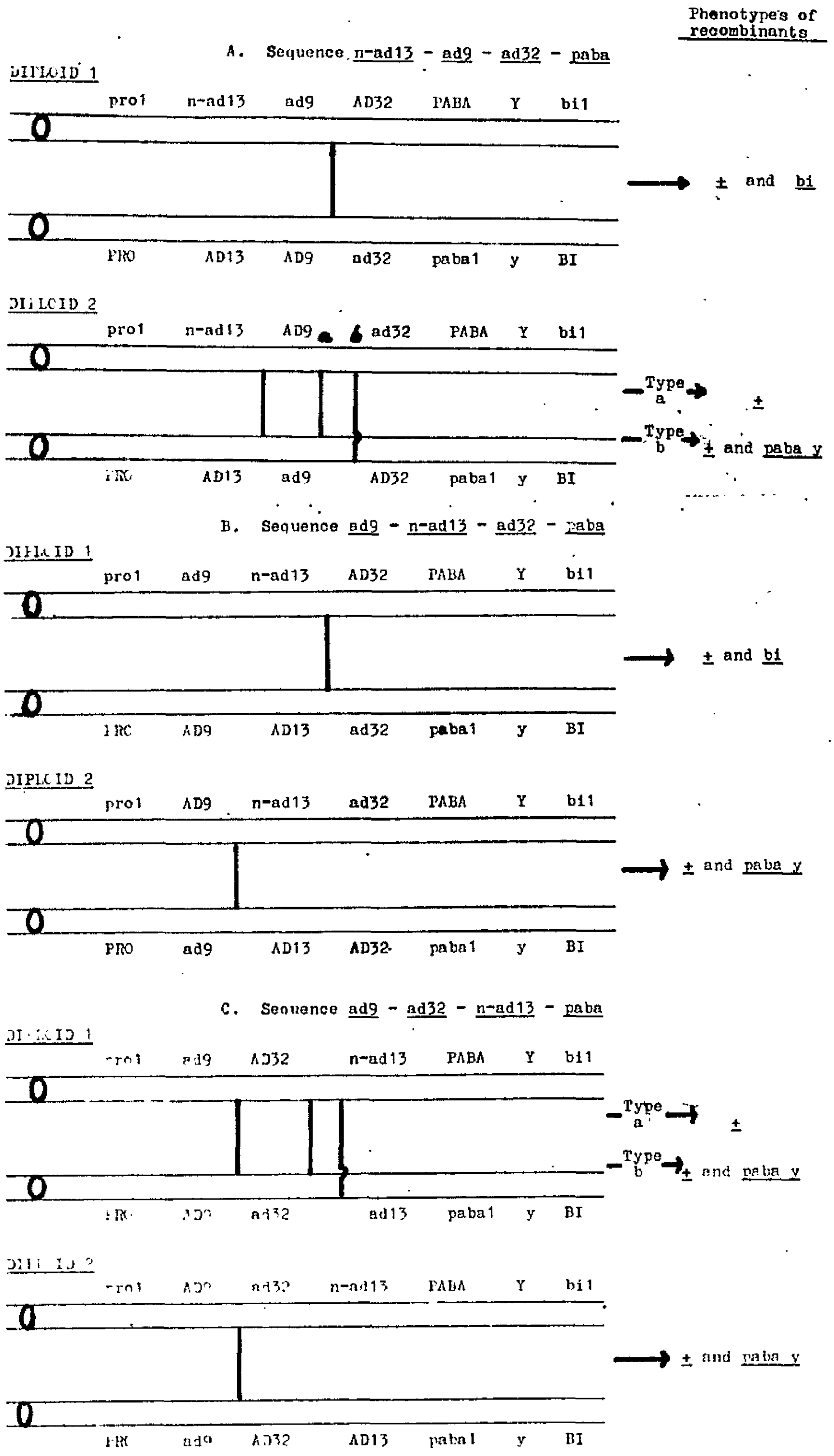
(2) $\frac{\text{pro1 n-ad13 AD9 ad32 PABA Y bi1}}{\text{PRO n-AD13 ad9 AD32 paba1 y BI}}\;\frac{\text{w3 acr2}}{\text{W ACR}}$

(3) $\frac{\text{PRO i-(ad32 AD9 ad13) paba1 y BI}}{\text{pro1 n-AD13 ad9 AD32 PABA Y bi1}}\;\frac{\text{w3 acr2}}{\text{W ACR}}$.

The origin of the strand carrying two adenineless alleles in diploids (1) and (2) has already been described (see pp. 99-100). The *i-ad13 ad32* component of diploid (3) was derived from a mitotic recombinant of *pro1 ad32 bi1* / *ad13 paba1 y; w3 acr2* (see class *iii*, Table 17).

The types of mitotic recombinants recovered from the three diploids are listed in Table 28, and the frequency with which they arise (as judged by the proportion of parental *ad*-requiring colonies producing at least one adenine-independent segregant) is given in Table 29.

The finding of an excess of the *bi* class for diploid (1) and the *paba y* class for diploid (2) is in



Expected Most Frequent Types of Adenine-independent Recombinants from Diploids 1 and 2 (see text), where the Position of the n-ad13 Site with respect to the ad9 - ad32 Sequence is Unknown

FIG. 46

agreement with a non-inverted sequence, either ad13 - ad9 - ad32 or ad9 - ad13 - ad32 (Fig. 46), and supports the contention that the strands carrying two ad alleles in each of these diploids is non-inverted.

The percentage of adenine-requiring colonies producing adenine-independent segregants for diploids (1) and (2), compared with the i-ad13 / ad9 , i-ad13 / ad32 and ad9 / ad32 combinations (Table 29) would indicate the first sequence of ad sites (A in Fig. 46). The lower frequency in the case of the n-ad13 ad32 / ad9 combination would be expected when two crossovers are required for adenine-independence.

The results obtained with diploid (3) are predicted by the assumption that the strand carrying the ad13 and ad32 alleles contained the inversion and that two-strand double crossovers (in the ad13 - ad9 and ad9 - ad32 intervals) were necessary for the production of adenine-independent recombinants since (a) the frequency of segregant production is only 2.7% (cf. other combinations in Table 29) and (b) the ratio of auxotrophic recombinants to prototrophic recombinants is only 0.48 as compared to 0.87 for diploid (2). (In the case of diploid (3) the selected crossovers within the inversion must be 2-strand doubles, hence giving rise to phenotypically wild-type diploids, while for diploid (2) the selected crossovers (see A in Fig. 56) can be either 2- or 3-stranded and hence can give rise to phenotypically auxotrophic diploids.) Although the numbers of *bi* and *paba y* segregants obtained from diploid (3) were too few to permit a comparison, the high ratio of other auxotrophic phenotypes to these classes is also consonant with the interpretation that the *ad13 ad32* component of diploid (3) possessed the *ad13* inversion.

c. Half-tetrad analysis

A random sample (Table 30) of mitotic recombinants from diploid (2), viz.

$$\frac{\text{pro1 n-ad13 AD9 ad32 PABA Y bi1}}{\text{PRO n-AD13 ad9 AD32 paba1 y BI}} \quad \frac{\text{w3 acr2}}{\text{W ACR}},$$

was analysed. The results, given in Table 31, confirm that *ad9* is located between the *ad13* and *ad32* sites in the non-inverted sequence. Class *i* $\left(\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad PABA Y bi1}}\right)$ and class *ii* $\left(\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad paba1 y BI}}\right)$ -- see Table 31 and

Fig. 46A -- are the consequence of the double crossover required to produce adenine-independent segregants from this diploid.

Assuming no chromatid interference and random segregation of chromatids, one would expect to find classes *i* and *ii* in the proportion of 3:1; the actual numbers found are not significantly different from this expectation.

The types of recombination required to produce the various classes of recombinants found in this experiment (Table 31) would indicate that high negative interference is associated with the selected recombinations within the *ad13* - *ad9* and *ad9* - *ad32* intervals.

C. *Summary of Evidence in Favour of the Inversion Hypothesis.*

We may sum up our main results and conclusions with regard to the inversion as follows: -

(1) A diploid with the constitution known to be either $\dfrac{\text{pro1 adx ADZ PABA Y bi1}}{\text{PRO ADX adz paba1 y BI}}$ or

$\frac{\text{pro1 ADZ adx PABA Y bi1}}{\text{PRO adz ADX paba1 y BI}}$ is expected to give rise by mitotic recombination to diploids which are adenine independent and hence which may be selected for by their ability to grow on adenineless medium. Among such diploids, the number which are *paba y* will exceed the number which are *bi* if *adx* is distal to *adz*, while the number which are *bi* will exceed the number which are *paba y* if *adx* is proximal to *adz*. Results of this kind were obtained for most of the pairs of *ad* alleles investigated, i.e. *ad33* / *ad13* , *ad33* / *ad9* , *ad33* / *ad32* , *ad33* / *ad17* , *ad33* / *ad15*, *ad13* / *ad17* , *ad9* / *ad32* , *ad9* / *ad17* and *ad9* / *ad15* .

(2) For certain pairs of alleles, i.e. *ad13* / *ad9* , *ad13* / *ad32* , *ad13* / *ad15* and *ad32* / *ad15* , the numbers of *paba y* and *bi* among the adenine-independent recombinants were equal (within the limits of sampling error).

(3) These anomalous results could be explained if there were an inversion spanning the two sites between which recombination was selected.

(4) In order to test the inversion hypothesis, the mitotic segregants from the diploids yielding anomalous results were subjected to half-tetrad analysis, i.e. the

diploid segregants were allowed to break down into their constituent haploids, the genotypes of which were then determined.

(5) The half-tetrad analyses suggest that no single crossovers giving rise to viable adenine-independent diploids occurred within the diploids ad9 / ad13 and ad32 / ad13, thus indicating that an inversion spanning the two sites, between which recombination had been selected, was present in one of the parental strands.

(6) Some of the haploid strands -- i -- extracted in the half-tetrad analyses are predicted to carry the inversion, others -- n -- to be non-inverted. These two types may be distinguished by making use of another strain -- s -- which has no inversion but carries an allele ad-c (distinct from adx and adz), the site of which lies within the inverted region in the i strands. The cross i // s should then yield no single crossover strands, whereas in the progeny of the cross n // s single crossovers should be the largest class. Similar differences should be observed between the mitotic recombination behaviour of the diploids i / s and n / s. These tests were carried out. All of the extracted

haploid strands behaved as expected, both in the meiotic and in the mitotic analyses.

(7) The experiments described above give the sequence of sites in the non-inverted strands as ad13 - ad9 - ad32 and by analogy the sequence in the inverted strands as ad32 - ad9 - ad13 .

D. Discussion and Conclusions.

It has been shown that ad13 strains carry an inversion spanning the sites of ad13, ad9 and ad32, but not ad33 and ad15. (The relation with respect to the ad17 site will be considered in part 5 of this discussion.) The non-inverted sequence of sites was shown to be

ad33 - n-ad13 - ad9 - ad32 .

1. Evidence that the inversion does not determine a mutant phenotype.

Were the ad13 phenotype due to an inversion within the cistron without, in addition, a localised mutant within the inversion, one would expect (a) to find no difference between the recombination behaviour of what have been termed i-ad13 ad32 and n-ad13 ad32, and (b) to be unable to separate ad13 from the inversion.

The data in Tables 28 and 29 clearly show that the mitotic recombination experiments involving i-ad13 ad32 / ad9 and n-ad13 ad32 / ad9 differ and that the results obtained are those predicted by the hypothesis that i-ad13 ad32 possesses the inverted sequence of the region containing the two point mutations, while n-ad13 ad32 has the non-inverted sequence.

Both the meiotic and mitotic recombination experiments involving n-ad13 ad9 and n-ad13 ad32 (Tables 24, 28, 29, 31) give the same pattern of recombination found in structural homozygotes which contrasts with that found in structural heterozygotes -- cf. for example the crosses ad9 // ad32 (Table 23) and n-ad13 ad9 // ad32 (Table 24) with i-ad13 // ad9 (Table 27) or the mitotic analyses of n-ad13 ad9 / ad32 (Table 28) and ad9 / ad32 (Table 12) with i-ad13 / ad9 (Table 13). The conclusion is justified that those strains designated as non-inverted are instances of ad13 having been separated from the i-ad13 inversion.

Because of the differences between the "i" and "n" double mutant strains and also because ad13 can be removed from the inversion, the hypothesis that the inversion is responsible for the adenine requirement of ad13 strains can be rejected.

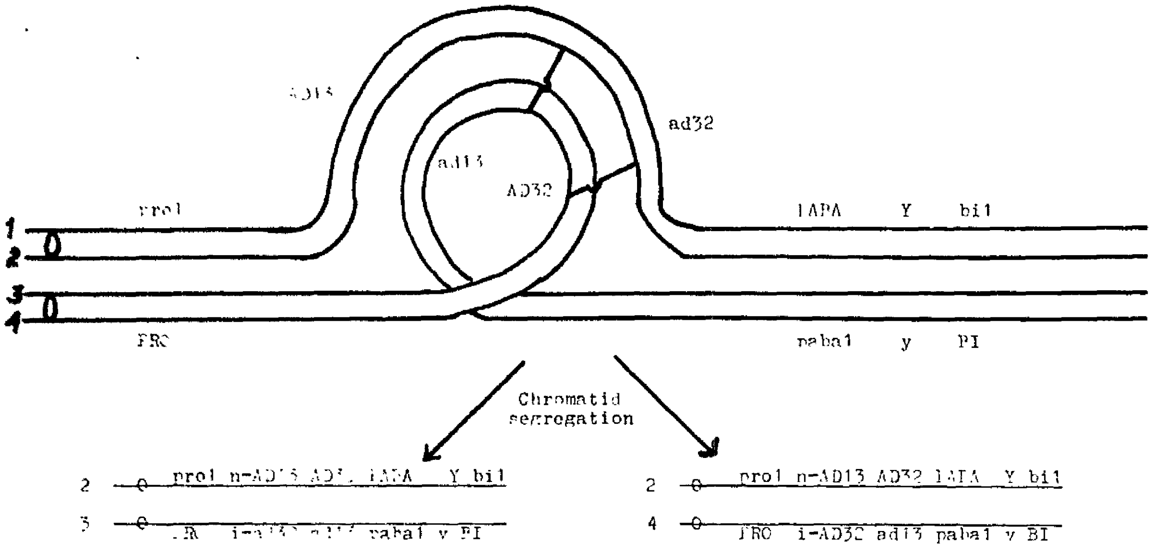
One could imagine, however, that the i-ad13 inversion produces a mutant phenotype and that i-ad13 strains also possess the ad13 point mutation. This scheme runs into the following difficulties when applied to the half-tetrad data of experiments involving the i-ad13 / ad9 and i-ad13 / ad32 combinations: -

(a) The coincidental recombination in both the pro - inversion and inversion - paba intervals (which must be invoked in connection with the recombinational origin of n-ad ad / i-AD AD recombinants) would then appear to occur more frequently than crossing-over in just one of these intervals.

(b) The number of recombinants showing this coincidental crossing-over would be at least as large as those resulting from only the selected crossing-over within the inversion.

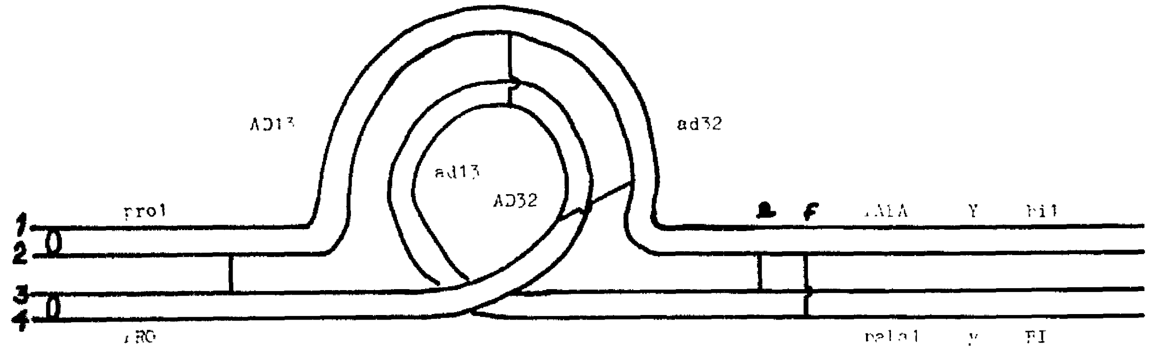
(c) The recombination fractions for the intervals between the inversion and the adjacent outside markers would then be greater than 50%.

Consider, for example, the half-tetrad analysis of the ad32 / i-ad13 diploid given in Table 17. If one



The only Type of Double Crossing-over within the Inversion that will Result in Adenine-independent Segregants from an Inversion Heterozygote, According to the Hypothesis that the Strain Possessing the Inversion is Doubly Mutant (i.e. due to the point mutation within the inversion and also to the inversion)

FIG. 47



TYPE OF CROSSOVER	CHROMATIDS TO SAME NUCLEUS	GENOTYPE	CLASS
e	3 + 2	PRO n-AD13 AD32 paba1 y BI pro1 i-ad32 ad13 PABA Y bi1	iii
e	3 + 1	PRO n-AD13 AD32 paba1 y BI pro1 -AD13 ad32 PABA Y bi1	i
f	3 & 1	PRO n-AD13 AD32 paba1 y BI pro1 r-AD13 ad32 PABA Y bi1	iv

Types of Crossing-over Postulated for the Recombinational Origin of Class iii and iv Half-tetrads (Table 17) on the Hypothesis that the ad13 Strain is Doubly Mutant (i.e. due to the inversion and also to the point mutation within the inversion

FIG. 48

assumes that *i-ad13* strains are doubly mutant due to the point mutation (i.e. *ad13*) *and also to the inverted sequence* of part of the cistron, adenine-independent recombinants can result from only one type of crossing-over within the inversion (see Fig. 47). The double recombination shown in Fig. 31A for the origin of class *iii* and *iv* diploids (Table 17) would not produce adenine-independent segregants according to this "mutant-inversion" hypothesis, for the *AD32 AD13* strand should have the inversion and would therefore be mutant. Instead, one would have to postulate four recombinations (two within the inversion and two outside -- see Fig. 48) for the origin of classes *iii* and *iv*. According to this scheme over 60% of the half-tetrads analysed would have been the consequence of an additional (i.e. non-selected) recombination in each of the *pro* - *inversion* and *inversion* - *paba* regions. Furthermore, crossovers in both these intervals would be more frequent than in just one of the intervals. On the other hand, if one assumes that the inversion does not determine a mutant phenotype, then the figure is 27% for unselected recombination in the *pro* - *paba* region, with a crossover in just one of the two intervals adjacent to the inversion occurring more frequently than coincidental recombination in both intervals.

It seems more reasonable to assume that the n-ad13 ad9 and n-ad13 ad32 haploids recovered in the half-tetrad analyses resulted from the recombination events postulated in Figs. 20A and 31A and hence that the AD9 AD13 and AD32 AD13 haploids obtained from these same half-tetrads have the inversion. It should be possible (but time-consuming) to test this assumption by the method suggested in Appendix B.

Another line of evidence would discount the "mutant-inversion" hypothesis. In part 5 of this discussion it is shown that ad17 strains possess an inversion identical with the i-ad13 inversion (in addition to a point mutation). Were the i-ad13 inversion to confer a mutant phenotype upon the strains which possessed it, then those strains would not revert to wild type. Yet revertants were recovered in experiments with ad17 (Table 7). Outcrossing (p.31) indicated that one of these "revertants" was either a very closely linked suppressor or a true reverse mutation.

Although the direct test has not been attempted (see Appendix B), it seems reasonable to conclude that the ad13 inversion does not produce a mutant phenotype.

2. Significance of the ad13 inversion in relation to gene function.

The nature of the ad13 inversion indicates (a) that the region in which the rearrangement is found does not have its information duplicated elsewhere in the cistron since mutant sites have been detected within it and (b) that strains possessing non-mutant sites of the ad9 cistron and the ad13 inversion are in all likelihood adenine-independent.

It appears, therefore, that the manner in which the ad9 cistron codes for the polypeptide chain (whose amino-acid sequence it supposedly determines, see Chapter II) permits some reshuffling of the genetic material without changing its information content with respect to the polypeptide chain -- or at least without altering it to such an extent that no active protein can be made.

One possibility is that the genetic information residing in the inverted region is not concerned with the composition of the polypeptide chain. The difficulty with this suggestion is that point mutations have been detected within the inverted region. One could imagine that these point mutations blocked the formation of the polypeptide through some repressor system or by interrupting the coding sequence specifying the amino-acid sequence.

A second interpretation is that the inverted region specifies a dispensable part of the amino-acid sequence. For example, a portion of the ribonuclease molecule can be cleaved without destroying its activity (Anfinsen, 1959; Hirs, 1960; Richards, 1958). It might be possible that a cistron having the rearrangement coded for only a portion of the wild-type polypeptide and that the shortening of the polypeptide chain did not destroy protein activity. The point mutations detected within the inverted region could bring about loss of activity by altering the active centre or by preventing polypeptide formation (as in the previous suggestion.)

A third suggestion is that the effect of the inversion is somewhat analogous to the polypeptide interaction suggested as a complementation mechanism (see Chapter II), except that in the case of the inversion the correction would take place within the one polypeptide chain. Suppose, for example, that the proximal break which led to the inversion produced one mutation and the distal break a second mutation. Suppose also that these mutations specify "new" amino-acids which are incorporated into the doubly-mutant polypeptide. In line with the Crick-Orgel theory (Chapter II) it could be envisaged that the two "new" amino-acids (or peptides) could interact in such a way to allow the protein to achieve an active conformation. One would have to assume, of course, that the rest of the rearrangement was either irrelevant with respect to protein activity or that it still produced a code that specified an amino-acid sequence compatible with protein activity.

A variant of this proposal is suggested by the work on the *in vitro* restoration of ribonuclease activity (Richards, 1958). It was found that two incomplete, individually inactive, portions of a *single polypeptide* chain could be mixed together to produce an active complex. Similarly, it could be thought that the break points of the inversion caused two incomplete parts of the polypeptide chain to be synthesised which then interacted in such a manner that the resulting polypeptide complex behaved as if it were an unbroken wild-type polypeptide chain.

In connection with the "self-complementation" idea it is worth noting that the discovery of revertants distinguishable from wild-type -- e.g. *am-2*^{1} and *am-1038* in *Neurospora* (Fincham, 1957; Pateman, 1957; Fincham and Pateman, 1957a; Pateman, 1960a), *ad-4*$^+$ revertants in *Neurospora* (Woodward *et al.*, 1960) and *P*$^+$ revertants in *E. coli* (discussion following Levinthal, 1959a; Garen, 1960) -- suggests that at least some revertants are not true reverse mutations. That is, the change is not from a mutant code to the wild-type code, but to another code which compensates in part for the "mistake" caused by the first mutation, with a further "mistake."

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A final possible explanation of the non-mutant phenotype associated with the rearrangement is that the inversion does not change the amino-acid sequence in the polypeptide chain from that specified by the wild-type cistron, even though the inverted region does contribute information for part of that sequence. Point mutations would be expected to occur within this inverted region which would lead to loss of activity. However, if one assumes that the arrangement of the genetic material and of the amino-acid sequence is co-linear (a corollary to present coding hypotheses), then the occurrence of identical polypeptide chains with both normal and rearranged wild-type cistrons would suggest that the inversion reads the same from right-to-left as from left-to-right. Such an interpretation conflicts with the current coding hypotheses utilising sequences of DNA or RNA bases (e.g. Crick et al., 1957; Brenner, 1957a; Crick, 1958; Sinsheimer, 1959; Golomb et al., 1958; Gamow et al., 1956; Freudenthal, 1958; Levinthal, 1959b; Sueoka et al., 1959).

It would be difficult to reconcile the "wild-type" inversion with these codes no matter which of the schemes suggested applies, unless one assumes that the inverted region is not concerned with specifying amino-acid sequences in the polypeptide. It seems improbable that the rearrangement would meet the following conditions: -
(1) that the end points of the inversion occur between "words," or -- should the breaks occur within "words" -- that the new "words" formed after the rearrangement has occurred code for the same or at least acceptable amino-

acids and (2) that the intervening "words" in the inverted region code for a sequence of amino-acids that would still allow the formation of a functional protein.

It seems more likely that a nonsense "word," or a miss-sense or "stop" combination would occur at the ends of the inversion or within it, thus interrupting the synthesis of the polypeptide chain. If, however, only the part of the cistron proximal to or distal to the inverted region coded for the indispensable portion of the polypeptide, these objections to co-linearity and coding hypotheses would not apply.

It should be mentioned that the extant coding hypotheses have been questioned on other grounds, viz. heterogeneity in DNA of various micro-organisms (Doty *et al*., 1959; Crick, 1959; Belozersky and Spirin, 1958; Sinsheimer, 1959; Marmur and Doty, 1959; Sueoka *et al*., 1959) and the correlation between RNA and protein of different RNA-containing viruses (Yčas, 1960). The tacit assumption that co-linearity exists between genetic material and amino-acid sequence has also been queried (see Chapter II and also Levinthal, 1959b).

3. Recombination in diploids and crosses heterozygous for the ad13 inversion.

It is of interest to contrast the effects of the *ad13* inversion on recombination with those observed in the classical studies of inversion heterozygotes in *Drosophila* species and in maize.

Firstly, let us consider recombination within the inverted region. Sturtevant and Beadle (1936), in their monumental study of the relations of inversions to crossing-over in the X-chromosome of *Drosophila melanogaster*, have shown that recombination is apparently reduced within inversions in inversion heterozygotes. In some cases, for very long inversions at least, the apparent reduction is due solely to the inability to recover single and certain types of double crossovers. However, for a large number of reported inversions -- e.g. *sc-7*, *dl-49* and *ClB* (Sturtevant and Beadle, 1936) and *roughest-3* (Grüneberg, 1935) in *D. melanogaster*, those in *D. subobscura* studied by Spurway and Philip (1952), those in *D. pseudoobscura* investigated by Dobzhansky and Epling (1948) and *In-3a* in maize (Rhoades and Dempsey, 1953) -- crossing-over within the inversion seems to be strongly depressed.

In this thesis evidence from the meiotic analysis of i-ad13 // ad9 crosses suggests that the inversion does not depress recombination within the ad13 - ad9 interval. Since the map distances (expressed as recombination fractions) for the proximal break - to - ad13 and ad32 - to - distal break intervals are not known, a number of assumptions must be made to estimate the expected frequency of adenine-independent recombinants from i-ad13 // ad9 crosses: -

(1) The coincidence of double crossovers within the inverted region is one.

(2) The proximal break of the inversion is very near the ad33 site. (This will give an over-estimate of the proximal break - to - ad13 interval and thus exaggerate any reduction of crossing-over in the following calculations.)

(3) The unselected crossover takes place in the "X", not the "Y", interval (see Fig. 45). (This assumption seems reasonable since the cistron map (Fig. 51) and the data in Table 27 suggest that the break - to - ad13 interval is much larger than the ad32 - to - break interval.*)

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* But note that in mitotic recombination, a crossover at "X" or "Y" seems equally likely (cf. half-tetrad analyses of ad9 /i-ad13 and ad32 /i-ad13).

(4) The map distance between ad13 and ad9 (cf. cistron map, Fig. 51) is similar to that between ad13 and ad32.

(5) The n-ad13 - ad32 map distance equals the difference between the ad33 - ad32 and the ad33 - n-ad13 intervals (see Fig. 51).

The estimated recombination fraction for the n-ad13 - ad9 interval is 0.007 (i.e. the n-ad13 - ad32 interval on the cistron map, Fig. 51) and for the ad33 - ad13 interval is 0.018 (see Fig. 51). The expected proportion of all double crossovers (calculated on the assumption that C = 1.0) in these two intervals is 0.000126. Since only the two-strand and one type of three-strand double crossover could give viable adenine-independent recombinants, the expected proportion is 0.000063. In other words, with no suppression of crossing-over, with no increase in the coincidence of double crossing-over above the random expectation and with no chromatid interference, 0.000063 is the expected proportion of double crossovers based on adenine-independent recombinants from i-ad13 // ad9 . It will be noted that this value is quite close to those obtained for i and ii (corrected) in Table 26, even though the break - to - ad13 interval has probably been grossly over-estimated (see i-ad13 // ad17 meiotic analysis in section III). One can conclude that the coincidence of double-crossovers within the inversion, in a cross heterozygous for the ad13 inversion, is not less than one, and may be more.

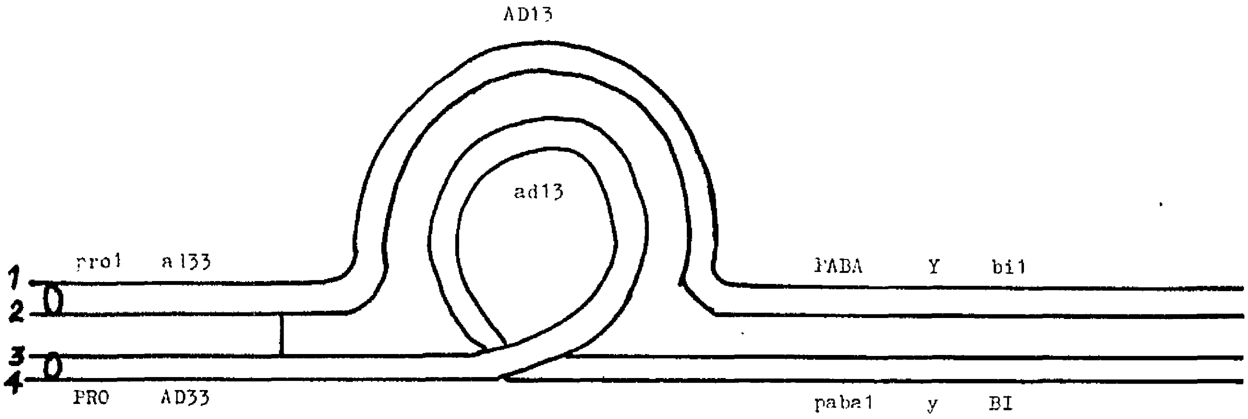
It should be emphasised that the ad13 inversion includes only part of the ad9 cistron, whereas all the other reported inversions are on a much larger scale, sometimes including practically a whole chromosome arm. Therefore, Dobzhansky's competitive pairing hypothesis (1931), invoked by Sturtevant and Beadle (1936) to explain the reduction of crossing-over within the shorter inversions they studied, is in conflict with the ad13 inversion data, as one would expect recombination within such a small inversion as i-ad13 to be very strongly suppressed.

Secondly, "classical" inversions generally suppress recombination in intervals adjacent to the inversion breakage points and sometimes throughout the rest of the chromosome arm, depending upon the size and type of rearrangement (cf. Sturtevant and Beadle, 1936; Grüneberg, 1935, Spurway and Philip, 1952, etc.). However, for certain inversion heterozygotes in maize and Drosophila there appears to be no reduction (Rhoades and Dempsey, 1953; Morgan, 1950; Russell and Burnham, 1950; Novitski and Braver, 1954). Rhoades and Dempsey (1953) found 16% recombination in the interval proximal to the In 3a inversion in maize (the Rg - to - inversion interval) when 4% was expected on the assumption that genetic recombination was effectively suppressed within the inversion and that the inversion permitted a normal exchange frequency in adjacent regions.

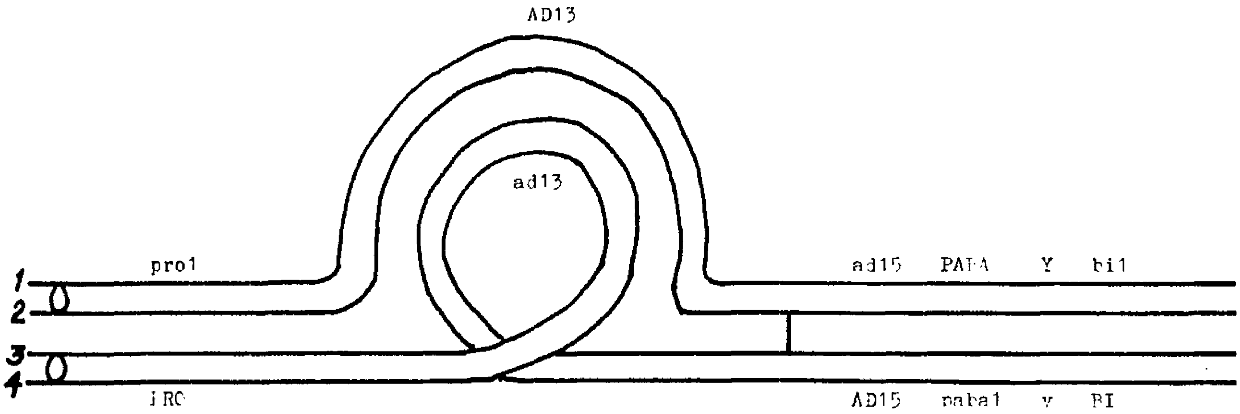
Non-selective meiotic analysis of a cross such as pro1 paba1 y // ad13 bi1 gave no indication of a change in recombination frequencies for the pro - ad and ad - paba intervals as compared to the "standard map" in Fig. 1 (Calef, unpublished; Martin-Smith, unpublished).

On the other hand, when selection is made for recombination within the ad13 inversion, in crosses or diploids heterozygous for i-ad13, there is a great increase of recombination in the regions adjacent to the inversion as compared to the "standard map" values (e.g. Tables 32 and 33). However, the pro - paba region is also greatly expanded in maps based on selective analyses not involving inversion heterozygotes (see section III). To ascertain the effect of the inversion on recombination in intervals

A. ad site outside inversion is proximally located



B. ad site outside inversion is distally located



Most Likely Types of Crossovers Selected in Diploids Heterozygous for i-ad13 and an Allele outside the Inversion (see text)

FIG. 49

adjacent to the inversion for the selected sample of the population (i.e. adenine-independent recombinants) it is necessary to compare the recombination fractions obtained from analysis of a normal cross (or a cross involving identical inversions) with a cross heterozygous for the inversion, where the selected crossovers take place in intervals of similar dimension and location with respect to outside markers. Consideration of this relation is deferred until the data relevant to negative interference is presented (see pp. 153, 160).

Thirdly, negative interference across an inversion was observed in mitotic recombination experiments and half-tetrad analyses of diploids heterozygous for the ad13 inversion when selection was made for recombination between an ad allele within the inversion and one outside it, e.g. in the ad33 / i-ad13 and ad15 / i-ad13 experiments.

In the case of ad33 / i-ad13, one would expect the most likely type of recombination between alleles to occur proximally to the inverted region, and in the case of ad15 / i-ad13, the expected crossover would be between the distal termination of the inversion and ad15 (Fig. 49). (Double crossovers would be required within the inverted region for the production of adenine-independent recombinants.) In both combinations the interval on the other side of the inverted region from the selected crossover, as well as the interval on the same side of the inversion as the selected recombination, shows an increase (Table 34).

The only reported case of negative interference across an inversion is that of In(X)1 in *Drosophila subobscura* (Spurway and Philip, 1952), where it was found that if a recombination occurred in one region immediately adjacent to the points of breakage, the coincidence of a second recombination in the other region was 26 times that expected on the basis of single crossovers observed in these regions. The In(X)1 inversion differs in many ways from i-ad13; it is about 100 map units long, covering about one third of the whole X-chromosome, recombination within the inversion is drastically reduced, and single crossing-over between the inversion and adjacent markers is also reduced as compared to the standard map. It seems reasonable to assume that the negative interference spanning i-ad13 has a different origin from that of In(X)1 in *D. subobscura* (see p.159; cf. Pritchard, 1960a).

4. Negative interference attributable to the ad13 inversion.

The question of whether selection for recombination between an allele within the inversion and one outside it is accompanied by an increased negative interference over and above the amount that could be attributed to the stringency of selection, can be attacked indirectly by a comparison of mitotic recombination data from ad33 / i-ad13 and ad33 / n-ad13 ad9 (or ad33 /n-ad13 ad32) diploids. Ideally, the map length of the ad33 - to - proximal break interval and the ad33 - to - n-ad13 interval should be identical in order to test the effect of the inversion on negative interference. For the former diploid it seems reasonable to assume that the selected crossovers are largely confined to the region proximal to the inversion. (Double crossovers, one on either side of the ad13 site, would be required within the inversion to give rise to adenine-independent progeny. It appears from the meiotic analyses described in the next section that the proximal - break - to - ad13 interval is only 1/1000th or less of the whole ad33 - n-ad13 interval. Thus, for the purposes of comparison, the intervals in which recombination is selected in the two diploids are approximately equal.

A comparison of the ratio of single to double and multiple crossover types would thus give an indication of the effect of the inversion on recombination in the unselected intervals. The actual comparison made was of the presumed single crossover class (bi or paba y, depending upon the parental diploid) -- which will include some double crossovers -- with the double and multiple classes of auxotrophs. This crude approximation will of course mask slight effects as the double crossover auxotrophs are underestimated and also the information from the prototrophic recombinants is not available without a half-tetrad analysis. Nevertheless, one finds an apparent association of high negative interference with the inverted sequence of the ad13 strand (Table 35).

Since the required meiotic analyses were not made it is not established that the effect is also found in meiosis (but see p.153).

5. A test for the existence of other intra-cistronic inversions.

Having found such a correlation between "additional" negative interference (i.e. that not attributable solely to the stringency of selection) and the ad13 inversion, a similar test was made in respect of the *ad13* / *ad17* combination.

For the purposes of this test the comparison was between *ad9* / *ad32*, *n-ad13 ad32* / *ad17* and *i-ad13* / *ad17* . Since it is not possible to obtain a *n-ad13* / *ad17* combination and an *i-ad13* / *ad17* diploid having the same map length for the selected interval, the *ad9* / *ad32* diploid was included as the "selection" negative interference control. The apparent map lengths of the *ad9* - *ad32* and *ad32* - *ad17* intervals are of the same order of magnitude (see Fig. 51), and the "true" map length of the latter interval is assumed to be larger (see section III). The results listed in Table 36 suggest that the *i-ad13* / *ad17* diploid owes its low negative interference, when compared with the *n-ad13 ad32* / *ad17* mitotic recombination results, to the *ad17* strain having a rearrangement identical to the *ad13* inversion.

Recombination analysis of *ad9* / *ad17* diploids (Table 12) suggests that a single crossing-over in the selected interval gives rise to viable recombinants. Thus, the *ad9* and *ad17* sites cannot *both* be within the inverted region. Since the *ad9* site is located within the *ad13* inversion one can conclude that the *ad17* site is outside the rearrangement, provided the ad13 and ad17 inversions are identical. In other words, it appears that the ad13 (and ad17) inversion terminates proximally to the ad17 site and that it spans the sites of ad13, ad9 and ad32.

It seems probable that the inversion arose prior to the induction of the ad13 and ad17 point mutations since both mutants were isolated from the progeny of a single sample of conidia exposed to UV irradiation (see Table 2).

It will be recalled that an inversion was postulated for ad15 strains (see also section III). This inversion apparently differs from the ad13 inversion, for the i-ad13 / ad15 combination gave the pattern of high nega-interference (Table 13) found with the n-ad13 ad32 / ad17 diploid (Table 36) -- i.e. the structural heterozygote -- and not the low level of interference associated with the i-ad13 / ad17 diploid (Table 36) -- i.e. the inversion homozygote. A control experiment to establish the amount of negative interference due to the stringency of selection was not performed. However, since the ad15 site is located distally to the ad17 site (see cistron map, Fig. 51), one would expect the selection in the case of the i-ad13 / ad17 diploid to be more stringent than that for the i-ad13 / ad15 diploid on the hypothesis that the ad13 and ad15 inversions are identical. Therefore, the i-ad13 / ad17 diploid acts as the control because negative interference becomes more intense when the selected interval becomes smaller (cf. Pritchard, 1960a). The hypothesis of identical inversions would require that the i-ad13 / ad17 diploid mitotic analysis showed at least as much "selective" negative interference as the i-ad13 / ad15 diploid analysis, yet the latter diploid was found to have the higher negative interference. It seems a justifiable conclusion that the ad13 and ad15 inversions are not identical.

The nature of the rearrangement carried by ad15 strains will be considered in the next section.

III. Meiotic Analyses and Construction of the Meiotic Map for the ad9 Cistron: -

A. Introduction.

The sequence of sites for the non-inverted arrangement of the cistron can be written as

ad33 - ad13 - ad9 - ad32 - ad17 - ad15 --- paba

on the basis of the data reported in sections I and II. (As the ad17 site appears to be located distally to the ad13 rearrangement -- see p. 131 -- it follows that it must also be located distally to the sites within the inversion. Hence, the ad17 site must be distal to the ad32 site in both the inverted and non-inverted sequences.)

The i-ad13 and ad17 strains would therefore have the following arrangement for the ad9 cistron:-

ad33 - ad32 - ad9 - ad13 - ad17 - ad15 --- paba .

The meiotic analysis results given below were obtained with three main purposes in mind: (1) to corroborate the linear orders shown above, (2) to determine the map distances between sites and to get a minimum estimate of the cistron size and (3) to ascertain the dimension of the ad13 inversion relative to that of the whole cistron. In addition, information pertaining to negative interference was obtained from some of the crosses made.

B. Results.

1. Crosses involving pairs of different *ad* alleles.

ad33 // n-ad13. The estimated recombination fraction for the *ad33* - *n-ad13* interval is 0.018 ± 0.011 (Table 37). Due to the extremely poor fertility of the cross, only three adenine-independent recombinants were found. Data with respect to negative interference was therefore unobtainable from this cross.

ad33 // ad32. Table 38 lists the data relevant to the estimation of the recombination fraction for this interval, which is 0.025 ± 0.00061. For technical reasons the phenotypic classification of recombinants was not made. However, *y* colonies were in excess of *Y* colonies, as would be expected for the *ad33* - *ad32* - *paba* sequence.

ad33 // ad17. Two replicate experiments give estimates for the *ad33* - *ad17* interval (Table 39) approximately 1/500th that obtained for the *ad33* - *ad32* interval, suggesting a reduction in the production of adenine-independent recombinants due to the rearrangement postulated for ad17 strains. It should be noted that even in the event that an inversion does not suppress crossing-over, the observable recombination within the rearrangement is virtually suppressed, since single crossovers would be inviable and double-crossovers within the inversion would still be adenine-requirers.

The phenotypic classification of recombinants confirms the ad33 - ad17 sequence because the pro paba y class is obviously the single (selected) crossover phenotype (Table 40). Calculation of recombination fractions for the unselected intervals (Table 41) indicates that the replicate experiments are heterogeneous with respect to recombination in the ad--paba interval. Both platings show a significant increase in recombination for the pro - ad interval (as compared to the standard map) and for the y - bi interval. Only in the second plating is there a significant expansion of the map in the ad - paba region. In both replicates the larger recombination fractions for the paba - y interval are not significantly different from the standard value.

ad33 // ad15. The recombination fraction estimate for the ad33 - ad15 interval (Table 42) is smaller than that for the ad33 - ad32 interval by a factor of 10. This finding is again consonant with the suggestion that ad15 strains possess a rearrangement for part of the cistron. Classification of recombinants (Table 43) corroborates the ad33 - ad15 - paba sequence established in the mitotic recombination studies. In contrast to the ad33 // ad17 cross, only the ad - paba interval appears to be expanded (although the increase is not significant) -- see Table 44.

ad9 // ad32. Data from these crosses have already been presented in the preceding section. Estimates for the ad9 - ad32 recombination fraction were given in Tables 22 and 24. Classification of recombinants from the ad9 // ad32 cross was listed in Table 23 and that from the n-ad13 ad9 // ad32 cross in Table 24. A comparison of recombination fractions for the unselected intervals in both crosses is given in Table 45. All platings gave significantly larger recombination fractions for the pro - ad and ad - paba intervals when compared with the standard values (Table 45). Recombination fractions for the unselected intervals, derived from the weighted and pooled data of Table 45, are shown in Table 46. The increases in intervals 1 (pro - ad) and 2 (ad - paba) are significant, while those in 3 (paba - y) and 4 (y - bi) are not, when comparison is made with the standard map (Table 46).

ad9 // ad15. This cross gives an estimated recombination fraction for the ad interval of 9.3×10^{-5} (Table 47). The phenotypic classification of recombinants (Table 47) indicates an ad9 - ad15 - paba sequence. Negative interference was detected in only the pro - ad interval. The sample size is too small to warrant any conclusion with respect to recombination in other intervals.

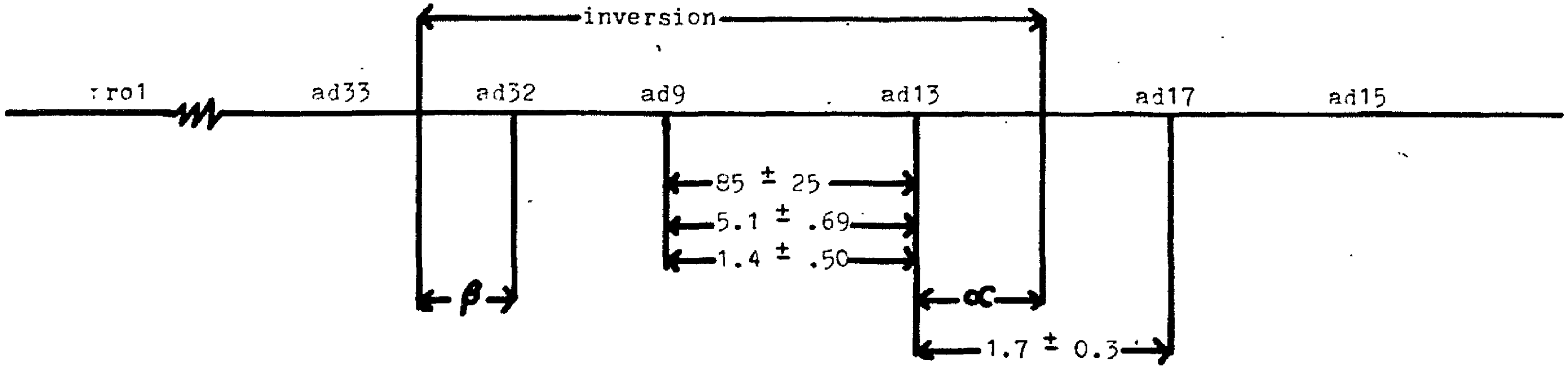
ad32 // ad17. Analysis of crosses between these alleles is technically difficult due to the fact that unreduced ascospores occur at a higher frequency than adenine-independent recombinants and grow equally well as the latter. Recombinants were detected on the basis of greater conidiation and differences in colour intensity and morphology of conidial heads. As it is likely that some recombinants were not discerned by this method, the estimated recombination fractions given in Table 48 may well be underestimates of the true value. Phenotypic classification of recombinants from reciprocal crosses (with respect to markers distal to the ad9 cistron on chromosome I), given in Table 49, suggest that viability disturbances have occurred in these analyses. In cross A, the unlinked PYRO marker is recovered in significant excess over pyro; in cross B, it is w (also unlinked) that is found in significant excess of W. The complete absence of the pro paba y class in Cross B recombinants is very puzzling, as is the great excess of the paba (y)-w class over paba y. Since it is impossible to choose between various possible explanations of these observations, one can regard the data as giving only tentative indication of high negative interference in the pro - ad and ad - paba intervals. Further, the data neither support nor contradict the ad32 - ad17 - paba sequence deduced from the inversion studies, apart from the excess of the paba over the bi marker among recombinants in Cross B being a possible indication that ad17 is the distal allele.

Employment of ad32 strains bearing another ad allele situated proximally to ad17 and ad32 would overcome the difficulties involved in analysing ad32 // ad17 crosses caused by the occurrence of non-adenine requiring ad32 / ad17 diploid ascospores. As recourse must be made to half-tetrad analysis to obtain such strains, only n-ad13 has so far been recovered in coupling with ad32. Now that the ad13 site has been shown to be proximal to ad32, such a strain could be utilised in meiotic analysis of ad32 // ad17.

ad32 // ad15. Estimates of the recombination fraction for this interval, obtained from two different crosses, proved to be homogeneous; the combined estimate is 0.000015 ± 0.0000013 (Table 50). Classification of recombinant phenotypes corroborates the previously assigned ad32 - ad15 - paba order (Table 51). The map lengths of the regions adjacent to the selected crossover are dramatically increased (Table 52), with the recombination fraction for the ad32 - paba1 interval a hundred-fold greater in the selective analysis of ad32 // ad15 than the value obtained by AD PABA selection from an ad32 // paba1 cross (Table 60).

ad17 // ad15. As Calef (1957) had carried out an intensive investigation of negative interference among the adenine-independent progeny of crosses involving these two alleles, the purpose of the present analysis was to obtain an estimate of the recombination fraction for the ad17 - ad15 interval, which is 0.000014 ± 0.0000018 (Table 53). The phenotypic classification of recombinants (Table 54) is consistent with the ad17 - ad15 - paba sequence previously established by Calef (1957). A comparison of recombination fractions derived from the data in Table 54 with the standard values for the unselected intervals (see Table 55) indicates a significant increase in the ad15 - paba region among the adenine-independent recombinants. Because of the small number of recombinants analysed, the increase in the paba1 - y interval is not significant.

A summary of Calef's (1957) results is given in Table 56, from which it can be seen that selective analysis apparently causes a lengthening of all four non-selected intervals. These data indicate in addition (a) that recombination fractions for the pro - ad interval, derived from non-selective analysis, are homogeneous and in agreement with the standard value published by Käfer (1958) for the pro1 - paba1 interval and (b) that recombination fractions for the paba - y and y - bi intervals, also from non-selective analysis, are heterogeneous and appear in some crosses to be markedly reduced



Meiotic Map of the i-ad13 Inversion
(recombination fractions X10^5)

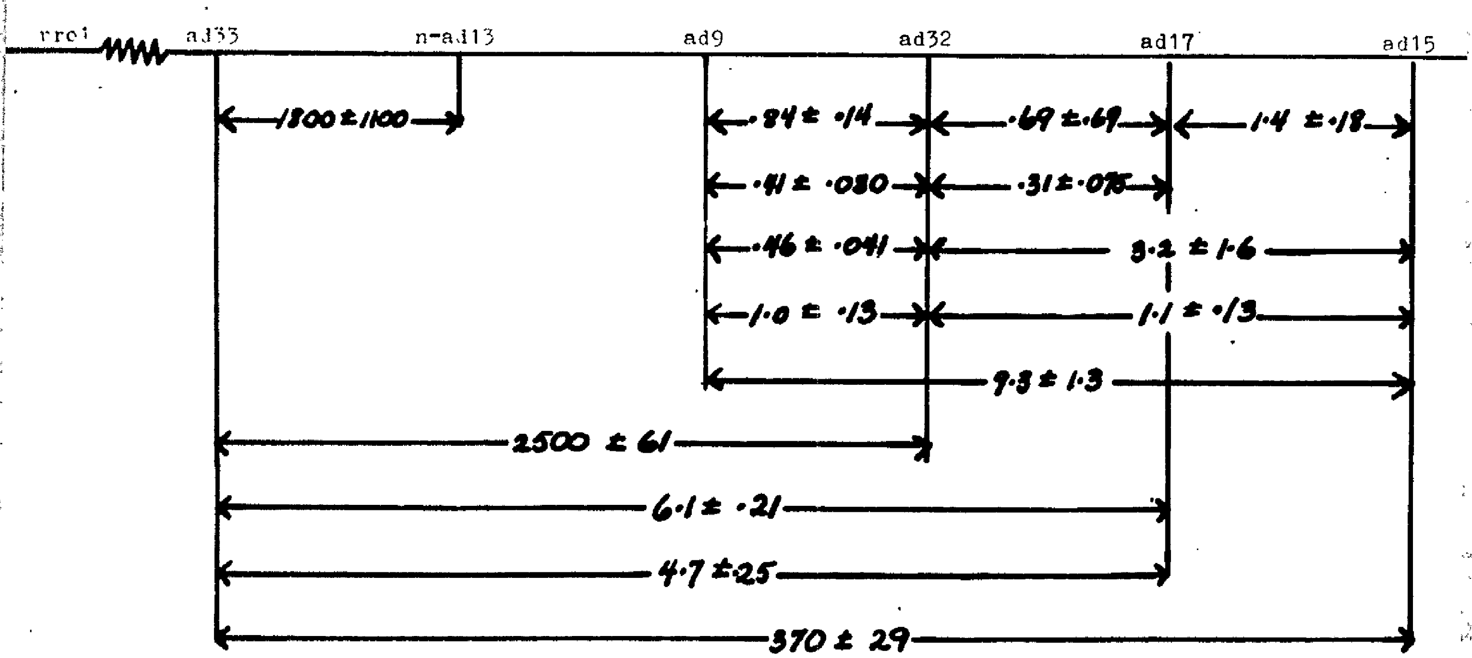
FIG. 50

as compared to the standard values.

This heterogeneity of recombination in the *paba* - *bi* region suggests that the recombination fraction estimate for the *ad17* - *ad15* interval (Table 53) might be an over-estimate. However, should the true recombination fraction for the *paba* - *bi* interval in this cross be reduced to as little as one third of the standard value (cf. Cross A, Table 56), the estimated recombination fraction would be reduced only from 14×10^{-6} to 4.7×10^{-6}.

i-ad13 // ad17. The estimated recombination fraction for the *i-ad13* - *ad17* interval (Table 57) is a measurement of the map "length" between the *ad13* and *ad17* sites in the *i-ad13* inverted sequence, granted that *ad17* strains also possess the *i-ad13* inversion. The maximum estimate of α (see Fig. 50), that part of the inversion corresponding to the interval between the proximal break point and the *ad13* site in the normal sequence, is then equivalent to the *i-ad13* - *ad17* interval recombination fraction.

The proximal break appears to have occurred very near to the *ad13* site, relative to the whole *ad33* - *n-ad13*



Meiotic Map of the ad9 Cistron
(recombination fractions X10^5)

FIG. 51

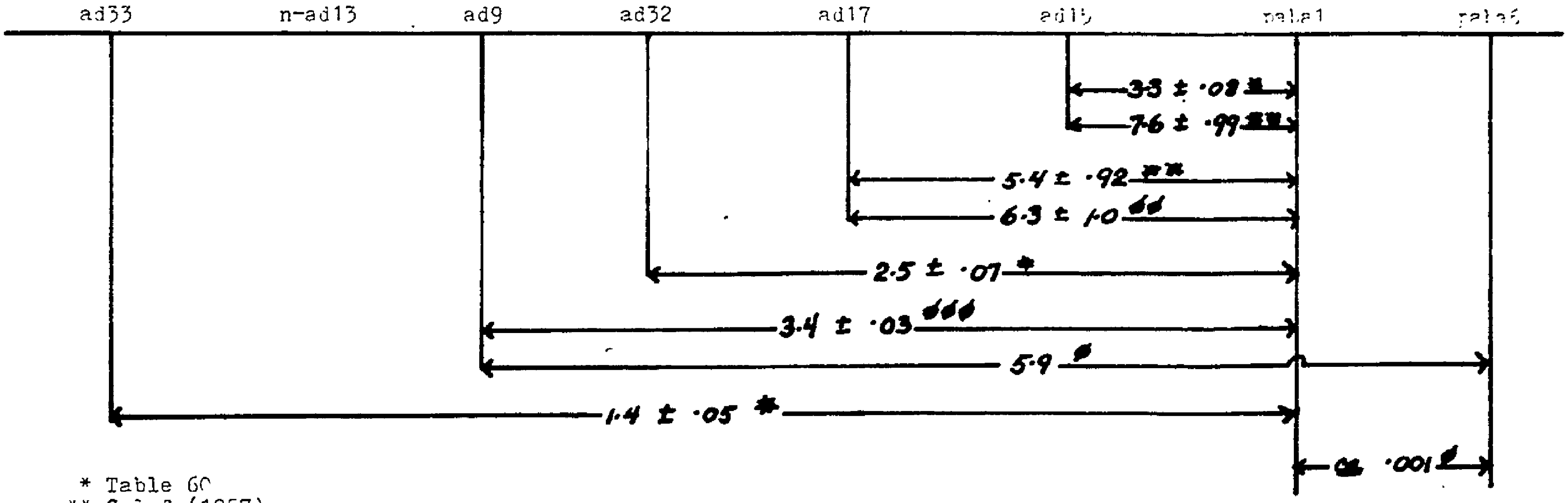
interval, as α is estimated to be $\leq$ 1/1000th the total ad33 - n-ad13 interval map distance.

The phenotypic classification of adenine-independent recombinants from this cross (Table 58) confirms the i-ad13 - ad17 - paba order derived from mitotic analysis. The small number of these recombinants permits only a rough comparison between recombination fractions for non-selected intervals with the standard values (Table 59). Even with this limited sample, however, the lengthening of the pro - i-ad13 interval is significant, and the data suggest that the ad17 - paba region has also been enlarged as compared to the standard map.

i-ad13 // ad9. Crosses involving these two alleles have already been considered in the previous section (see Tables 26, 27 and 32).

2. ad // paba1 crosses.

Owing to the non-additivity of recombination fractions obtained for the ad33 - ad32, ad33 - ad17 and ad33 - ad15 intervals (Fig. 51) -- due presumably to rearrangements present in the ad17 and ad15 strains -- no estimation of the length of the ad9 cistron can be made



* Table 60
** Calef (1957)
Ø Siddiqi (unpublished)
ØØ Elliott (1960b)
ØØØ Pritchard (1956)

<u>Meiotic Map of the ad - paba Intervals</u>
(recombination fractions X10^3)

FIG. 52

from the foregoing analyses. However, the cistron appears to be larger than the ad15 - paba1 interval (cf. recombination fraction of 0.025 for the ad33 - ad32 interval and Calef's (1957) estimate of 0.0076 for the ad15 - paba1 interval). It was expected that selective analysis of ad // paba1 crosses should be sensitive enough to provide at least a crude estimate of the minimum length of the ad9 cistron.

Table 60 gives the estimated recombination fractions for the ad33 - paba1, ad32 - paba1 and ad15 - paba1 intervals (see also Fig. 52). The three estimates are significantly different from one another, but, contrary to expectation, the ad33 - paba1 interval has the smallest recombination fraction.

The estimate of the ad33 - ad32 interval is about 20 times as large as that for the ad33 - paba1 interval. This discrepancy cannot be explained by possible technical errors due to contamination and/or viability disturbances in the determination of viable ascospores of hybrid origin (the *h* fraction, Table 4). Admittedly, the *h* fraction derived from the ad33 // n-ad13 ad32 cross (Table 37) is extremely low and the recombinants from the ad33 // n-ad13 ad32 and ad33 // ad32 crosses were not classified. However, the recombination fractions determined from these two crosses are additive (Fig. 51), and it seems unlikely that this is fortuitous.

In the case of the ad // paba1 crosses, an overestimation of the h fraction would result in an underestimation of the recombination fractions. This is also unlikely to be the source of disagreement, as the fractions derived from the present experiments are of the same order as those obtained by other workers (see Fig. 52).

Neither does possible variability of recombination in the paba - bi region account for the discrepancies. Departures from the standard value of the magnitude required to bring the intra-cistron and ad - paba maps into line have not been detected in non-selective analysis of ad // paba1 crosses (cf. Calef, 1957; Elliott, 1960b).

Classification of the AD - PABA segregants from the three crosses is given in Table 61, and the recombination fractions derived therefrom are in Table 62. In all three crosses the unselected intervals are expanded as compared to the standard map (Table 62). The standard recombination fraction given for interval a (pro - ad) is that for the pro1 - paba1 interval, which is necessarily physically larger than the various pro - ad intervals. However, examination of non-selective analysis data for the pro1 - ad17 and pro1 - ad15 intervals obtained by Calef (1957) and Elliott (1960b) indicates that the genetic lengths of those intervals do not differ significantly from the pro1 - paba1 value. Indeed, the majority of determinations made by Calef and Elliott suggest a slightly higher value for the two pro - ad intervals. Thus, in each cross selective analysis is associated with an increase in interval a of about 0.05*, in interval c of about 0.04 - 0.07 and in interval d of about 0.03 - 0.04, as compared to the standard recombination fractions.

As non-selective analyses were not made of these crosses it might be argued that the apparent increases due to selective analysis merely reflect a total increase among all the progeny of these crosses as compared to the average found among the crosses upon which the standard values are based. However, Calef (1957) and Elliott (1960b) found similar increases when comparisons were made

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* This holds for the ad33 // paba1 cross only if 0.025, the map distance between ad33 and ad32, is deducted from the standard pro1 - paba1 estimate to obtain an approximation of the standard pro1 - ad33 value.

between data obtained in selective and non-selective analyses of one population of ascospores (Table 63), thus suggesting that the present data reflect heterogeneity _within_ populations (viz. the _AD - PABA_ sample is not equivalent to the whole population of "hybrid" ascospores as regards recombination in intervals _a_, _c_ and _d_) rather than heterogeneity _between_ populations (viz. variation in recombination for _a_, _c_ and _d_ when "hybrid" progeny of different crosses are compared).

C. Discussion and Conclusions.

1. Estimates of recombination between ad alleles and paba1 (Fig. 52).

The recombination fractions for the _ad33_ - _paba1_, _ad32_ - _paba1_ and _ad15_ - _paba1_ intervals (Table 60) are significantly different and vary inversely to the length of the _ad_ - _paba1_ interval. Similarly, Calef (1957) obtained a larger value for the _ad15_ - _paba1_ interval than for the _ad17_ - _paba1_ interval, although the difference is not significant ($X_1^2 = 2.6626$*). In addition, the

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* The equation for determining the standard errors of Calef's estimates was kindly derived by Dr. J.S. Gale.

recombination fraction for the ad33 - paba1 interval is about 1/20th that of the ad33 - ad32 interval. Two hypotheses could be advanced to account for these results: -

(a) The larger recombination fraction for the intra-cistronic interval, as compared to the ad33 - paba1 estimate, might be thought to favour a branched chromosome model. On this model, recombination between ad mutants and paba1 would occur on the main chromosome axis, while intra-cistronic recombination would occur on the branches (cf. Schwartz, 1955, 1958; Taylor, 1957, 1958b; Roman and Jacob, 1958). Thus, one would expect to find no differences between recombination fractions for the various ad - paba intervals. Nor should there be any correlation between intra-cistronic recombination and recombination between outside markers.

This hypothesis must be rejected, as the existence of a branched chromosome is disproved by the fact that the sites within the ad9 cistron can be arranged in a linear array on the basis of the distribution of outside markers among the recombinants from inter-allelic crosses and heterozygous diploids (cf. Pritchard, 1960a and b).

(b) An assumption that the paba1 strains possess a rearrangement proximal to paba1 and including some portion of the ad33 - paba1 interval would explain the discrepancy between the ad9 cistron map and the ad - paba1 map. It is tempting to think that the inverse relationship observed in the ad // paba1 crosses is an expression of the effects of structural heterozygosity, were the paba1 strains to have an inversion located proximally to the ad15 site.

It will be recalled that paba1 has been used in all the mitotic and meiotic mapping experiments described in this thesis. The possibility therefore exists that the inversion proposed for paba1 strains was introduced into some of the ad strains in conjunction with the paba1 marker. However, there is no indication from the mitotic and half-tetrad analyses of any rearrangement associated with the paba1 marker. Indeed, the ad13, ad15 and ad17 inversions were shown to be independent of the arrangement of outside markers.

2. The meiotic map of the ad9 cistron (Fig. 51).

The sequence established by mitotic means was corroborated by the meiotic analyses, except for the relation of ad32 with respect to ad17. In the latter case no order could be determined from the ad17 // ad32 crosses (Table 49).

The apparent reduction of inter-allelic recombination in the ad33 // ad17 and ad33 // ad15 crosses, as compared to the ad33 // n-ad13 and ad33 // ad32 crosses, is consistent with the view that the ad17 and ad15 strains possess rearrangements for part of the ad9 cistron. Because of this reduction, however, an estimate of the minimum length of the cistron must be based on the recombination fraction for the ad33 - ad32 interval, viz. 0.025.

Owing to the complications involved in the ad // paba1 crosses, no information was obtainable with reference to the size of the ad32 - ad15 interval relative to the proximal portion of the ad9 cistron. However, Siddiqi's analysis (unpublished) of a cross involving ad9 and paba6 suggests that the largest part of the cistron lies between the ad33 and ad9 sites (see Fig. 52). Thus, 0.025 can be taken as a rough estimate of the minimum size of the cistron.

The ad13 (and ad17) inversion appears, therefore, to occupy a considerable portion of the cistron. Taking 0.025 to be the minimum cistron length and 0.007 the size of the n-ad13 - ad32 interval (see Fig. 51), the ad13 inversion would be about 0.3 times the minimum cistron length if α and β (i.e. the terminal portions of the inversion, see Fig. 50) are negligible in comparison to the marked part of the inversion. It does seem from the i-ad13 // ad17 data that the proximal break occurred relatively close to the ad13 site. Siddiqi's unpublished data (for ad9 // paba6) would indicate that the β interval is also probably small in relation to the ad13 - ad32 segment.

It is of interest that the ad9 cistron is apparently at least ten times larger than the estimated lengths of other cistrons investigated in Aspergillus nidulans (cf. Pritchard, 1960a). Whether this difference reflects differences in the amount of genetic material contained in the ad9 cistron on the one hand and the "typical" cistrons on the other, is an open question.

Let us assume, for a moment, that the number of amino-acid residues coded by two cistrons, differing greatly in genetic length, is known. If the two cistrons were found to determine polypeptide chains containing approximately the same number of amino-acids, one would probably conclude that genetic length is not necessarily a reflexion of the amount of genetic material in a cistron.

On the other hand, if a correlation were found between amino-acid residues and genetic length, one would conclude that genetic lengths do reflect the amount of genetic material in a cistron. However, there are several possibilities to be considered which could obscure the true relationship:- (1) non-random distribution of recombination, which might give rise to recombinational "hot spots," (2) presence of undetectable portions of some cistrons due to the absence of analysed mutants bounding them and (3) the existence of genetic material in some cistrons having some function other than coding for amino-acid sequences in the polypeptide chains supposedly made by those cistrons.

3. The ad15 inversion.

The apparent reduction of recombination in the ad33 - ad15 interval confirms the prediction that ad15 strains carry a rearrangement for part of the ad9 cistron (see p.136). It is an obvious deduction from the classification of recombinants of the ad9//ad15, ad32//ad15 and ad17//ad15 crosses that the ad15 inversion does not include both the ad15 site and the ad9, ad32 or ad17 site. Moreover, the mitotic recombination data from ad33 / ad15 (Table 12) and the half-tetrad analysis of the ad32 / ad15 and i-ad13 / ad15 diploids (Tables 64 and

20) suggest that single crossovers between the ad32 (or ad13) and ad15 alleles produce viable recombinants.

One can conclude, therefore, that the ad15 inversion does not span both the ad15 site and any of the other five mutant sites (viz. ad33, ad13, ad32, ad9, ad17). Apart from an indication that the ad15 and ad13 inversions are not identical (see pp. 131-132), the extent and position of the ad15 inversion are unknown.

4. Negative interference and the effective pairing hypothesis.

In all the crosses analysed the genetic length of linkage group I (Fig. 1) appeared to be expanded for the adenine-independent recombinants (from ad // ad crosses) and the adenine-, p.a.b.a.-independent recombinants (from ad // paba crosses) when compared to the standard map. For the most part the increases seemed to be confined to those regions adjoining the selected intervals. However, in most cases the paba - y and y - bi intervals were also lengthened (usually not significantly).

The ranking of the available data according to the recombination fractions obtained for the selected intervals shows a general trend towards increased length of adjacent intervals with decreased length of the selected interval (Tables 65 and 66). Pritchard (1960a) found a similar relationship for his ad8 cistron studies.

The relationships for the distal interval (Table 66) appear to be complicated. The data are too meagre to justify any detailed interpretation; nevertheless, the larger increases observed in the ad32 // ad15 and ad17 // ad15 crosses (as compared to those found for the ad9 // ad32 crosses, for example) would be consistent with the view advanced in connection with mitotic recombination concerning inversion effects on negative interference (see pp. 128-132). That is, inversion heterozygotes capable of producing viable single crossovers (in the selected interval) appear to produce a higher level of negative interference than structural homozygotes having a selected interval of the same dimension.

A comparison of the i-ad13 // ad9 (structurally heterozygous) and i-ad13 // ad17 (structurally homozygous) crosses would suggest that, while there might be an added negative interference operating over the pro - ad interval in the inversion heterozygote, the inversion effect does not extend into the distal interval. This sort of finding is predicted by the effective pairing hypothesis (Pritchard, 1955; 1960a, b) and will be considered later.

The coincidence of recombination between very closely linked markers and further recombination in adjacent intervals at a greater than random frequency appears to be a common phenomenon, having been observed, for example, in _Aspergillus_ (Pritchard, 1955, 1960a and b; Calef, 1957; Siddiqi, unpublished), in _Neurospora_ (St. Lawrence, 1956; Mitchell, 1956; Freese, 1957a and b; DeSerres, 1958; Pateman, 1960b), in yeast (Leupold, 1958), in bacteriophage, (Streisinger and Franklin, 1956; Chase and Doermann, 1958) and in _Drosophila_ (Chovnick, 1958; Green, 1960). As the various hypotheses which have been advanced to explain this localised negative interference have been discussed recently in some detail (see Pritchard, 1960a and b), it will suffice to summarise briefly the three types of mechanism that have been proposed and to consider their applicability to the data obtained in respect of the _ad9_ studies.

a. _Gene conversion_. Gene conversion in the Lindegren sense of directed mutation (Lindegren, 1955) can be disregarded, for the intensity of negative interference shows a correlation with the recombination fraction of the selected interval and not with particular alleles (cf. Pritchard, 1960a). In an analysis of tetrads from two crosses involving either ad15 or ad17 (Strickland, 1958a and b) there is no evidence for a directed-mutation type of conversion. Indeed, Strickland's data indicate that deviation from 2:2 allele ratios in *Aspergillus* tetrads is a rare event; of the 17 abnormal asci found in a sample of 1642 fully classified tetrads only three could not be accounted for by contamination. Strickland (1958b) concludes that two of these abnormal asci could have resulted from an extra mitotic division followed by loss or degeneration of some of the ascospores, while the third is an example of "conversion" in *Aspergillus nidulans* (see also Pontecorvo, 1958a).

Gene conversion by a non-reciprocal replication mechanism is favoured by the correlation found in *Neurospora* tetrad analysis between recombination and 3:1 allele ratios (e.g. Freese, 1957a; DeSerres, 1958 ; Case and Giles, 1958a and b). Pritchard (1955, 1960a and b) concludes that such a mechanism occurs at too low a frequency in the ad8 cistron to account for the intense negative interference observed. Strickland's studies (1958b) suggest the same interpretation in respect of the ad9 cistron. Although one tetrad from an ad17 cross apparently had a portion of the ad17 parental chromosome (from the centromere distal to a crossover in the ad17 - paba1 interval) represented three times, no instance of recombination in the pro - ad interval associated with conversion was found in 765 tetrads from crosses involving ad15 or ad17. In the ad15 cross, for example, 80 tetrads (out of 577) were recombinant in this interval (Strickland, 1958a). This would indicate that the high negative interference observed in the pro1 - ad17 region among adenine-independent progeny of ad15 // ad17 crosses (Table 66) could not be explained by "copy-choice" conversion. (Too few asci were recombinant in the ad - paba1 interval in Strickland's analysis to allow one to make a direct comparison between his data and the negative interference studies of the ad9 cistron.)

b. Discontinuous pairing. The existence of discontinuously paired chromosome regions to which recombination is confined has been advanced by Pritchard (1955, 1960a, b), Calef (1957) and Chase and Doermann (1958) as the probable basis of localised negative interference. The argument is as follows: -

The observation is that, compared with the total progeny population, that part of the population which is recombinant for a very small interval b are also more often recombinant for intervals a and c, which are adjacent to interval b. This is the consequence of only a small proportion of the total meioses from which the total population is derived having "effective pairing segments" in intervals a and c, whereas the selected sample arose from those meioses in which the effective pairing occurred in interval b. Pairing in b sometimes extends into interval a and/or interval c. As pairing is a limiting factor in recombination, recombinants for interval b are more likely to be recombinant also for interval a or c than is a progeny sample upon which less or no restriction is placed upon the types of pairing segments permitted. Moreover, the smaller the interval b, the greater the restriction placed upon the types of effective pairing segments and consequently the greater is the increase in intervals a and c among the selected sample.

Pritchard (1960a) has elegantly demonstrated that effective pairing is one of the limiting factors in observable recombination, that the segments have a mean length of 0.4 map unit and a variable (probably random) distribution. Calef (1957), on the other hand, postulates that the effective pairing segments are highly variable in length and that some are at least 15 map units long. Pritchard's data (see 1960a) are not in agreement with effective pairing segments of this dimension. The disagreement between these estimates can be overcome by application of a third type of hypothesis (*vide infra*).

c. *Pooling of heterogeneous data.* Sturtevant (1955) suggested that negative interference could be simulated by pooling data from a heterogeneous population in which some nuclei showed a high frequency of crossing-over per cell and others a low frequency. High coincidence values would be found when the selected sample (selected because of one recombination) was compared with the total population. One would not expect to find, were such heterogeneity the only factor involved, negative interference localised to the intervals adjacent to the selected recombination.

That a non-localised negative interference does occur in *Aspergillus* has been demonstrated by Elliott (1960b), who found (1) that selection for recombination in the ad17 - paba1 interval is associated not only with an increase in adjacent intervals but also with smaller increases in a non-adjacent interval and an interval on another chromosome and (2) that selection for recombination between loosely linked markers produces a slight lengthening of the genetic map. Elliott's studies suggest that there is a weak correlation between recombination throughout the whole nucleus. Interference over long distances found in other organisms, e.g. *E. coli* (Rothfels, 1952; Cavalli-Sforza and Jinks, 1956) and in an inversion heterozygote of *D. subobscura* (Spurway and Philip, 1952; see p. 127, this thesis), may also have the same basis.

One can conclude in respect of the ad9 investigations that population heterogeneity is, first, a minor factor involved in the production of the high negative interference associated with selection for rare recombinant types and, second, probably responsible for the slight increases observed for the regions into which the selected effective pairing segments of the dimension proposed by Pritchard would not extend.

The meiotic data presented in this section are generally consistent with the effective pairing hypothesis.

However, it appears that inversions are also a factor to be considered. The comparisons of negative interference in structurally homozygous diploids with that in heterozygous diploids (Tables 35 and 36, see pp. 128 - 132) lend support to the contention that an inversion within an effectively paired region can have a positive influence on recombination. (See p. 153 for discussion of the comparison of meiotic data.) This contention is compatible with the effective pairing hypothesis: - If one accepts that recombination takes place at the time of chromosome replication (cf. Belling, 1931), it does not seem unreasonable that a prolongation of contact between homologues might result when an effective pairing segment includes a region of structural heterozygosity. The apparent absence of unequal crossing-over in Aspergillus (cf. Pritchard, 1955) suggests that pairing is exact. A point-for-point alignment of an inverted region with the non-inverted region of the homologous chromosome would produce a loop configuration which could possibly favour more switches from one template to the other than in the case of structurally homozygous effective pairing segments, by virtue either of longer contact or of alteration in the speed and ease of replication.

It should be pointed out that the much smaller increase in the ad - paba interval for the i-ad13 // ad9 cross as compared with the ad32 // ad15 cross (Table 66, see also p. 153) does not detract from the proposed effect of inversions on negative interference. Firstly, in the former cross double crossovers in the inversion - ad13 and ad13 - ad9 intervals were selected, thus shifting the mid-point of the mean effective pairing segment proximally as compared to the latter cross. Secondly, the selected interval in the former cross is probably much larger than the latter since the "recombination estimates" for the i-ad13 // ad9 crosses are actually estimates of the frequency with which adenine-independent recombinants arise as the result of double crossing-over within the inversion (see pp. 101-103).

Maguire (1960) questions the validity of the effective pairing hypothesis on the grounds that there is a strong correlation between reduction in chiasma frequency and reduced cytologically-observable pairing in structural heterozygotes (Dobzhansky, 1934). She states that it is not obvious "...why such regions should tend to escape segmental pairing of the sort postulated by

Pritchard."

The test of the hypothesis is not to be found at the cytological level (cf. Pritchard, 1960b). The relevant question is whether or not pairing is repressed at the genetic level. Apparently some large ("macro") inversions do suppress recombination, both inside and outside the inverted regions (see pp. 121 - 127). It has already been pointed out that much of the apparent reduction within many "macro" inversions is due to the inability to recover single crossovers and certain types of double crossovers. Moreover, suppression of extra-inversion recombination varies from species to species and from one inversion to another. Novitski and Braver (1954) have shown that this suppression can be reversed by changing the environment of the inversion, in their experiments by supplying heterochromatin. This suggests that the distribution of different effective pairing segments within a cell is easily influenced by factors not directly resulting from structural heterozygosity (cf. effect of temperature on non-localised negative interference in Aspergillus, Elliott, 1960b).

A consideration of "macro" inversions is therefore really an investigation of what effect they, plus other factors which may have been introduced in conjunction with inversion formation, exert on the distribution of unrelated effective pairing segments. Intra-cistron inversions, on the other hand, provide a means of testing whether or not pairing is inhibited in a portion of a segment <u>potentially</u> able to pair -- i.e. that which would have a high probability of pairing in a <u>selected</u> sample from a structurally homozygous population. The comparison is made between two samples selected for recombination in an interval shorter than the effective pairing segment. One sample is derived from a population heterozygous for an inversion which either includes the selected interval or is less than an effective-pairing length away from it. The other is from a structurally homozygous population. If pairing is not inhibited, the "inverted" sample should not show less recombination in the intervals adjacent to the selected region than the control sample. Indeed, this is what was found with respect to the <u>ad9</u> cistron inversions (see pp.128-132).

5. <u>The relation between negative interference in meiosis and mitosis and its bearing on the effective pairing hypothesis.</u>

The pattern of negative interference in mitotic recombination is similar to that observed in meiosis (cf. Pritchard, 1955). The much lower frequency of effective pairing during the parasexual cycle could account for the great difference in recombination frequency in the mitotic as compared to the meiotic system.

Half-tetrad analysis of two diploids, one of which has the selected interval shifted distally with respect to the other (Table 67), indicates that when the selection is moved away from the pro - ad interval, negative interference in that interval is reduced, while that in the distal interval (i.e. ad - paba1) is increased. This sort of result is consonant with a discontinuous pairing model (cf. Pritchard, 1960a).

The difference in interval lengths on the mitotic and meiotic chromosome maps (Pontecorvo and Käfer, 1958) can be interpreted as the result of differing distributions of effective pairing segments during meiosis and mitosis.

The non-localised negative interference associated with selection for mitotic recombination in large intervals (cf. Pontecorvo and Käfer, 1958) probably reflects the heterogeneity in the amount of mitotic crossing-over per nucleus. This heterogeneity is magnified when a comparison is made with meiotic recombination because of the greater rarity of effective pairing segments in the parasexual cycle.

IV. Summary: -

1. Mitotic recombination analyses of all non-complementing allele combinations permitted the ordering of some of the alleles. However, a unique linear sequence could not be established, as the four combinations, ad13 / ad9, ad13 / ad32, ad13 / ad15 and ad15 / ad32, gave anomalous results.

2. The anomalous results could be explained if inversions were present in some of the strains from which the four combinations were derived.

3. It was concluded from half-tetrad analyses and meiotic studies that ad13 strains apparently possess an inversion spanning the sites of ad13, ad9 and ad32, with the non-inverted sequence of these sites as ad13 - ad9 - ad32.

4. After elucidation of the ad13 inversion it was possible to establish the unique linear sequence

   ad33 - ad13 - ad9 - ad32 - ad17 - ad15

   for non-inverted strains.

5. ad17 strains apparently contain an inversion identical with the ad13 inversion.

6. ad15 strains probably have an inversion. It may differ from the ad13 inversion. The extent

of the ad15 inversion could not be determined as the ad15 site, on the one hand, and the rest of the mutant sites, on the other, cannot both be within the inversion.

7. The ad13 inversion does not seem to produce a detectable mutant phenotype.

8. The allelic sequence suggested by the mitotic recombination and inversion studies was corroborated by the meiotic analyses. It was found that the intra-cistronic inversions reduce the apparent map length of the ad9 cistron, although they may not reduce the frequency of crossing-over.

9. Data from ad // paba1 crosses suggested that the paba1 strains used carry a rearrangement which includes part of the ad9 cistron.

10. The localised negative interference observed following meiotic recombinant selection is consistent with the effective pairing hypothesis (Pritchard, 1955).

11. Inversions were shown to increase negative interference in mitotic and possibly also in meiotic recombination. It was suggested that this relationship is compatible with the effective pairing hypothesis.

APPENDIX A.

TABLES 1 - 67 (INCLUSIVE)

Table 1

Meaning of mutant symbols

Symbol of mutant	Symbol of wild type	Phenotype determined by mutant
y	Y	yellow conidia
w	W	white conidia (epistatic to Y / y)
acr	ACR	recessive resistance to acriflavine
ad	AD	adenine requirement
bi	BI	biotin requirement
paba	PABA	p-aminobenzoic acid requirement
pro	PRO	proline or arginine requirement
pyro	PYRO	pyridoxin requirement

Table 2

Origin of adenine mutants used

Mutant ø	Strain irradiated with UV	References and remarks ** (see also Käfer, 1958)
ad9	bi1	(1); self-sterile and largely sterile in crosses with ad13, ad15 and ad17 (3).
ad13	"	an allele of ad9 (1), (2); self-sterile and largely sterile in crosses with ad9, ad15 and ad17 (3).
ad15	"	an allele of ad9 (1), (2), self-sterile and largely sterile in crosses with ad9 and ad13 (3); ad15 // ad17 fertile (2).
ad17	"	"
ad32 øø	bi1; w3	S9C39 isolated 1954 following starvation (4); closely linked with paba1 (3).
*ad33	"	S9C8 isolated 1954 following starvation (4).

* Not listed in Käfer (1958)

** Reference numbers refer to:- (1) Pritchard, 1956; (2) Calef, 1957; (3) Calef, unpublished; (4) Sneath, unpublished.

ø ad9, -13, -15 and -17 were isolated from one starvation experiment and ad32 and -33 from a second starvation experiment.

øø Käfer (1958) incorrectly states that either Calef (1957) or Pritchard (unpublished) found ad32 to be allelic with ad9 -- see Chapter II, this thesis.

Table 3

Determination of colour genotype of white haploids obtained after haploidisation of adenine-independent diploid recombinants

I. ad haploids

Colour of known ad component of 'reconstituted' diploid	Colour of 'reconstituted' diploid	Colour sectors on 'reconstituted' diploid	Colour marker in ad haploid
yellow	yellow	-----	y
yellow	green	-----	Y
green	green	yellow	y
green	green	no yellow	Y *

II. AD haploids

Colour allele carried by ad component of recombinant diploid ∅	Colour of recombinant diploid	Colour sectors on recombinant diploid	Colour marker in AD haploid
-----	yellow	-----	y
y	green	-----	Y
Y	green	yellow	y
Y	green	no yellow	Y *

* checked by outcrossing

∅ ad component of recombinant diploid as determined in I.

Table 4

Indirect method of estimating the recombination fraction between selected markers in recombinant selection experiments (after Pritchard, 1955)

n = No. of ascospores plated on medium selecting
PABA BI recombinants.

a = No. of colonies produced by n ascospores.

m = No. of ascospores plated on medium selecting
AD (or AD PABA) recombinants.

b = No. of colonies produced by m ascospores.

x = Recombination fraction between paba1 and bi1
= 0.22 (Käfer, 1958; Fig. 1, this thesis).

h = Fraction of ascospores viable and from hybrid asci
= 2a/nx

$$S.E._h = \sqrt{h(2-hx)/nx}$$

q = Recombination fraction between ad (or ad and paba)
mutants = nbx/ma

$$S.E._q = \sqrt{q\left[nx(2-hq) + mq(2-hx)\right] / mnhx}$$

Table 5

Optimal concentration and range of concentrations of adenine supporting growth of ad9 alleles, and effect of temperature on adenine requirement

adenine hydrochloride conc. (mg./ml. MM)	temperature	Growth response of all ad9 alleles *
0	25°,37°,43°	none
0.001	25°,37°,43°	"
0.01	37°	spidery aconidiate colonies
0.025	"	"
0.05	"	"
0.075	"	sporulating colonies which grow more slowly than those on fully supplemented MM
0.1	25°,37°,43°	maximum growth and sporulation
0.125	37°	"
0.2	"	"
0.4	"	"
0.8	"	"
1.0	"	"

* after two days' incubation

Table 6

Summary of available data with respect to back-mutation in conidia of the ad9 alleles

Strain	No. conidia plated on adenineless medium***	Average no. of conidia per plate	No. of AD colonies
*ad9 bi1	3.2×10^8	varied between 4×10^6 and 5×10^7	0
ad9 paba1 y)	1.09×10^9	7×10^7	1
)	3.6×10^7	2.4×10^6	0
*ad13 bi1	3.2×10^8	varied between 4×10^6 and 5×10^7	0
ad13 paba1 y; pyro4	5.8×10^8	2.6×10^7	0
*ad15 bi1	3.2×10^8	varied between 4×10^6 and 5×10^7	0
**ad15 (strain not specified)	ca. 10^9	?	1
pro1 ad15 paba1 y)	1.0×10^8	5.7×10^6	0
)	4.6×10^7	4.0×10^7	3
*ad17 bi1	3.2×10^8	varied between 4×10^6 and 5×10^7	0

Table 6 (Continued)

Strain	No. conidia plated on adenineless medium***	Average no. of conidia per plate	No. of AD colonies
**ad17 (strain not specified)	ca. 10^9	?	0
ad17 paba1 y bi1	7.5×10^8	3.8×10^7	5
ad17 bi1	7.8×10^7	5.6×10^6	1
ad32 bi1	7.5×10^8	3.8×10^7	0
pro1 ad33 bi1	3.9×10^8	3×10^7	1

* unpublished data of Calef.

** Calef (1957).

*** haemocytometer estimate.

Table 7

Reconstruction experiments testing for inhibition of an adenine-independent strain by an adenine-requiring strain

I. Conidia plated on MM + p.a.b.a. (added to molten medium and poured as a top layer). Estimated number of paba1 plated per dish = 33.

Estimated no. conidia per dish from ad9 paba1 y	Average no. colonies (all green)
1.00×10^5 (two dishes)	19.5
5.34×10^5 (two dishes)	30.5
1.00×10^6 (two dishes)	17.0
5.34×10^6 (two dishes)	21.5
1.00×10^7 (two dishes)	20.0
5.34×10^7 (one dish)	27
1.00×10^8 (two dishes)	24.0
5.34×10^8 (two dishes)	19.5

II. Conidia plated on MM + biotin (added to molten medium and poured as top layer). Estimated number of bi1; w3 plated per dish = 94.

Table 7 (Continued)

Estimated no. conidia per dish from ad32 bi1		Average no. colonies (all white)
0		58.2
5.28×10^4	(four dishes)	56.5
1.00×10^5	"	52.5
5.28×10^5	"	45.8
1.00×10^6	"	54.5
6.33×10^6	(two dishes)	50.0
1.02×10^7	(four dishes)	54.2
5.08×10^7	"	48.2
1.02×10^8	"	54.2

Table 8

Data from mitotic recombination experiments comparing spontaneous mutation frequencies with mitotic recombination frequencies

Type of diploid	No. adenine-independent segregants*	No. adenine-requiring colonies (approximate)
ad9/ad9	0	1,000
ad13/ad13	0	3,740
ad15/ad15	0	525
ad32/ad32	0	1,500
ad33/ad33	0	1,050
ad9/ad13	492	3,175
ad9/ad32	152	1,185
ad13/ad32	792	2,940
ad13 /ad33	150	1,050
ad13/ad15	262	650

* Experimental conditions as described on pp. 18-20.

Table 9

Reported cases of intra-cistronic complementation

Explanation of table:- "Mutants tested" specifies the number of mutants tested in all possible combinations, while "complement" indicates the number of mutants which complement in at least one combination. The symbolism of the complementation maps is discussed in the text (pp.37-38). Note that the number of mutants found in each complementation group is indicated above the horizontal lines which represent complementation groups.

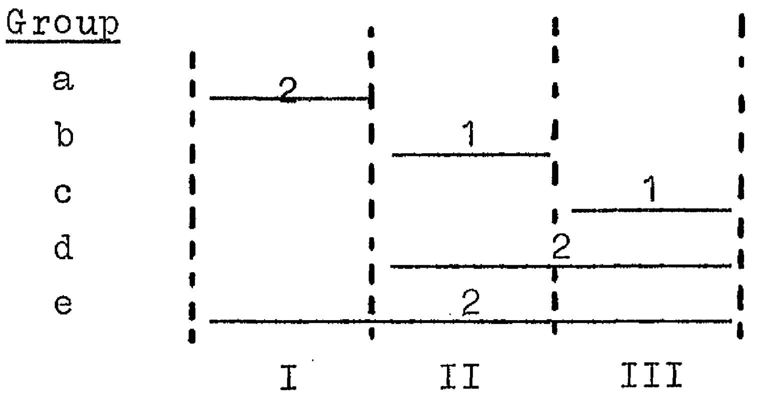
Phage T4

rII cistrons:- partial complementation with some leaky mutants, not investigated further (Benzer, 1959).

Escherichia coli K-12

Gal (A,B,C) cistron:- 8 mutants tested, 6 complement (Lederberg, 1960; Morse, 1959).

Complementation map



Escherichia coli B/r

Table 9 (Continued)

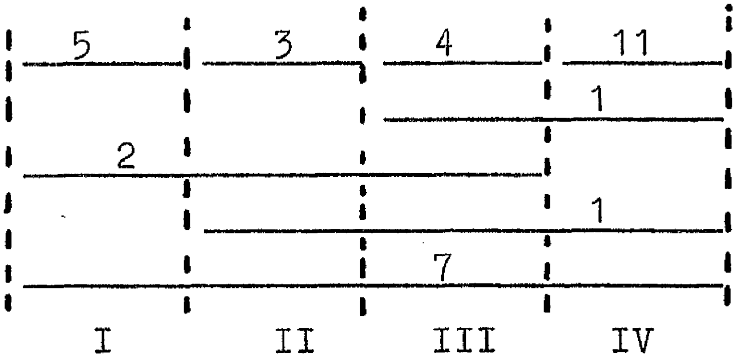
ara (C) cistron:- 5 mutants tested, 0 complement (Gross and Englesberg, 1959).

Salmonella typhimurium

hisA cistron:- 9 mutants tested, 0 complement (Hartman *et al*., 1960a and b).

hisB cistron:- 34 mutants tested, 27 complement (Hartman *et al*., 1960a and b).

Complementation map



hisC cistron:- 13 mutants tested, 0 complement (Hartman *et al*., 1960a and b).

hisD cistron:- 52 mutants tested, 33 complement (Hartman *et al*., 1960a and b).

Complementation map

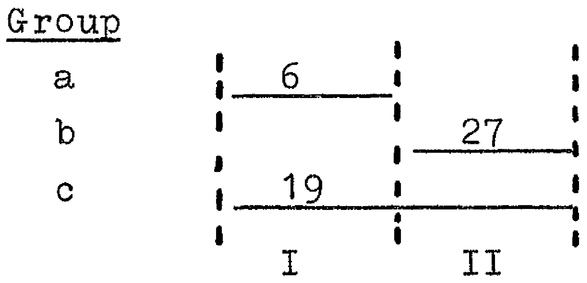
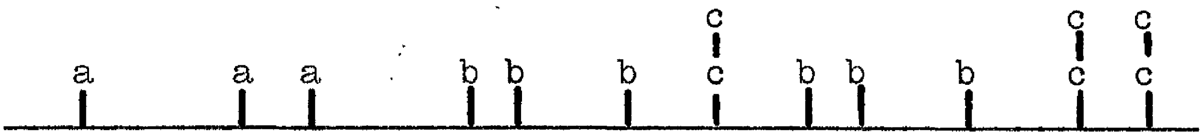


Table 9 (Continued)

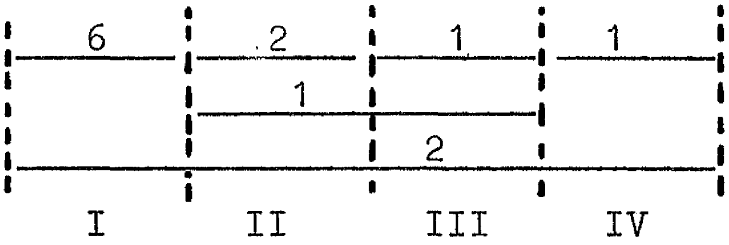
hisD cistron (continued):-

Comparison of genetic and complementation maps
(3 group *a* mutants, 6 group *b* and 6 group *c* mutants mapped)



hisE cistron:- 13 mutants tested, 11 complement (Hartman *et al.*, 1960a and b).

Complementation map



hisF cistron:- 11 mutants tested, 0 complement (Hartman *et al.*, 1960a and b).

hisG cistron:- 7 mutants tested, 0 complement (Hartman *et al.*, 1960a and b).

hisH cistron:- 2 mutants tested, 0 complement (Hartman *et al.*, 1960a and b).

tryC cistron:- 6 mutants tested, 0 complement (Balbinder, 1960).

tryD cistron:- 18 mutants tested, representing 15 distinguishable sites, 0 complement (Balbinder, 1960).

Neurospora crassa

ad3 cistrons:- ad3A -- 13 mutants tested, 0 complement

Table 9 (Continued)

ad3 cistrons(continued): -
(deSerres, 1960).

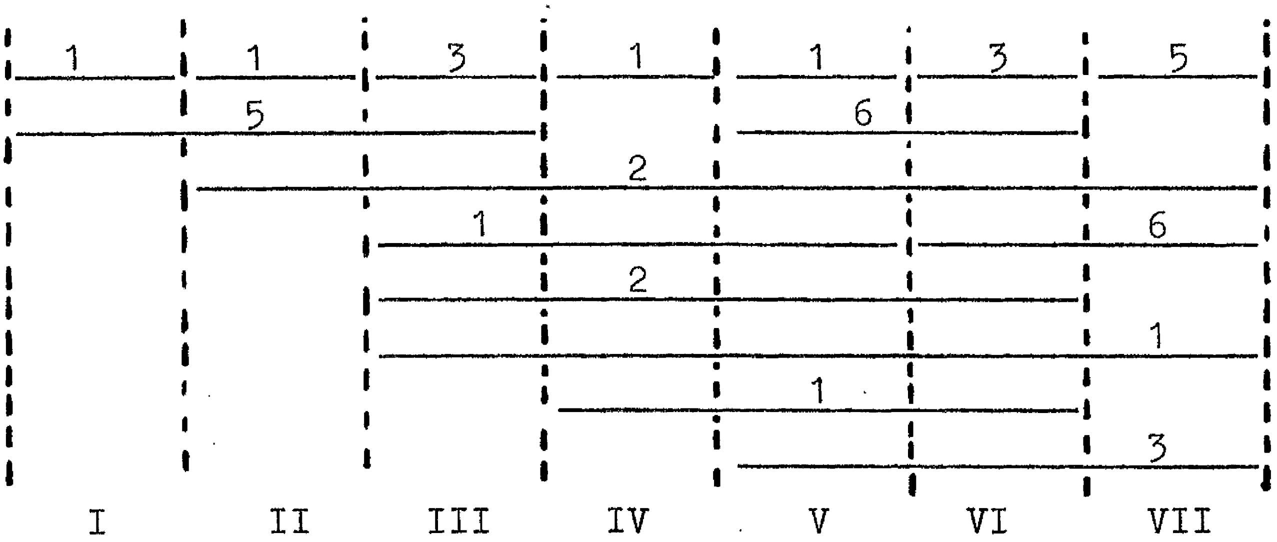
ad3B -- 21 mutants tested, 0 complement (deSerres, 1956).

rare instances of complementation, no details given (deSerres, unpublished, cited in Case and Giles, 1960).

ad4 cistron:- 123 mutants tested, 51 complement (Woodward *et al*., 1958).

Complementation Map

(42 complementing mutants only; cf. Fig. 1 in Woodward *et al*., 1958)



ad5 cistron:- ? mutants tested, 13 complement (Giles, 1958).

Complementation map

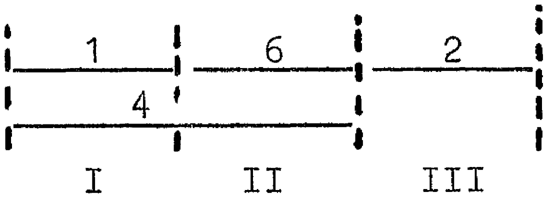
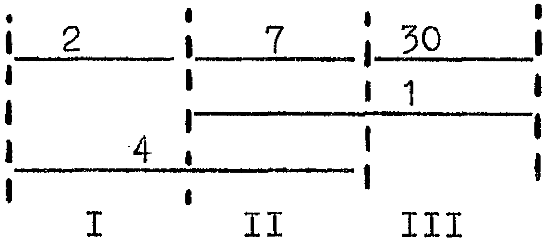


Table 9 (Continued)

adE cistron:- ? mutants tested, 44 complement (Giles, (1958).

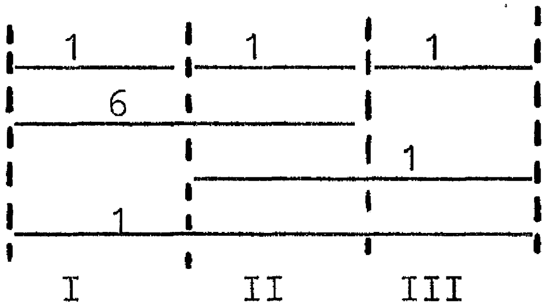
Complementation map



ad8 cistron:- 306 mutants tested (including 64 secondary mutants), 106 primary and 19 secondary complement (Ishikawa, 1960), no details given.

am cistron:- 11 mutants (including 2^1, a "revertant"), 10 complement (Fincham, 1958).

Complementation map



arg1 cistron:- 40 mutants tested, 12 complement (Catcheside and Overton, 1958).

Complementation map

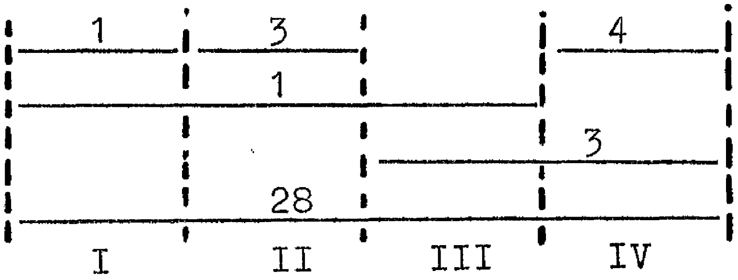


Table 9 (Continued)

arg2 cistron: - 63 mutants tested, 0 complement (Catcheside, 1960a and personal communication).

arg3 cistron: - 33 mutants tested, 0 complement (Catcheside, 1960a and personal communication).

arg5 cistron: - 20 mutants tested, 0 complement (Catcheside, 1960a and personal communication).

arg6 cistron: - no data, some mutants complement (Catcheside, 1960a).

arg10 cistron:- 13 mutants tested, 2 complement (Catcheside, 1960a).

arom3 cistron:- 11 mutants tested, some complement (no data) to give 5 complementation units (Gross and Fein, 1960).

cys1 cistron: - 22 mutants tested, 0 complement (Catcheside and Overton, 1958).

cys2 cistron: - 21 mutants tested, 0 complement (Catcheside and Overton, 1958).

his1 cistron: - 54 mutants tested, 15 complement; non-linear complementation map (Catcheside, 1960b).

Complementation map

(Note that Group D shows a discontinuity in a one-dimensional representation.)

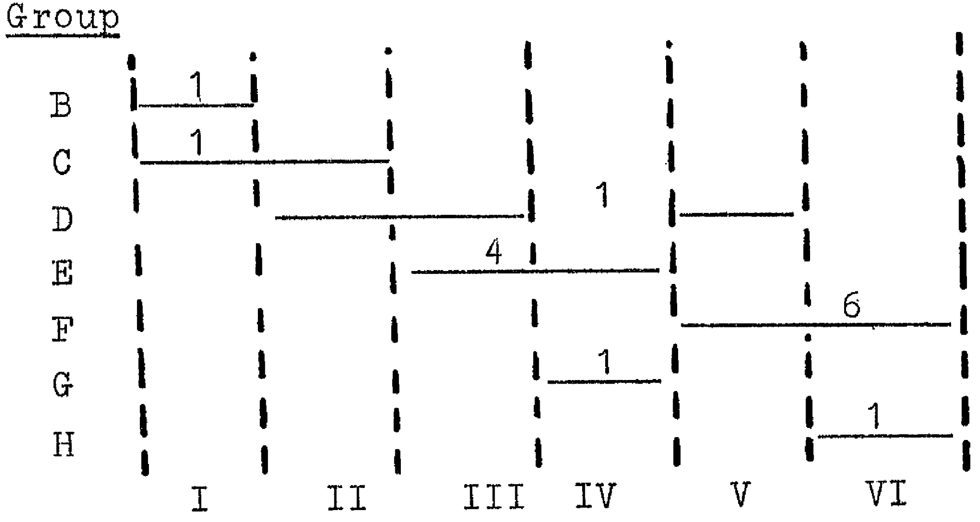
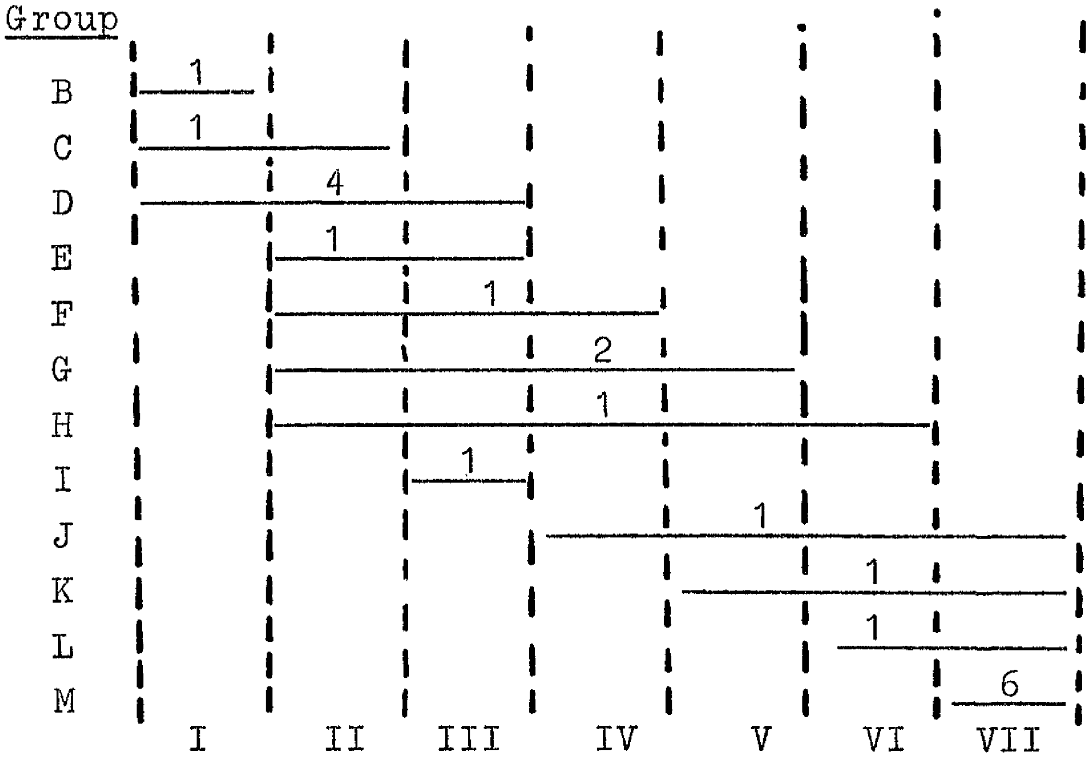


Table 9 (Continued)

his2 cistron: - 74 mutants tested, 21 complement (Catchside, 1960b).

Complementation map



his3 cistron: - 96 mutants tested, 44 complement (Catchside, 1960a).

Complementation map

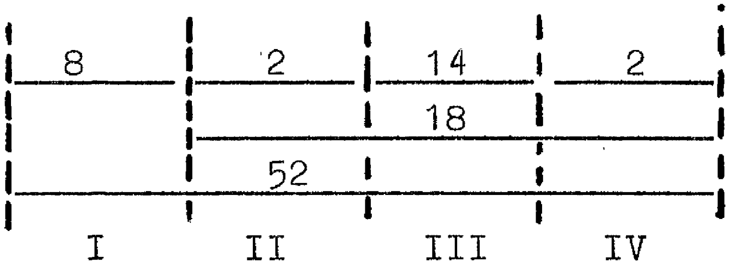
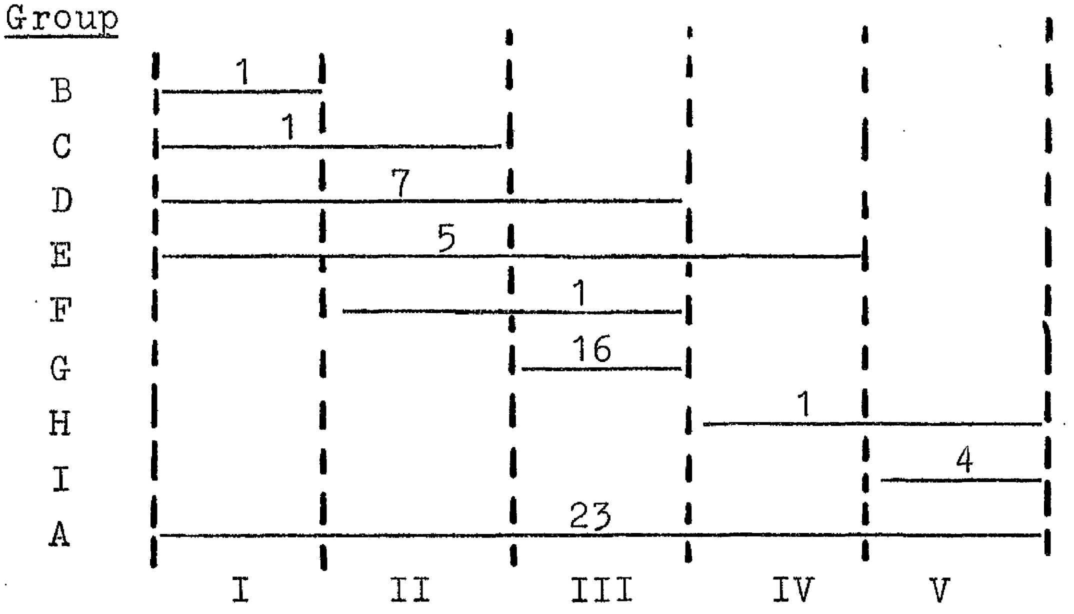


Table 9 (continued)

his-3 cistron (continued):- ? mutants tested, ? complement, giving 11 complementation units; genetic and complementation maps "roughly co-linear." (Webber, 1959).

his5 cistron: - 59 mutants tested, 36 complement (Catcheside, 1960b).

Complementation map



his6 cistron: - 99 mutants tested, 0 complement (Catcheside, 1960b).

his7 cistron: - 7 mutants tested, 0 complement (Catcheside, 1960b).

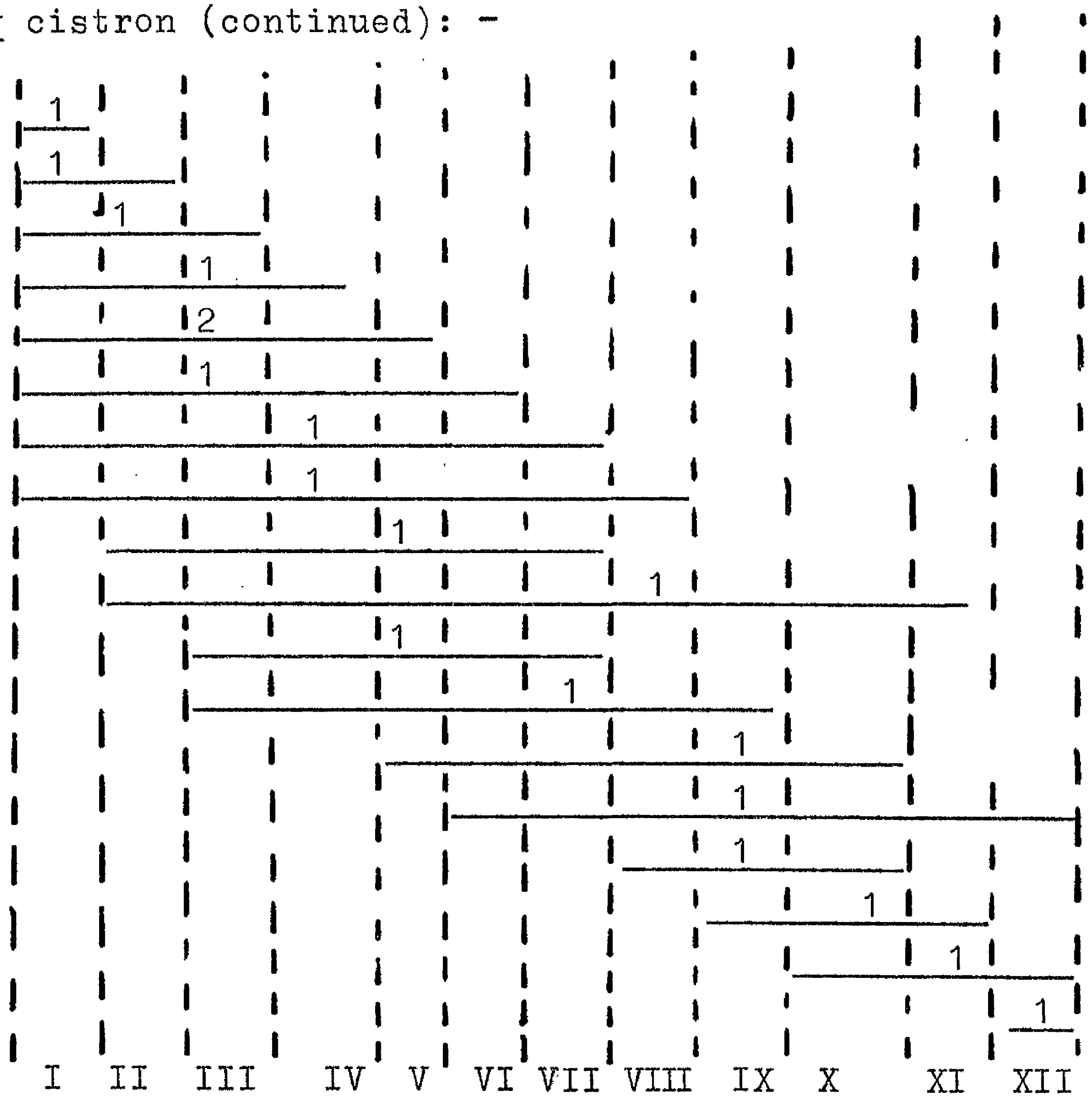
iv cistron: - ? mutants tested, 19 complement (Wagner et al., 1960).

Complementation map

(see page 187)

Table 9 (Continued)

iv cistron (continued): -



lys? cistron: - 46 mutants tested, some complement, giving 6 - 7 complementation groups and 3 - 4 complementation units (Catcheside and Overton, 1958). Larger scale experiments reveal a possibly non-linear complementation map (Catcheside, personal communication).

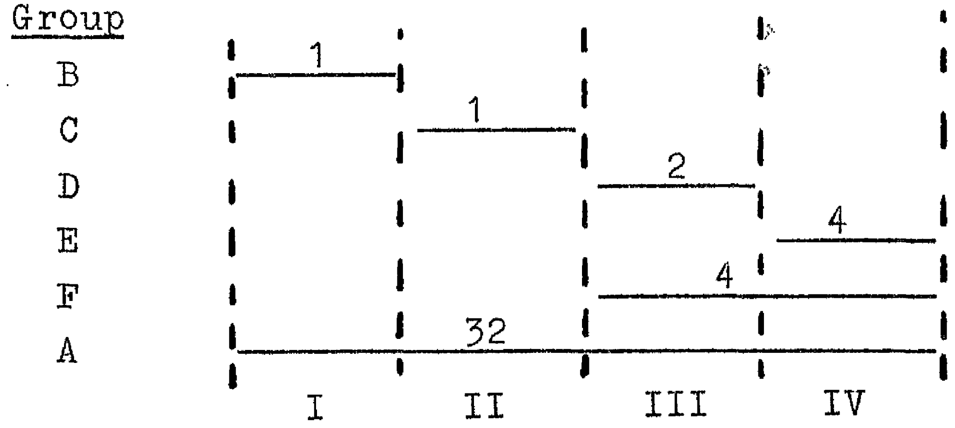
lys1 cistron: - 8 mutants tested, 0 complement (Catcheside and Overton, 1958).

Table 9 (Continued)

lys3 cistron: - 5 mutants tested, 0 complement (Catcheside and Overton, 1958).

me2 cistron: - 44 mutants tested, 12 complement; genetic and complementation maps apparently co-linear (Murray, 1960b).

Complementation map

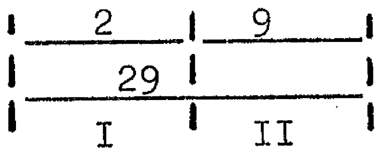


Genetic map

me8 cistron: - 35 mutants tested, some complementation (no data) (Murray, 1960a).

orn2 cistron: - 40 mutants tested, 11 complement (Catcheside and Overton, 1958).

Complementation Map



pan2 cistron: - 75 mutants tested, 23 complement (Case and Giles, 1960); see Fig. 8 of this thesis for complementation and genetic maps;

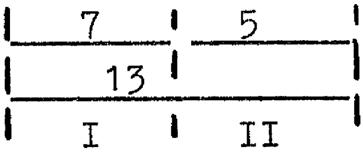
Table 9 (Continued)

pan2 cistron (continued): - complementation and genetic maps not strictly co-linear.

pyr3 cistron: - 52 mutants tested, some complementing, giving six complementation units; genetic and complementation maps not co-linear (Woodward and Cook, 1960).

tryp1 cistron: -25 mutants tested, 12 complement (Ahmad and Catcheside, 1960).

Complementation map

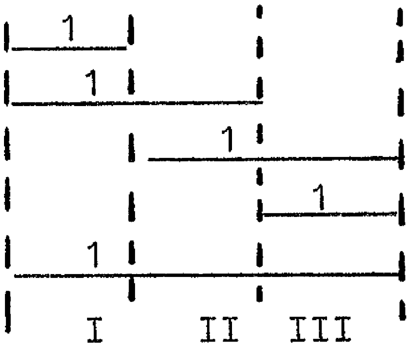


tryp2 cistron:- 9 mutants tested, 0 complement (Ahmad and Catcheside, 1960).

tryp3 (= *td*) cistron: - 44 mutants tested, 10 complement (Ahmad and Catcheside, 1960); 5 mutants tested, 4 complement (Lacy and Bonner, 1958; details in Catcheside, 1960a).

Complementation maps

(Lacy and Bonner)



(Ahmad and Catcheside)

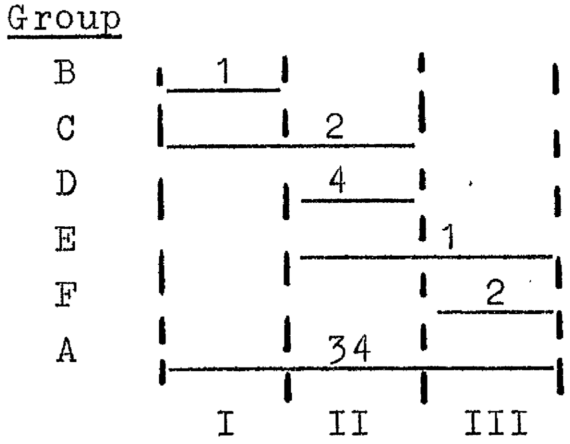


Table 9 (Continued)

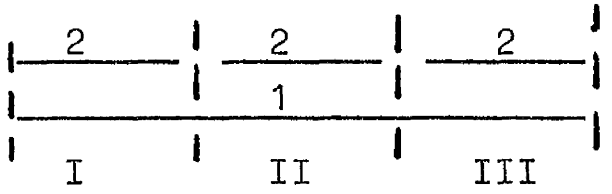
tryp4 cistron: - 4 mutants tested, 0 complement (Ahmad and Catcheside, 1960).

Aspergillus nidulans

ad9 cistron: - 6 mutants tested, 3 complement (Martin-Smith, 1957); see Fig. 7, this thesis, for complementation map and Fig. 51, this thesis, for genetic map. Complementation and genetic maps not co-linear.

sb3 cistron: - 7 mutants, 6 complement (Roberts, personal communication)

Complementation map



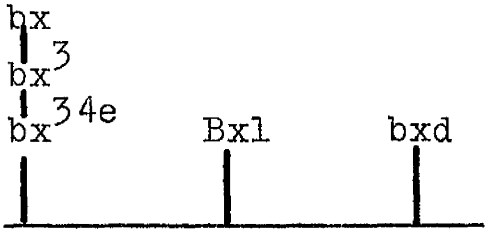
Schizosaccharomyces pombe

ad8 cistron: - 24 mutants tested, some complement (no data) (Megnet, 1959).

Drosophila melanogaster

bithorax cistron: - 5 mutants tested, 4 complement (Lewis, 1951).

Genetic map



Complementation map

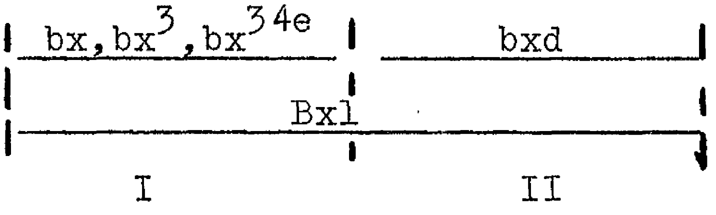
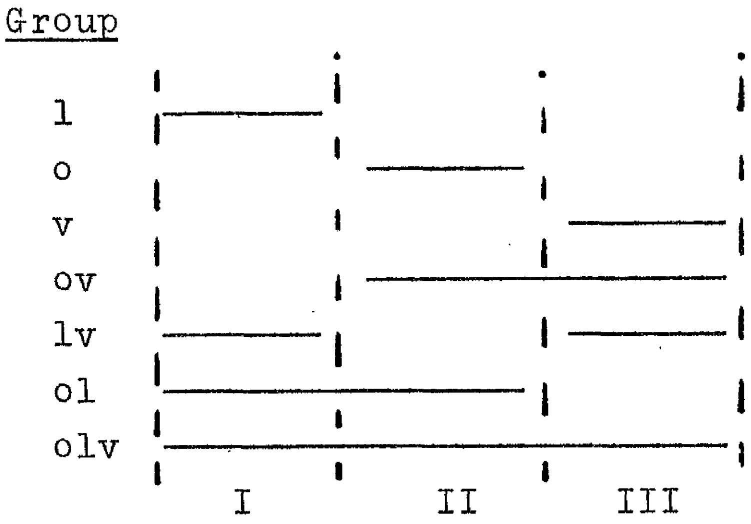


Table 9 (Continued)

dumpy cistron: - 7 mutant phenotypes tested, 6 complement (Carlson, 1959a); complementation map appears to be non-linear.

Complementation map

(Note that *lv* is discontinuous in a one-dimensional representation)



Genetic map

ma-l cistron: - ? mutants tested, 2 complement to give wild type eye colour but less than normal amount of xanthine dehydrogenase (Glassman, 1960).

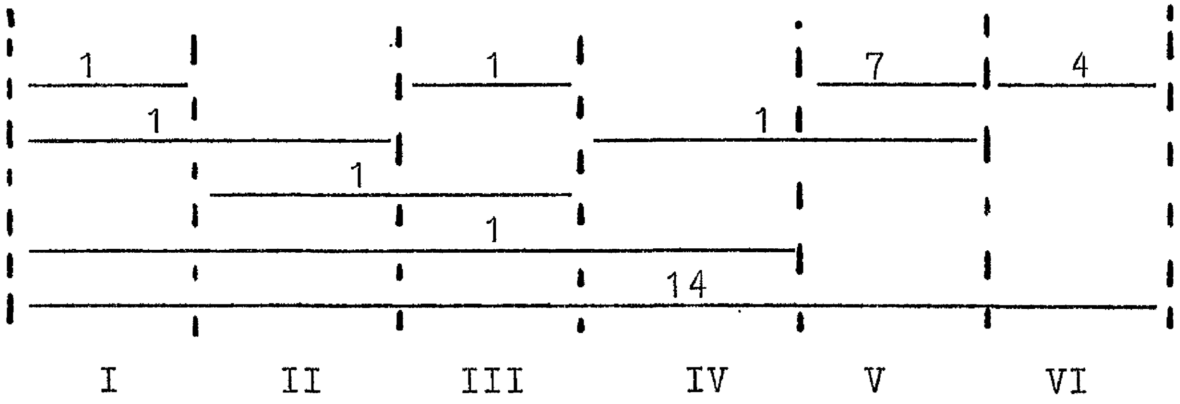
mch cistron: - some complementation, no data given (Fahmy and Fahmy, 1959).

Notch locus: - complementation observed between some recessive visibles, no data given (Welshons and von Halle, 1960).

Table 9 (Concluded)

r cistron: - 31 mutants tested, 17 complement (Fahmy and Fahmy, 1959).

Complementation map



ry cistron: - complementation observed, no data given (Glassman, 1960).

t cistron: - some complementation, no data (Fahmy and Fahmy, 1959).

Table 10

Types of complementation tests applicable to different organisms

Test	Example of organism and type of mutant	Reference
cis-trans	phage *T4B* and *T4D*, *rII* mutants	Benzer, 1955; Edgar, 1958a & b
cis-trans	phage λ, *c* mutants	Kaiser, 1957
cis-trans	phage *T2* and *T4*, *c* mutants	Brenner, 1957b
phenotypic mixing	phage *T2*, *h* mutants	Streisinger and Franklin, 1956
phenotypic mixing	phage λ, *h* mutants	Edgar, 1959
abortive transduction	*E. coli*, *ara* mutants	Gross and Englesberg, 1959
abortive trandsuction	*Salmonella typhimurium*, various (but not all) types of nutritional mutants	Demerec and Ozeki, 1959; Hartman *et al.*, 1960b.
heterogenotes: cis-trans	*E. coli K-12*, *Gal* mutants	Lederberg, 1952; Morse, 1959; Lederberg, 1960.
diploids: cis-trans	*Schizosaccharomyces pombe*, nutritional and mating type mutants	Leupold, 1958
heterokaryons	*Neurospora crassa*, many kinds of heterokaryon-compatible mutant strains	e.g. deSerres, 1960; Case and Giles, 1960; Catcheside, 1960 a & b
pseudo-wild types	*N. crassa*, *pan-2* mutants	Case and Giles, 1960

Table 10 (Concluded)

Test	Examples of organism and type of mutant	Reference
heterokaryons and *trans* diploids	*Aspergillus nidulans*, all types	e.g. Pontecorvo *et al*., 1953
diploids: cis-trans	*Aspergillus*, *ad-8* mutants	Pritchard, 1955
cis-trans	*Drosophila melanogaster*, *Star-asteroid*, *dumpy*, etc.	Lewis (1951), Carlson (1959a & b)
trans-diploid pollen	*Oenothera organensis*, *S* mutants	Lewis (1958, 1960)

Table 11

Cis-trans tests (Lewis effect) of some *ad9* alleles

Trans diploid*	Adenine phenotype	*Cis* diploid*	Adenine phenotype
$\frac{\text{n-(AD13 ad32)}}{\text{i-(AD32 ad13)}}$	mutant	$\frac{\text{i-(AD32 AD13)}}{\text{n-(ad13 ad32)}}$	wild
$\frac{\text{n-(AD13 ad32)}}{\text{i-(AD32 ad13)}}$	"	$\frac{\text{n-(AD13 AD32)}}{\text{i-(ad32 ad13)}}$	"
$\frac{\text{n-(AD13 ad9)}}{\text{i-(AD9 ad13)}}$	"	$\frac{\text{i-(AD9 AD13)}}{\text{n-(ad13 ad9)}}$	"
$\frac{\text{n-(AD13 ad9)}}{\text{i-(AD9 ad13)}}$	"	$\frac{\text{n-(AD13 AD9)}}{\text{i-(ad9 ad13)}}$	"
$\frac{\text{AD33 ad13}}{\text{ad33 AD13}}$	"	$\frac{\text{AD33 AD13}}{\text{ad33 ad13}}$	"
$\frac{\text{ad13 AD15}}{\text{AD13 ad15}}$	"	$\frac{\text{AD13 AD15}}{\text{ad13 ad15}}$	"

* "n-" = non-inverted order of *ad13*, *ad9* and *ad32* sites

"i-" = inverted order of *ad13*, *ad9* and *ad32* sites
= *ad32* - *ad9* - *ad13*.

Details of the inversion are given in Chapter III, section II.

Table 12

Mitotic recombination analyses providing information with respect to the linear order of the ad9 alleles

---

I. ad33 / ad13

Trans diploid A: $\frac{\text{PRO ad33 AD13 PABA Y bi1}}{\text{PRO AD33 ad13 paba1 y BI}}\ \frac{\text{w3 ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
174	46	27	16

bi ≠ paba y (i.e. bi > paba y)

$X_1^{2}\ ^{*} = 4.4952 \quad P < 0.05$

Trans diploid B: $\frac{\text{pro1 AD33 ad13 PABA Y bi1}}{\text{PRO ad33 AD13 paba1 y BI}}\ \frac{\text{W ACR}}{\text{w3 acr2}}$

Diploid recombinants:

+	bi	paba y	other
158	25	45	23

paba y ≠ bi (i.e. paba y > bi)

$X_1^{2}\ ^{*} = 5.7142 \quad P < 0.02$

Combined data from A and B:

bi(A) + paba y(B)	paba y(A) + bi(B)
91	52

$X_1^{2}\ ^{*} = 10.636 \quad P < 0.01$

SEQUENCE: ad33 - ad13-paba

---

* Chi square test that the two classes do not differ significantly from 1:1

Table 12 (Continued)

II. ad33 / ad9

Trans diploid A: $\frac{\text{pro1 ad33 AD9 PABA Y bi1}}{\text{PRO AD33 ad9 paba1 y BI}} \; \frac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
106	41	18	9

bi ≠ paba y (i.e. bi > paba y)

X_1^2 * = 8.9662 $P < 0.01$

SEQUENCE: ad33 - ad9 - paba

III. ad33 / ad32

Trans diploid A: $\frac{\text{pro1 ad33 AD32 PABA Y bi1}}{\text{PRO AD33 ad32 paba1 y BI}} \; \frac{\text{W ACR}}{\text{w3 ACR}}$

Diploid recombinants:

+	bi	paba y	other
150	51	26	7

bi ≠ paba y (i.e. bi > paba y)

X_1^2 * = 8.1169 $P < 0.01$

SEQUENCE: ad33 - ad32 - paba

---

* Chi square test that the two classes do not differ significantly from 1:1

Table 12 (Continued)

IV. ad33 / ad17

Trans diploid A: $$\frac{\text{pro1 ad33 AD17 PABA Y bi1}}{\text{PRO AD33 ad17 paba1 y BI}}\quad\frac{\text{W ACR}}{\text{w3 ACR}}$$

Diploid recombinants:	+	bi	paba y	other
	131	51	12	8

bi $\neq$ paba y (i.e. bi $>$ paba y)

X_1^{2} * $= 24.143$ P $\ll 0.01$

SEQUENCE: ad33 - ad17 - paba

V. ad33 / ad15

Trans diploid A: $$\frac{\text{PRO ad33 AD15 PABA Y bi1}}{\text{PRO AD33 ad15 paba1 y BI}}\quad\frac{\text{w3 ACR}}{\text{W acr2}}$$

Diploid recombinants:	+	bi	paba y	other
	98	35	15	2

bi $\neq$ paba y (i.e. bi $>$ paba y)

X_1^{2} * $= 8.0000$ P < 0.01

SEQUENCE: ad33 - ad15 - paba

---

* Chi square test that the two classes do not differ significantly from 1:1

Table 12 (continued)

VI. ad13 / ad17

Trans diploid A: $\frac{\text{pro1 ad13 AD17 PABA Y bi1}}{\text{PRO AD13 ad17 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
79	38	9	9

bi ≠ paba y (i.e. bi > paba y)

χ^2_1 * $= 17.894 \quad P \ll 0.01$

SEQUENCE: ad13 - ad17 - paba

VII. ad9 / ad32

Trans diploid B: $\frac{\text{pro1 AD9 ad32 PABA Y bi1}}{\text{PRO ad9 AD32 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{w3 ACR}}$

Diploid recombinants:

+	bi	paba y	other
116	18	48	11

paba y ≠ bi (i.e. paba y > bi)

χ^2_1 * $= 13.636 \quad P < 0.01$

SEQUENCE: ad9 - ad32 - paba

---

* Chi square test that the two classes do not differ significantly from 1:1

Table 12 (continued)

VIII. ad9 / ad17

Trans diploid A: $\frac{\text{pro1 ad9 AD17 PABA Y bi1}}{\text{PRO AD9 ad17 paba1 y BI}} \; \frac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
87	36	21	9

bi $\neq$ paba y (i.e. bi $>$ paba y)

$X_1^{2\,*} = 3.9474 \quad P < 0.05$

Trans diploid B: $\frac{\text{pro1 AD9 ad17 PABA Y bi1}}{\text{PRO ad9 AD17 paba1 y BI}} \; \frac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
87	14	30	15

paba y $\neq$ bi (i.e. paba y $>$ bi)

$X_1^{2\,*} = 5.8183 \quad P < 0.02$

Combined data from A and B:

bi(A) + paba y(B)	paba y (A) + bi(B)
66	35

$X_1^{2\,*} = 9.736 \quad P < 0.01$

SEQUENCE: ad9 - ad17 - paba

---

* Chi square test that the two classes do not differ significantly from 1:1

Table 12 (concluded)

IX. ad9 / ad15

Trans diploid A: $\dfrac{\text{pro1 ad9 AD15 PABA Y bi1}}{\text{PRO AD9 ad15 paba1 y BI}}\ \dfrac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
120	61	40	10

bi $\neq$ paba y (i.e. bi $>$ paba y)

X_1^2 * = 4.3664 $P < 0.05$

Trans diploid B: $\dfrac{\text{pro1 AD9 ad15 PABA Y bi1}}{\text{PRO ad9 AD15 paba1 y BI}}\ \dfrac{\text{W ACR}}{\text{w3 ACR}}$

Diploid recombinants:

+	bi	paba y	other
88	16	39	7

paba y $\neq$ bi (i.e. paba y $>$ bi)

X_1^2 * = 9.6184 $P < 0.01$

Combined data from A and B:

bi(A) + paba y(B)	paba y (A) + bi (B)
100	35

X_1^2 * = 12.411 $P < 0.01$

SEQUENCE: ad9 - ad15 - paba

---

* Chi square test that the two classes do not differ significantly from 1:1

Table 13

Mitotic recombination analyses providing no information with respect to the linear order of the ad9 alleles

---

I. ad13 / ad9

Trans diploid A: $\frac{\text{pro1 ad13 PABA Y bi1}}{\text{PRO ad9 paba1 y BI}}$ $\frac{\text{W ACR}}{\text{w3 ACR}}$

Diploid recombinants:

+	bi	paba y	other
175	52	46	10

bi = paba y $X^2_1 = 0.368$ $P > 0.50$

Trans diploid B: $\frac{\text{pro1 ad9 PABA Y bi1}}{\text{PRO ad13 paba1 y BI}}$ $\frac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
92	26	27	12

paba y = bi $X^2_1 = 0.019$ $P > 0.80$

Combined data from A and B:

bi(A) + paba y(B)	paba y(A) + bi(B)
79	72

$X^2_1 = 0.324$ $P > 0.50$

Table 13 (Continued)

II. ad13 / ad32

Trans diploid A: $\dfrac{\text{pro1 ad13 PABA Y bi1}}{\text{PRO ad32 paba1 y BI}} \quad \dfrac{\text{W ACR}}{\text{w3 ACR}}$

Diploid recombinants:

+	bi	paba y	other
152	102	136	16

paba y = bi $\quad X_1^2 = 4.8571 \quad P < 0.05$

Trans diploid B: $\dfrac{\text{PRO ad32 PABA Y bi1}}{\text{PRO ad13 paba1 y BI}} \quad \dfrac{\text{w3 ACR}}{\text{W ACR}}$

Diploid recombinants

+	bi	paba y	other
85	33	43	8

paba y = bi $\quad X_1^2 = 1.3858 \quad P > 0.20$

Combined data from A and B:

bi(A) + paba y(B)	paba y(A) + bi(B)
155	169

$X_1^2 = 1.8344 \quad P > 0.10$

III. ad13 / ad15

Trans diploid A: $\dfrac{\text{pro1 ad13 AD15 PABA Y bi1}}{\text{PRO AD13 ad15 paba1 y BI}} \quad \dfrac{\text{W ACR}}{\text{w3 acr2}}$

Diploid recombinants:

+	bi	paba y	other
133	91	101	20

Table 13 (Continued)

III. ad13 / ad15 (continued)

paba y = bi $X_1^2 = 0.521$ $P > 0.30$

Trans diploid B: $\frac{\text{pro1 AD13 ad15 PABA Y bi1}}{\text{PRO ad13 AD15 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{W ACR}}$

Diploid recombinants:

+	bi	paba y	other
134	52	56	19

paba y = bi $X_1^2 = 0.148$ $P = 0.70$

Combined data from A and B:

bi(A) + paba y (B)	paba y(A) + bi(B)
147	153

$X_1^2 = 0.120$ $P > 0.70$

IV. ad32 / ad15

Trans diploid A': $\frac{\text{PRO ad32 PABA Y bi1}}{\text{PRO ad15 paba1 y BI}} \quad \frac{\text{w3 ACR}}{\text{W acr2}}$

Diploid recombinants:

+	bi	paba y	other
103	38	48	11

paba y = bi $X_1^2 = 1.1628$ $P > 0.20$

Trans diploid A'': $\frac{\text{pro1 ad32 PABA Y bi1}}{\text{PRO ad15 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{w3 acr2}}$

Diploid recombinants:

+	bi	paba y	other
79	76	72	17

Table 13 (Concluded)

IV. ad32 / ad15 (continued)

bi = paba y $\quad X^2_1 = 0.1081 \quad P > 0.70$

Trans diploid B: $\dfrac{\text{pro1 ad15 PABA Y bi1}}{\text{PRO ad32 paba1 y BI}} \quad \dfrac{\text{W ACR}}{\text{w3 ACR}}$

Diploid recombinants:

+	bi	paba y	other
118	61	61	24

bi = paba y $\quad P = 1.0$

Combined data from A', A'' and B:

bi(A'+A") + paba y(B)	paba y(A'+A")+bi(B)
175	181

$X^2_1 = 0.101 \quad P > 0.70$

Table 14

The genotypes of wild-type diploids derived from

$$\frac{\text{pro1 ad13 PABA Y bi1}}{\text{PRO ad9 paba1 y BI}} \quad \frac{\text{W}}{\text{w3}}$$

Class No.	Genotype of Chromosome I * ∅	No. obtained
i	$\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 ad13 PABA Y bi1}}$	1
ii	$\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 AD9 ad13 PABA Y bi1}}$	4
iii	$\frac{\text{pro1 AD9 AD13 PABA Y bi1}}{\text{PRO ad9 AD13 paba1 y BI}}$	8
iv	$\frac{\text{PRO AD9 AD13 PABA Y bi1}}{\text{pro1 AD9 ad13 paba1 y BI}}$	1
v	$\frac{\text{PRO AD9 AD13 PABA Y bi1}}{\text{pro1 ad9 AD13 paba1 y BI}}$	1
vi	$\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 AD13 PABA Y bi1}}$	1

* all diploids still heterozygous W/w3

∅ For the purposes of this table, the arbitrary order of ad9 - ad13 - paba is used.

Table 15

The genotypes* of wild-type diploids derived from

$$\frac{\text{pro1 ad9 PABA Y bi1}}{\text{PRO ad13 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{w3 acr2}}$$

Group No.	Genotype of Chromosome I ∅	No. obtained
A	$\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad PABA Y bi1}}$	12
B	$\frac{\text{pro1 AD PABA Y bi1}}{\text{PRO ad paba1 y BI}}$	10
C	$\frac{\text{pro1 AD paba1 y BI}}{\text{PRO ad PABA Y bi1}}$	4
D	$\frac{\text{pro1 AD PABA y BI}}{\text{PRO ad paba1 Y bi1}}$	1
E	$\frac{\text{PRO AD PABA Y bi1}}{\text{pro1 ad paba1 y BI}}$	2
F	$\frac{\text{PRO AD paba1 y BI}}{\text{pro1 AD PABA Y bi1}}$	1

* Only the ad genotypes of the Group A diploids were determined and are given in Table 16.

∅ All diploids still heterozygous W ACR / w3 acr2

Table 16

The genotypes of Group A recombinant diploids

(see Table 15)

Class No.	Genotype of Chromosome I * ø	No. Obtained
1	$\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 ad13 PABA Y bi1}}$	1
2	$\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 ad9 AD13 PABA Y bi1}}$	10
3	$\frac{\text{PRO AD9 AD13 paba1 y BI}}{\text{pro1 AD9 ad13 PABA Y bi1}}$	1

---

* For the purposes of this table, the order of ad sites is arbitrarily taken as ad9 - ad13 - paba.

ø all diploids still heterozygous W ACR / w3 acr2

Table 17

The genotypes of wild-type diploids derived from

$$\frac{\text{pro1 ad32 PABA Y bi1}}{\text{PRO ad13 paba1 y BI}} \quad \frac{\text{W ACR}}{\text{w3 acr2}}$$

Class No.	Genotype of Chromosome I * ∅	No. Obtained
i	$\frac{\text{pro1 AD13 AD32 PABA Y bi1}}{\text{PRO ad13 ad32 paba1 y BI}}$	1
ii	$\frac{\text{pro1 AD13 AD32 PABA Y bi1}}{\text{PRO ad13 AD32 paba1 y BI}}$	12
iii	$\frac{\text{PRO AD13 AD32 paba1 y BI}}{\text{pro1 ad13 ad32 PABA Y bi1}}$	1
iv	$\frac{\text{PRO AD13 AD32 paba1 y BI}}{\text{pro1 AD13 ad32 PABA Y bi1}}$	10
v	$\frac{\text{pro1 AD13 AD32 paba1 y BI}}{\text{PRO ad13 ad32 PABA Y bi1}}$	4
vi	$\frac{\text{pro1 AD13 AD32 paba1 y BI}}{\text{PRO ad13 AD32 PABA Y bi1}}$	1
vii	$\frac{\text{PRO AD13 AD32 PABA Y bi1}}{\text{pro1 ad13 AD32 paba1 y BI}}$	1
viii	$\frac{\text{pro1 AD13 AD32 PABA y BI}}{\text{PRO ad13 AD32 paba1 Y bi1}}$	1
ix	$\frac{\text{pro1 AD13 AD32 paba1 Y bi1}}{\text{PRO AD13 ad32 PABA y BI}}$	1
x	$\frac{\text{PRO AD13 AD32 PABA y BI}}{\text{pro1 AD13 ad32 PABA Y bi1}}$	1
		33

---

* All diploids still heterozygous W ACR / w3 acr2

∅ For the purposes of this table, the arbitrary order of ad13 - ad32 - paba is used.

Table 18

Classification of mitotic recombinants in Table 17 according to number of crossovers

No. of crossovers	Classes		No. obtained
1	-		0 *
2	i, ii, iii, iv		24
3	(a) intervals adjacent to inversion: v, vi, vii	6	7
	(b) *paba* - *y* interval: viii	1	
4	ix, x (including one cross-over in *paba* - *y* interval)		2

* Class *v* regarded as a triple recombinant, see text.

Table 19

Classification of a further 17 wild-type diploids from

pro1 ad32 PABA Y bi1 W ACR
PRO ad13 paba1 y BI w3 acr2

Type	No. of crossovers required	Genotype of Chromosome I * ∅	No. obtained
A	2	pro1 AD13 AD32 PABA Y bi1 PRO ad13 AD32 paba1 y BI	5
B	2	PRO AD13 AD32 paba1 y BI pro1 ad PABA Y bi1	4
C	3	pro1 AD13 AD32 paba1 y BI PRO ad PABA Y bi1	6
D	4	pro1 AD13 AD32 paba1 Y bi1 PRO ad PABA y BI	1
E	5	pro1 AD13 AD32 paba1 y BI PRO AD13 AD32 PABA Y bi1	1

* all still heterozygous W ACR / w3 acr2

∅ order arbitrarily written as ad13 - ad32 - paba for the purposes of this table

Table 20

The genotypes of wild-type diploids derived from

pro1 ad13 AD15 PABA Y bi1 / PRO AD13 ad15 paba1 y BI W ACR / w3 acr2

Class No.	Genotype of Chromosome I *	No. obtained
i	PRO AD13 AD15 PABA Y bi1 / pro1 ad13 ad15 paba1 y BI	4
ii	PRO AD13 AD15 PABA Y bi1 / pro1 ad13 AD15 paba1 y BI	2
iii	pro1 AD13 AD15 PABA Y bi1 / PRO AD13 ad15 paba1 y BI	3
iv	PRO AD13 AD15 paba1 y BI / pro1 ad13 AD15 PABA Y bi1	6

* all diploids still heterozygous W ACR / w3 acr2

Table 21

The genotypes of wild-type diploids derived from

PRO ad33 AD13 paba1 y BI w3 acr2
pro1 AD33 ad13 PABA Y bi1 W ACR

Class No.	Genotype of Chromosome I *	No. obtained
i	pro1 AD33 AD13 paba1 y BI PRO ad33 ad13 PABA Y bi1	1
ii	PRO AD33 AD13 paba1 y BI pro1 AD33 ad13 PABA Y bi1	3
iii	pro1 AD33 AD13 PABA Y bi1 PRO ad33 AD13 paba1 y BI	7
iv	pro1 AD33 AD13 paba1 y BI PRO ad33 AD13 PABA Y bi1	1
v	PRO AD33 AD13 paba1 y BI pro1 ad33 AD13 PABA Y bi1	2
vi	PRO AD33 AD13 PABA Y bi1 pro1 ad33 AD13 paba1 y BI	1
vii	PRO AD33 AD13 PABA Y bi1 pro1 AD33 ad13 paba1 y BI	1

* all still heterozygous for w3 acr2 / W ACR

Table 22

Estimation of recombination in a cross involving ad9 and ad32

Cross: $\frac{\text{pro1 ad9 AD32 PABA Y bi1}}{\text{PRO AD9 ad32 paba1 y BI}}$

	Selection for				
	PABA BI		AD9 AD32		
Plating	Spores plated	Colonies	Spores plated	Colonies	Recombination fraction*
i	6.35×10^4	1284	3.35×10^8	142	0.0000046 ± 0.00000041
ii	1.83×10^4	265	1.08×10^8	29	0.0000041 ± 0.00000080
i and ii (pooled and weighted) **					0.0000045 ± 0.00000036
iii	1.80×10^4	168	1.23×10^8	44	0.0000084 ± 0.0000014

* Estimated by the method described in Table 5 and Chapter I.

** Recombination fraction estimates from platings i and ii are homogeneous:-

Homogeneity tests

i and ii $X_1^2 = 0.31$ P 0.50

i and iii $X_1^2 = 6.79$ P 0.01

ii and iii $X_1^2 = 7.11$ P 0.01

Table 23

Classification of adenine-independent colonies obtained in the ad9 // ad32 cross (Table 22)

Intervals: 1 0 2 3 4

Cross:	pro1	ad9	AD32	PABA	Y	bi1
	PRO	AD9	ad32	paba1	y	BI

Phenotypes	Crossover types	No. found in platings i	ii	iii
bi	0	69	9	22
pro bi	1,0	22	11	5
paba y	0,2	9	2	7
y	0,3	21	3	3
+	0,4	7	0	1
pro paba y	1,0,2	4	2	2
pro y	1,0,3	1	0	3
pro	1,0,4	2	0	0
paba bi	0,2,3	3	1	0
paba y bi	0,2,4	1	0	1
y bi	0,3,4	2	0	0
pro paba bi	1,0,2,3	0	1	0
pro paba y bi	1,0,2,4	0	0	0
pro y bi	1,0,3,4	1	0	0
paba	0,2,3,4	0	0	0
pro paba	1,0,2,3,4	0	0	0
: Totals		142	29	44

Table 24

Estimation of recombination in a cross involving n-ad13 ad9 and ad32

Inter-vals:		1		0		2		3		4			
Cross*:	pro1		(n-ad13 ad9)		AD32		PABA		Y		bi1	w3	acr2
	PRO		(n-AD13 AD9)		ad32		paba1		y		BI	W	ACR

	Selection for				
	PABA BI		AD13 AD9 AD32		
Plat-ing	Spores plated	Colonies	Spores plated	Colonies	Recombin-ation fraction
i	3.63×10^4	839	5.77×10^7	61	0.000010 ± 0.0000013

Classification of adenine-independent recombinants

Phenotype	Crossover type	No. obtained	
bi	0	14	26
bi w	0	12	
pro bi	1,0	4	11
pro bi w	1,0	7	
paba y	0,2	3	10
paba(y) w	0,2	7	
y	0,3	5	9
+	0,4	2	
w	0,3 or 0,4	2	
pro y	1,0,3	3	5
pro w	1,0,3 or 1,0,4	2	
		Total	61

* This cross does not distinguish between n-ad13 - ad9 - ad32 - paba and n-ad9 - ad13 - ad32 - paba.

Table 25

Estimation of recombination in a cross involving ad9 and n-ad13 ad32

Cross: $\dfrac{\text{pro1 n-ad13 AD9 ad32 PABA Y bi1 w3 acr2}}{\text{PRO n-AD13 ad9 AD32 paba1 y BI W ACR}}$

	Selection for				
	PABA BI		AD13 AD9 AD32		
Plat-ing	Spores plated	Colonies	Spores plates	Colonies	Recombin-ation frequency ø
1	5.59×10^4	195	2.68×10^7	0	---
2	5.43×10^4	307	1.56×10^7	1 øø	0.0000025 ± 0.0000025
3	Not done		3.90×10^7	2 øø	0.0000026 *
4	"		5.05×10^7	0	---
5	"		4.28×10^7	0	---
6	"		1.345×10^8	0	---
7	"		3.12×10^7	3 øøø	0.0000048 *
8	"		1.78×10^7	0	---
9	1.35×10^5	1162	1.10×10^7	0	---
10	Not done		1.01×10^7	0	---
Total:			3.79×10^8	6	0.00000072 **

ø i.e. the proportion of progeny estimated to be recombinant in the ad13 - ad9 and ad9 - ad32 intervals

øø all bi

øøø 1 pro y, 1 pro bi, 1 pro w

* An h value (fraction of spores viable and from hybrid meioses) of 0.04 was assumed, on the basis of platings 1 and 2

** An h value of 0.04 was assumed for platings 3-7, and a

Table 25 (concluded)

** (continued) : - value of 0.078 for platings 8 and 10. This latter value was derived in plating 9. Platings 1-7 and 8-10 were made from two different sets of crosses.

Table 26

Estimation of recombination in crosses involving ad9 and i-ad13

Cross A: $\frac{\text{pro1} \quad \text{AD13 ad9} \quad \text{PABA} \quad \text{Y bi1}}{\text{PRO} \quad \text{i-(AD9 ad13)paba1} \quad \text{y BI}} \quad \frac{\text{PYRO}}{\text{pyro4}}$

Cross B: $\frac{\text{pro1} \quad \text{i-(AD9 ad13)} \quad \text{PABA} \quad \text{Y bi1}}{\text{PRO} \quad \text{AD13 ad9} \quad \text{paba1} \quad \text{y BI}} \quad \frac{\text{W}}{\text{w3}}$

	Selection for					
	PABA BI		AD13 AD9			
Plating, Cross	Spores plated	Colonies	Spores plated	Colonies	Recombination frequency *	
i(A)	4.22×10^4	323	3.73×10^7	66	0.000051 ± 0.0000069	
ii(A)	4.65×10^4	17**	2.98×10^7	42	0.00085 ± 0.00025	**
ii(A)	-- corrected				0.000040 ± 0.0000066	**
iii (B)	2.19×10^4	490	5.64×10^6	8	0.000014 ± 0.0000050	

* i.e. the proportion of progeny estimated to be recombinant for the ad13 - ad9 interval and also a second interval within the ad13 inversion via 2-strand double crossovers

** This unusually low frequency of PABA BI colonies (in ii, uncorrected) suggests a technical fault. The recombination freq. is recalculated on the basis of the h value derived in plating i, as platings i and ii were made from the same cross.

Homogeneity tests

i and ii(uncorrected): $X_1^2 = 10.6$ $P < 0.01$

i and ii (corrected): $X_1^2 = 1.2$ $P > 0.20$

i and iii: $X_1^2 = 18.8$ $P < 0.01$ ii and iii: $X_1^2 = 11.2$ $P < 0.01$

Table 27

Classification of adenine-independent colonies obtained from ad9 // i-ad13 crosses (see Table 26)

Intervals:		1		0		2		3		4		
Cross A:	pro1		AD13		ad9		PABA		Y		bi1	PYRO
	PRO		i-(AD9		ad13)		paba1		y		BI	pyro4
Cross B:	pro1		i-(AD9		ad13)		PABA		Y		bi1	W
	PRO		AD13		ad9		paba1		y		BI	w3
Cross B':					"							W
												W

Phenotype of Chromosome I		Possible crossovers*	No. obtained in platings **			
Cross A	Crosses B, B'		i	ii	iii	iv
paba y	pro bi	XO	19	30	1	1
pro bi	paba y	OY	0	1	0	0
øpro paba y	bi	1,XO (OY,2)	25	3	6	4
øøbi	pro paba y	XO,2 (1,OY)	1	1	0	1
paba bi	pro y	XO,3	5	6	0	15
paba y bi	pro	XO,4	0	0	0	1
pro paba bi	y	1,XO,3 (OY,2,3)	6	1	1	2
pro paba y bi	+	1,XO,4 (OY,2,4)	6	0	0	1
y	pro paba bi	XO,2,3 (1,OY,3)	1	0	0	0
+	pro paba y bi	XO,2,4 (1,OY,4)	0	0	0	0
paba	pro y bi	XO,3,4	2	0	0	0
pro paba	y bi	1,XO,3,4 (OY,2,3,4)	1	0	0	0
			66	42	8	25

See next page for footnotes.

Table 27

(concluded)

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* "X" and "Y" are positions of the second crossover within the inversion -- see Fig. 45. "X" is proximal to _ad13_ in the non-inverted sequence, and "Y" is distal to _ad9_ in the non-inverted sequence. _OY_ possibilities are bracketed as they appear to be less likely than _XO_ possibilities.

** Platings _i_ and _ii_ were from cross A, _iii_ from cross B, and _iv_ from cross B'.

ø Singles class if no inversion and if sequence were _ad13_ - _ad9_ - _paba_.

øø Singles class if no inversion and if sequence were _ad9_ - _ad13_-_paba_.

Table 28

Mitotic recombination analysis of diploids trebly heterozygous for ad13, ad9 and ad32

---

1. n-ad13 ad9 / ad32

Trans diploid:

pro1	n-ad13	ad9	AD32	PABA	Y	bi1	w3	acr2
PRO	AD13	AD9	ad32	paba1	y	BI	W	ACR

Diploid recombinants:

+	bi	paba y	other
268	168 +2 * 170	83	25

bi ≠ paba y (i.e. bi > paba y)

$X_1^{2**} = 29.917 \quad P \ll 0.01$

---

2. n-ad13 ad32 / ad9

Trans diploid:

pro1	n-ad13	AD9	ad32	PABA	Y	bi1	w3	acr2
PRO	AD13	ad9	AD32	paba1	y	BI	W	ACR

Diploid recombinants:

+	bi	paba y	other
191 +5 ∅ 196	49	84	28

paba y ≠ bi (i.e. paba y > bi)

$X_1^{2**} = 9.2106 \quad P < 0.01$

---

* bi w

** Chi square test that the two classes do not differ significantly from 1:1

∅ w (presumably Y; w / y; w or Y; w / Y; w genotype for colour loci).

Table 28 (Concluded)

3. i-ad13 ad32 / ad9

Trans diploid:

PRO	i-ad32	AD9	ad13	paba1	y	BI	w3	acr2
pro1	AD13	ad9	AD32	PABA	Y	bi1	W	ACR

Diploid recombinants:

+	bi	paba y	other
31	5	3	7

Table 29

Frequency with which adenine-independent segregants arise from diploids heterozygous for ad13, ad9 and ad32 (see pp. 104-105)

EXPERIMENTAL CONDITIONS: - The incubation period was the same in all experiments. Only one segregant was counted per colony. Segregants included haploids and aneuploids in addition to diploids.

	Frequency of recombination			
	pro adx bi / adz paba y		pro adz bi / adx paba y	
Alleles in adx / adz ø parental diploids	No. ad colonies	No. & % giving segregants	No. ad colonies	No. & % giving segregants
i-ad13 / ad9	1141	296 (26%)	459	166 (36%)
i-ad13 / ad32	775	407 (61%)	404	169 (42%)
ad9 / ad32	---	---	810	196 (24%)
n-ad13 ad9 / ad32	2806	552 (20%)	---	---
n-ad13 ad32 / ad9	4158	380 (9%)	---	---
i-ad13 ad32 / ad9	---	---	1797	49 (2.7%)

ø for full genotypes, see Table 13 for 1st and 2nd diploids, Table 12 for 3rd diploid and Table 28 for 4th - 6th diploids.

Table 30

A comparison of phenotypes of a sample of mitotic recombinants subjected to half-tetrad analysis with the total recombinants obtained from diploid 2 (see Table 28)

Phenotype	Half-tetrad sample*		Total in recombina-ation experiment ø	
	No.	%	No.	%
+	23	46	191	53
paba y	16	32	84	23.5
bi	5	10	49	14
paba	2	4	11	3.1
y	2	4	7	2
paba bi	2	4	6	1.7
w	0	0	5	1.4
pro	0	0	2	0.6
pro paba y	0	0	1	0.3
pro paba bi	0	0	1	0.3
Total	50		357	

* Table 31

ø Table 28

Table 31

Half-tetrad analysis of fifty mitotic recombinants from

$$\frac{\text{pro1 n-ad13 AD9 ad32 PABA Y bi1}}{\text{PRO AD13 ad9 AD32 paba1 y BI}}\quad\frac{\text{w3}}{\text{W}}\quad\frac{\text{acr2}}{\text{ACR}}$$

Class	Pheno-type	Genotype (*ad* not determined)*	No. obtained	Type of recombination required ø
i	+	$\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad PABA Y bi1}}$	14	selected double
ii	paba y	$\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad paba1 y BI}}$	7	"
iii	+	$\frac{\text{pro1 AD paba1 y BI}}{\text{PRO ad PABA Y bi1}}$	2	triple (*pro* - *ad*)
iv	paba y	$\frac{\text{pro1 AD paba1 y BI}}{\text{PRO ad paba1 y BI}}$	6	"
v	bi	$\frac{\text{PRO AD PABA Y bi1}}{\text{pro1 ad PABA Y bi1}}$	1	triple (*ad* - *paba*)
vi	+	$\frac{\text{PRO AD PABA Y bi1}}{\text{pro1 ad paba1 y BI}}$	0	"
vii	bi	$\frac{\text{PRO AD paba1 Y bi1}}{\text{pro1 ad PABA Y bi1}}$	1	triple (*paba* - *y*)
viii	y	$\frac{\text{PRO AD paba1 y BI}}{\text{pro1 ad PABA y BI}}$	1	"
ix	+	$\frac{\text{pro1 AD PABA Y bi1}}{\text{PRO ad paba1 y BI}}$	4	quadruple (*pro* - *ad*, *ad* - *paba*)
x	bi	$\frac{\text{pro1 AD PABA Y bi1}}{\text{PRO ad PABA Y bi1}}$	1	"
xi	+	$\frac{\text{PRO AD paba1 y BI}}{\text{PRO ad PABA Y bi1}}$	2	quadruple ** (*centromere* - *pro*, *pro* - *ad*)

Table 31 (Concluded)

Class	Phenotype	Genotype (*ad* not determined)*	No. obtained	Type of recombination required ø
xii	bi	pro1 AD paba1 Y bi1 / PRO ad PABA Y bi1	1	quadruple (*pro* - *ad*, *paba* - *y*)
xiii	y	pro1 AD PABA y BI / PRO ad paba1 y BI	1	"
xiv	paba	pro1 AD paba1 Y bi1 / PRO ad paba1 y BI	2	"
xv	+	PRO AD PABA y BI / pro1 ad PABA Y bi1	1	quadruple (*ad* - *paba*, *paba* - *y*)
xvi	bi	PRO AD PABA Y bi1 / pro1 ad paba1 Y bi1	1	"
xvii	paba y	pro1 AD paba1 y BI / PRO AD paba1 y BI	3	quintuple øø (4 crossovers in *ad* intervals, *pro* - *ad* interval)
xviii	paba bi	PRO AD paba1 Y bi1 / pro1 ad paba1 Y bi1	1	quintuple (*ad* - *paba*, 2 in *paba* - *y*)
xix	paba bi	pro1 AD paba1 Y bi1 / PRO ad paba1 Y bi1	1	sextuple (*pro* - *ad*, *ad* - *paba*, 2 in *paba* - *y*)

* all still heterozygous *acr2 w3* / *ACR W*

ø Selected crossovers are in *ad13* - *ad9* and *ad9* - *ad32* intervals. Intervals in which unselected crossovers occurred are shown in brackets.

** or non-disjunction following selected recombinations

øø Could also result from the *AD* strand being replicated twice, coincidental with a crossing over in the *pro* - *ad* interval (cf. Strickland, 1958b).

Table 32

Comparison of the "standard" meiotic map with recombination fractions for unselected intervals based on selective analysis of crosses involving ad9 and i-ad13

(Data for the ad9 // i-ad13 crosses is taken from Table 27; the assumption is made that the second crossover within the inversion occurred at "X" -- see Fig. 45.)

Interval	Cross and plating	Recombination fractions		X_1^2 **
		Selective analysis	Standard map*	
1 pro - inversion	i(A)	0.58 ± 0.061	0.079 ± 0.012	64.9
	ii(A)	0.10 ± 0.046	"	0.2
	iii(B)	0.67 ± 0.17	"	12.2
	iv(B')	0.32 ± 0.093	"	6.55
2 inversion - paba1	i(A)	0.03 ± 0.021	0.0034 ± 0.000082	1.60
	ii(A)	0.02 ± 0.022	"	0.57
	iii(B)	---		
	iv(B')	0.04 ± 0.039	"	0.88
3 paba - y	i(A)	0.23 ± 0.052	0.157 ± 0.01	1.9
	ii(A)	0.17 ± 0.058	"	0.05
	iii(B)	0.17 ± 0.13	"	0.01
	iv(B')	0.68 ± 0.093	"	30.9
4 y - bi	i(A)	0.14 ± 0.043	0.057 ± 0.004	3.69
	ii(A)	---		
	iii(B)	---		
	iv(B')	0.08 ± 0.054	"	0.18

See next page for footnotes.

Table 32 (Concluded)

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* Value for interval 1 from Käfer (1958) -- this is the standard map value for the pro1 - paba1 interval.

Value for interval 2 from Pritchard (1956) for the larger ad9 - paba1 interval.

Values for intervals 3 and 4 from Käfer (1958).

** X_1^2 values having a probability of less than 0.01 are underscored.

Table 33

Fraction of half-tetrads recombinant for unselected intervals

(see p. 125)

Note that the frequency of meiotic recombination under conditions of no selection is ca. 10^4 greater than that of mitotic recombination (see pp. 68-69).

	Recombination fractions		
	Selective mitotic analysis		Standard meiotic values*
Interval	ad9 / i-ad13	ad32 / i-ad13	
(pro - inversion	0.125	0.18	
(pro - paba1			0.079
(inversion - paba	0.125	0.06	
(ad9 - paba1			0.0034
paba1 - y	--	0.09	0.157
y - bi1	--	--	0.057
(Total no. of half-tetrads)	(16)	(33)	

* As in Table 32.

Table 34

Negative interference spanning the ad13 inversion

	Recombination fraction		Interval position with respect to the selected recombination and inversion	
	Diploid I *	Diploid II *		
Interval	ad33 / i-ad13	ad15 / i-ad13	I	II
pro - ad	0.44	0.20	same side	across
ad - paba	0.75	0.53	across	same side
(Total no. of half-tetrads)	(16)	(15)		

* Data for I from Table 21; data for II from Table 20.

Table 35

Comparison of mitotic recombination in diploids heterozygous for ad33 and i-ad13 or for ad33 and n-ad13 ad32

I. ad33 / n-ad13 ad32

Trans diploid: -

$$\frac{\text{pro1 AD33 n-ad13 ad32 PABA Y bi1}}{\text{PRO ad33 AD13 AD32 paba1 y BI}} \quad \frac{\text{acr2 w3}}{\text{ACR W}}$$

Diploid recombinants: -

+	paba y	bi	pro	y	paba	paba bi	pro bi
178	92 +2* 94	32	1	3	7	7	1

pro paba y
1

Ratio of presumptive single recombinants (S) to doubles (D) plus multiples (M) : -

S	D + M
94	52

$S \neq D + M$ (i.e. $S > (D + M)$)

X_1^2 ** $= 12.082 \quad P < 0.01$

II. ad33 / i-ad13 (data from Table 12)

Trans diploid A : -

$$\frac{\text{PRO ad33 AD13 PABA Y bi1}}{\text{PRO AD33 i-ad13 paba1 y BI}} \quad \frac{\text{ACR w3}}{\text{ACR W}}$$

---

* paba w, presumably of y;w / y;w genotype with respect to colour loci.

** Chi square test of the hypothesis that S = D + M

Table 35
(concluded)

Ratio of presumptive single recombinants (S) to doubles (D) plus multiples (M) : -

S	D + M
46	43

$S = D + M \quad X_1^2 = 0.101 \quad P > 0.70$

Trans diploid B : -

$$\frac{\text{pro1 AD33 i-ad13 PABA Y bi1}}{\text{PRO ad33 AD13 paba1 y BI}} \quad \frac{\text{ACR W}}{\text{acr2 w3}}$$

Ratio of presumptive single recombinants (S) to doubles (D) plus multiples (M) : -

S	D + M
45	48

$S = D + M \quad X_1^2 = 0.097 \quad P > 0.70$

Combined data from II A and B: -

S	D + M
91	91

$S = D + M$

Table 36

Effect of ad13 inversion on negative interference:- a comparison of ad17 / i-ad13 and ad17 / n-ad13 ad32

I. ad9 / ad32 -- Control (see p. 130)

Data from Table 12

Singles(S)	Doubles(D) + Multiples(M)
48	29

$S \neq D + M$ (i.e. $S > [D + M]$)

($P < 0.05$ that $S = D + M$)

II. ad17 / i-ad13 -- Inversion homozygote

Data from Table 12

Singles(S)	Doubles(D) + Multiples(M)
38	18

$S \neq D + M$ (i.e. $S > [D + M]$)

($P < 0.01$ that $S = D + M$)

III. ad17 / n-ad13 ad32 -- Inversion heterozygote

Trans diploid: -

pro1	n-ad13	ad32	AD17	PABA	Y	bi1	acr2	w3
PRO	i-AD32	AD13	ad17	paba1	y	BI	ACR	W

Diploid recombinants: -

+	paba y	bi	paba	y	paba bi	pro bi w
168 +3 w 171	25	23	6	3	7	1

Presumptive singles = 25
Presumptive doubles + multiples = 40

$S \leq D + M$ ($P > 0.05$ that $S = D + M$)

Table 37

Estimation of recombination between *ad* alleles in a cross involving *ad33* and *n-ad13*

Cross: $\dfrac{\text{pro1 AD33 n-ad13 ad32 PABA Y bi1}}{\text{PRO ad33 AD13 AD32 paba1 y BI}}\ \dfrac{\text{acr2 w3}}{\text{ACR W}}$

Selection for				
PABA BI		AD33 AD13 AD32		
Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
3.12×10^5	15	7.50×10^5	3	0.018 ± 0.011

Table 38

Estimation of recombination between ad alleles in a cross involving ad33 and ad32

$$\text{Cross: } \frac{\text{pro1 AD33 ad32 PABA Y bi1}}{\text{PRO ad33 AD32 paba1 y BI}}$$

Selection for				
PABA BI		AD33 AD32		
Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
1.06×10^6	4,856	4.94×10^6	2,611	0.025 ± 0.00061

Table 39

Estimation of recombination between ad alleles in a cross involving ad33 and ad17

Cross: $\dfrac{\text{pro1 AD33 ad17 PABA Y bi1}}{\text{PRO ad33 AD17 paba1 y BI}}$

	Selection for				
	PABA BI		AD33 AD17		
Plating	Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
1	1.44×10^5	1,509	6.38×10^8	1,843	0.000061 ± 0.0000021
2	3.40×10^5	2,652	2.40×10^8	402	0.000047 ± 0.0000025

Homogeneity test of recombination fraction estimates: -

$X_1^2 = 18.39$ $P < 0.01$ that the estimates are homogeneous.

Table 40

Classification of a random sample of adenine-independent recombinants from a cross involving ad33 and ad17

Intervals: 1 0 2 3 4

Cross: $\frac{\text{pro1}}{\text{PRO}}\ \frac{\text{AD33}}{\text{ad33}}\ \frac{\text{ad17}}{\text{AD17}}\ \frac{\text{PABA}}{\text{paba1}}\ \frac{\text{Y}}{\text{y}}\ \frac{\text{bi1}}{\text{BI}}$

Phenotypes	Cross-overs	No. colonies in platings*	
		1	2
pro paba y	0	143	174
paba y	1,0	86	104
pro bi	0,2	1	9
pro paba bi	0,3	39	47
pro paba y bi	0,4	20	17
bi	1,0,2	1	5
paba bi	1,0,3	19	21
paba y bi	1,0,4	6	16
pro y	0,2,3	0	0
pro	0,2,4	0	1
pro paba	0,3,4	3	6
y	1,0,2,3	0	1
+	1,0,2,4	0	0
paba	1,0,3,4	2	1
pro y bi	0,2,3,4	0	0
y bi	1,0,2,3,4	0	0
		320	402

* Same platings as in Table 39.

Table 41

Comparison of recombination fractions for unselected intervals derived from a cross involving ad33 and ad17

(Data from Table 40)

	Recombination fractions		
	Selective analysis		
Interval	Plating 1	Plating 2	Non-selective analysis*
1 (pro-ad)	0.36 ± 0.027	0.37 ± 0.024	0.079 ± 0.012
2 (ad-paba)	0.0063 ± 0.0044	0.040 ± 0.0071	0.0054 ± 0.00099
3 (paba-y)	0.20 ± 0.022	0.19 ± 0.020	0.157 ± 0.010
4 (y-bi)	0.097 ± 0.017	0.10 ± 0.015	0.057 ± 0.004

Homogeneity tests of platings 1 and 2: -

Interval	X^2_1	P
1	0.11	>0.70
2	16.12	<0.01
3	0.07	>0.70
4	0.05	>0.80

Pooled and weighted estimates from platings 1 and 2: -

(1) 0.37 ± 0.018
(2) not homogeneous
(3) 0.19 ± 0.014
(4) 0.099 ± 0.011

See next page for footnote.

Table 41 (concluded)

Homogeneity tests of recombination fractions derived from selective analysis and the standard values* : -

Interval	Plating 1		Plating 2		Pooled estimates	
	X_1^2	P	X_1^2	P	X_1^2	P
1	90.45	<0.01	117.6	<0.01	180.94	<0.01
2	2.42	>0.10	22.99	<0.01	—	
3	3.17	>0.05	2.18	>0.10	3.68	>0.05
4	5.25	<0.05	7.67	<0.01	12.88	<0.01

---

* The recombination fraction for interval 1 is for the pro1 - paba1 interval (Käfer, 1958), for interval 2 is derived from an ad17 // paba1 cross (Calef, 1957), for interval 3 and interval 4 from Käfer (1958).

Table 42

Estimation of recombination between ad alleles in a cross involving ad33 and ad15

Cross: $\dfrac{\text{pro1 AD33 ad15 PABA Y bi1}}{\text{PRO ad33 AD15 paba1 y BI}}$

Selection for				
PABA BI		AD33 AD15		
Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
1.36×10^6	783	2.13×10^7	209	0.0037 ± 0.00029

Table 43

Classification of adenine-independent recombinants from a cross involving ad33 and ad15

(from Table 42)

Intervals: 1 0 2 3 4

Cross: $\dfrac{\text{pro1}\quad\text{AD33}\quad\text{ad15}\quad\text{PABA}\quad\text{Y}\quad\text{bi1}}{\text{PRO}\quad\text{ad33}\quad\text{AD15}\quad\text{paba1}\quad\text{y}\quad\text{BI}}$

Phenotypes	Crossovers	No. colonies found
pro paba y	0	164
paba y	1,0	6
pro bi	0,2	1
pro paba bi	0,3	23
pro paba y bi	0,4	4
bi	1,0,2	0
paba bi	1,0,3	7
paba y bi	1,0,4	0
pro y	0,2,3	0
pro	0,2,4	1
pro paba	0,3,4	1
y	1,0,2,3	0
+	1,0,2,4	2
paba	1,0,3,4	0
pro y bi	0,2,3,4	0
y bi	1,0,2,3,4	0
		209

Table 44

Comparison with the standard map of recombination fractions for unselected intervals derived from a cross involving ad33 and ad15

(from Table 43)

Interval	Recombination fractions	
	Selective analysis	Standard*
1 (pro-ad)	0.072 ± 0.018	0.079 ± 0.012 (a) 0.100 ± 0.013 (b)
2 (ad-paba)	0.019 ± 0.0095	0.0033 ± 0.000082
3 (paba-y)	0.15 ± 0.025	0.157 ± 0.010
4 (y-bi)	0.038 ± 0.013	0.057 ± 0.004

Homogeneity tests: -

Interval	X_1^2	P
1 (a)	0.10	>0.50
1 (b)	1.62	>0.20
2	2.73	>0.05
3	0.07	>0.70
4	1.95	>0.10

---

* The standard recombination fractions were obtained from the following:-
(1a) Käfer (1958) for the pro1 - paba1 interval
(1b) Table 62 for pro1 - ad33 interval when selection was made for AD PABA from ad33 // paba1
(2) Table 60 for ad15 - paba1 interval
(3) Käfer (1958)
(4) Käfer (1958).

Table 45

Comparison of recombination fractions for unselected intervals derived from ad9 // ad32 and n-ad13 ad9 // ad32 crosses

(Data from Tables 23 and 24)

Interval	Recombination fractions				
	Selective analysis				
	ad9//ad32			n-ad13 ad9//ad32	
	i	ii	iii	iv	Standard*
1	0.21 ± 0.034	0.48 ± 0.093	0.23 ± 0.063	0.26 ± 0.055	0.079 ± 0.012
2	0.12 ± 0.027	0.21 ± 0.075	0.23 ± 0.063	0.16 ± 0.047	0.0025 ± 0.000068
3	0.20 ± 0.033	0.17 ± 0.070	0.14 ± 0.052	0.25 ±** 0.078	0.157 ± 0.010
	0.09 ± 0.024	--	0.04 ± 0.031	0.065 ±** 0.044	0.057 ± 0.004

Homogeneity tests of values derived from selective analysis: - The value in ii for interval 1 is heterogeneous when tested against those obtained in i, iii and iv, as shown in the following:

Pair tested	X^2_1	P
i & ii	7.45	< 0.01
ii & iii	5.20	< 0.05
ii & iv	4.16	< 0.05

Homogeneity tests of selective values with the standard map values: - (continued next page)

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* (1) from Käfer (1958) for pro1 - paba1 interval
(2) from Table 60 for ad32 - paba1 interval
(3) and (4) from Käfer (1958)

** Based on W recombinants only.

Table 45 (concluded)

Homogeneity tests of selective values with the standard map values: -

	i		ii		iii		iv	
Interval	X_1^2	P	X_1^2	P	X_1^2	P	X_1^2	P
1	13.20	<0.01	18.29	<0.01	5.54	<0.02	10.34	<0.01
2	18.56	<0.01	7.74	<0.01	12.98	<0.01	11.23	<0.01
3	1.56	>0.20	0.03	>0.90	0.10	>0.70	1.40	>0.20
4	1.84	>0.10	--		0.30	>0.50	0.03	>0.80

Table 46

Combined estimates of recombination fractions in Table 45

(Homogeneous estimates weighted and pooled)

	Recombination fractions		
Interval	Selective analysis	Standard	X_1^2 *
1 (pro-ad)	0.23 ± 0.026	0.079 ± 0.012	27.806
2 (ad-paba)	0.15 ± 0.021	0.0025 ± 0.000068	49.333
3 (paba-y)	0.19 ± 0.025	0.157 ± 0.01	1.502
4 (y - bi)	0.070 ± 0.017	0.057 ± 0.004	0.562

* Testing homogeneity of selective and standard recombination fractions. Chi square values having probabilities of less than 0.01 are underscored.

Table 47

Estimation of recombination in a cross involving ad9 and ad15

Intervals:	1	0	2	3	4	
Cross:	pro1	ad9	AD15	PABA	Y	bi1
	PRO	AD9	ad15	paba1	y	BI

I.

Estimation of recombination between ad alleles

Selection for				
PRO PABA		AD9 AD15		
Spores plated	Colonies	Spores plated	Colonies	Recombination fraction*
7.86×10^4	1,186	3.01×10^6	53	0.000093 ± 0.000013

II.

Classification of adenine-independent recombinants

Phenotypes	Crossovers	No. colonies found
bi	0	42
pro bi	1,0	10
pro	1,0,4	1

* Because PRO PABA selection was used, x = 0.08 (the standard value for the pro - paba interval, Fig. 1) was employed in the calculation of the recombination fraction (cf. Table 4).

Table 47 (concluded)

III.

Recombination fractions for unselected intervals

Interval	Recombination fractions	
	Selective analysis	Standard ∅
1 (pro-ad)	0.21 ± 0.056 **	0.079 ± 0.012
2 (ad-paba)	---	0.0033 ± 0.000082
3 (paba-y)	---	0.157 ± 0.010
4 (y-bi)	0.019 ± 0.019	0.057 ± 0.004

∅ Interval (1) from Käfer (1958) for pro1 - paba1 interval, interval (2) from Table 60 for ad15 - paba1 interval, intervals (3) and (4) from Käfer (1958).

** Heterogeneous when tested against standard recombination fraction: - $X_1^2 = 5.28$, $P < 0.05$.

Table 48

Estimate of recombination between ad alleles in crosses involving ad32 and ad17

Cross A: $$\frac{\text{pro1 AD32 ad17 paba1 y BI}}{\text{PRO ad32 AD17 PABA Y bi1}} \quad \frac{\text{PYRO}}{\text{pyro4}}$$

Cross B: $$\frac{\text{pro1 AD32 ad17 PABA Y bi1}}{\text{PRO ad32 AD17 paba1 y BI}} \quad \frac{\text{W}}{\text{w3}}$$

	Selection for				
	PABA BI		AD32 AD17		
Cross and plating	Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
A(i)	1.29×10^4	398	1.03×10^6	1	0.0000069 ± 0.0000069
A(ii)	1.13×10^4	208	2.10×10^6	1	0.0000057 ± 0.0000057
B(i)	6.14×10^4	1816	4.08×10^7	17	0.0000031 ± 0.00000075
Pooled and weighted estimate *					0.0000032 ± 0.00000074

* The three estimates are homogeneous.

Table 49

Classification of adenine-independent recombinants from crosses involving ad32 and ad17

Intervals: 1 0 2 3 4

Cross A: $\frac{pro1 \quad AD32 \quad ad17 \quad paba1 \quad y \quad BI}{PRO \quad ad32 \quad AD17 \quad PABA \quad Y \quad bi1} \quad \frac{PYRO}{pyro4}$

Cross B: $\frac{pro1 \quad AD32 \quad ad17 \quad PABA \quad Y \quad bi1}{PRO \quad ad32 \quad AD17 \quad paba1 \quad y \quad BI} \quad \frac{W}{w3}$

Cross-over type	Cross A		Cross B*	
	Phenotype	No. Colonies	Phenotype	No. Colonies
0	pro bi (PYRO (pyro	6 0	pro paba y (W (w	0 0
1,0	bi (PYRO (pyro	1 2	paba y (W (w **	5 33
0,2	pro paba y (PYRO (pyro	1 1	pro bi (W (w	1 1
0,3	pro y (PYRO (pyro	0 0	pro paba bi (W (w	0 0
0,4	pro (PYRO (pyro	0 0	pro paba y bi (W (w	0 0
1,0,2	paba y (PYRO (pyro	4 1	bi (W (w ø	2 7
1,0,3	y (PYRO (pyro	0 0	paba bi W	3
1,0,4	+ (PYRO (pyro	0 0	paba y bi W	0
1,0,3 or 1,0,4			paba bi w	2
1,0,2,3	paba bi (PYRO (pyro	2 0	y W	0
1,0,2,3, or 1,0,2,4			w	2
		18		56

See next page for footnotes

Table 49 (concluded)

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* Pooled data of two platings, one of which was B(i) in Table 48.

** Presumed to carry y, these colonies were not outcrossed to verify the allele present at the y locus.

ø Presumed to carry Y, these colonies were not outcrossed to verify the allele present at the y locus.

Table 50

Estimation of recombination between ad alleles in crosses involving ad32 and ad15

Cross A: $\dfrac{\text{pro1 AD32 ad15 paba1 y BI}}{\text{PRO ad32 AD15 PABA Y bi1}} \quad \dfrac{\text{W}}{\text{w3}}$

Cross B: $\dfrac{\text{pro1 AD32 ad15 paba1 y BI}}{\text{PRO ad32 AD15 PABA Y bi1}} \quad \dfrac{\text{PYRO}}{\text{pyro4}}$

	Selection for				
	PABA BI		AD32 AD15		
Cross	Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
A	2.48×10^4	86	7.82×10^6	4	0.000032 ± 0.000016
B	4.08×10^5	3,171	1.79×10^8	72	0.000015 ± 0.0000013
Pooled and weighted estimate *					0.000015 ± 0.0000013

* Homogeneity test of recombination fraction estimates from A and B gave: - $X_1^2 = 1.71 \qquad P > 0.10$

Table 51

Classification of adenine-independent recombinants from a cross involving ad32 and ad15

Intervals: 1 0 2 3 4

Cross B: $\frac{\text{pro1 \quad AD32 \quad ad15 \quad paba1 \quad y \quad BI}}{\text{PRO \quad ad32 \quad AD15 \quad PABA \quad Y \quad bi1}}$ $\frac{\text{PYRO}}{\text{pyro4}}$

Phenotype	Crossover type	No. colonies found	
pro bi) pro bi pyro)	0	15) 12)	27
bi) bi pyro)	1,0	5) 5)	10
pro paba y) pro paba y pyro)	0,2	6) 6)	12
pro y) pro y pyro)	0,3	4) 2)	6
pro) pro pyro)	0,4	2) 1)	3
paba y) paba y pyro)	1,0,2	3) 2)	5
y) y pyro)	1,0,3	0) 3)	3
+) + pyro)	1,0,4		0
pro paba bi) pro paba bi pyro)	0,2,3	2) 1)	3
pro paba y bi) pro paba y bi pyro)	0,2,4		0
pro y bi) pro y bi pyro)	0,3,4		0
paba bi) paba bi pyro)	1,0,2,3	0) 2)	2
paba y bi) paba y bi pyro)	1,0,2,4		0
y bi) y bi pyro)	1,0,3,4		0

Table 51 (concluded)

Phenotype	Crossover type	No. colonies found	
pro paba) pro paba pyro)	0,2,3,4	1) 0)	1
	TOTAL		72

Table 52

Comparison with the standard map of recombination fractions for unselected intervals derived from a cross involving ad32 and ad15

(from Table 51)

Interval	Recombination fractions	
	Selective analysis	Standard *
1 (pro-ad)	0.28 ± 0.053	0.079 ± 0.012
2 (ad-paba)	0.32 ± 0.055	0.0033 ± 0.000082
3 (paba-y)	0.21 ± 0.048	0.157 ± 0.010
4 (y-bi)	0.056 ± 0.027	0.057 ± 0.004

Homogeneity tests of the values derived from selective analysis and the standard recombination fractions: -

Interval	X_1^2	P
1	13.69	<0.01
2	33.16	<0.01
3	1.17	>0.20
4	0.001	>0.95

---

* (1) from Käfer (1958) for the pro1 - paba1 interval,
(2) from Table 60 for the ad15 - paba1 interval,
(3) and (4) from Käfer (1958).

Table 53

Estimation of recombination between *ad* alleles in a cross involving *ad15* and *ad17*

Cross: $\frac{\text{PRO AD17 ad15 paba1 y BI}}{\text{pro1 ad17 AD15 PABA Y bi1}}\ \frac{\text{acr2 w3}}{\text{ACR W}}$

Selection for				
PABA BI		AD17 AD15		
Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
8.67×10^4	5,196	1.59×10^7	61	0.000014 ± 0.0000018

Table 54

Classification of adenine-independent recombinants from a cross involving ad15 and ad17

(from Table 53)

Intervals:		1		0		2		3		4			
Cross:	PRO		AD17		ad15		paba1		y		BI	acr2	w3
	pro1		ad17		AD15		PABA		Y		bi1	ACR	W

Phenotype	Crossover type	No. colonies found	
bi	0	12	28
bi w *		16	
pro bi	1,0	0	4
pro bi w *		4	
paba y	0,2	6	12
paba w **		6	
y	0,3	6	11
+	0,4	1	
w	0,3 or 0,4	4	
paba bi	0,2,3	1	4
paba bi w *		3	
y bi	0,3,4	1	1
(y) bi w ø		?	
pro paba bi	1,0,2,3	1	1
pro paba bi w		0	
			61

* Presumed to carry Y

** Presumed to carry y

ø Not distinguishable from type 0 crossover.

Table 55

Comparison with the standard map of recombination fractions derived from data in Table 54

	Recombination fractions	
Interval	Selective analysis	Standard *
1 (pro-ad17)	0.082 ± 0.033	(i) 0.079 ± 0.012 (ii) 0.086 ± 0.011
2 (ad15-paba1)	0.28 ± 0.057 ø	(i) 0.0033 ± 0.000082 (ii) 0.0076 ± 0.00099
3 (paba1-y)	0.32 ± 0.088** øø	0.157 ± 0.010
4 (y-bi1)	0.071 ± 0.049**	0.057 ± 0.004

* Sources are as follows: -

(1-i) Käfer (1958) for <u>pro1</u> - <u>paba1</u> interval
(1-ii) calculated from pooled data of crosses A, B and A", non-selective analysis, of Calef (1957)
(2-i) Table 60
(2-ii) Calef (1957) after correction of arithmetical error
(3) Käfer (1958)
(4) " "

** Based on <u>W</u> recombinants only.

ø Homogeneity tests with standard values (i) and (ii) give X^2_1 values of 23.57 ($P < 0.01$) and 24.66 ($P < 0.01$), respectively.

øø Homogeneity test with standard recombination fraction gives X^2_1 value of 3.39 ($P > 0.05$).

Table 56

Comparison between recombination fractions based on selective analysis and those based on non-selective analysis of ad17 // ad15 crosses

(From Calef, 1957, with minor corrections)

Intervals:		1		0		2		3		4		
Cross A:	PRO pro1		ad17 AD17		AD15 ad15		paba1 PABA		y Y		BI bi1	w3 W
Cross A':	PRO PRO		ad17 AD17		AD15 ad15		paba1 PABA		y Y		BI bi1	
Cross A":	PRO pro1		ad17 AD17		AD15 ad15		paba1 PABA		y Y		BI bi1	
Cross B :	PRO pro1		AD17 ad17		ad15 AD15		paba1 PABA		y Y		BI bi1	w3 W

	Cross A			Cross A'			Cross A"			Cross B		
	Recombination fractions			Recombination fractions			Recombination fractions			Recombination fractions		
Interval	Selective	Non-selective	X_1^2 ∅	Selective	Non-selective	X_1^2 ∅	Selective	Non-selective	X_1^2 ∅	Selective	Non-selective	X_1^2 ∅
1	0.167 ± 0.025	0.075 ± 0.066	6.98	--	--	--	0.296 ± 0.037	0.089 ± 0.015	18.1	0.146 ± 0.026	0.082 ± 0.018	4.11
2	0.310 ± 0.031	0.0076 ± 0.00099 *	95.1	0.388 ± 0.030	0.0076 ± 0.00099 *	160.6	0.324 ± 0.038	0.0076 ± 0.00099 *	69.3	0.298 ± 0.034	0.0076 ± 0.00099 *	53.7
3	0.153 ± 0.024	0.056 ± 0.018	27.0	0.240 ± 0.027	0.117 ± 0.028	12.7	0.222 ± 0.033	0.156 ± 0.019 **	3.2	0.191 ± 0.029	0.102 ± 0.019	5.46
4	0.056 ± 0.0156	0.019 ± 0.011 ∅∅	3.25	0.098 ± 0.0185	0.031 ± 0.02	6.28	0.136 ± 0.028	0.040 ± 0.010	16.6	0.084 ± 0.021	0.055 ± 0.014	1.32

Homogeneity tests of non-selective recombination fractions for intervals 3 and 4 with standard values of Käfer (1958): -

	X_1^2 ∅			
Interval	Cross A	Cross A'	Cross A"	Cross B
3	24.1	1.81	0.002	6.56
4	10.5	2.58	2.37	0.002

* from ad17 // paba1 cross where selection was made for AD PABA (Calef, 1957)

∅ Chi square values corresponding to probabilities of less than 0.05 are underscored.

** Heterogeneous when tested against non-sel. values obtained in Crosses A & B

∅∅ Heterogeneous when tested against non-sel. value obtained in Cross B.

Table 57

Estimation of recombination between ad alleles in a cross involving i-ad13 and ad17

Cross: $\frac{\text{pro1 i-AD13 ad17 PABA Y bi1}}{\text{PRO i-ad13 AD17 paba1 y BI}}$ $\frac{\text{W}}{\text{w3}}$

Selection for				
PABA BI		AD13 AD17		
Spores plated	Colonies	Spores plated	Colonies	Recombination fraction
1.15×10^4	611	8.41×10^6	34	0.000017 ± 0.0000030

Table 58

Classification of adenine-independent recombinants from a cross involving i-ad13 and ad17

(from Table 57)

Intervals: 1 0 2 3 4

Cross: $\frac{\text{pro1 \quad i-AD13 \quad ad17 \quad PABA \quad Y \quad bi1}}{\text{PRO \quad i-ad13 \quad AD17 \quad paba1 \quad y \quad BI}}$ $\frac{\text{W}}{\text{w3}}$

Phenotype*	Crossover type	No. colonies found	
pro paba y	0	2	15
pro paba w		13	
paba y	1,0	6	10
paba w		4	
pro bi	0,2		0
pro bi w			
pro paba bi	0,3	1	7
pro paba bi w	0,3 or 0,4	3	
pro paba y bi	0,4	3	
bi	1,0,2	0	1
bi w		1	
paba bi	1,0,3		1
paba bi w	1,0,3 or 1,0,4		0
			34

* Those w phenotypes listed as crossover types 0 and 1,0 are presumed to carry y; those listed as types 0,2 and 1,0,2 are presumed to carry Y.

Table 59

Comparison with the standard map of recombination fractions derived from data in Table 58

Interval	Recombination fractions Selective analysis	 Standard*	X^2_1 **
(pro1 - iad13)[1]	0.35 ± 0.082	0.079 ± 0.012	10.63
(ad17-paba1)[2]	0.029 ± 0.029	0.0054 ± 0.00092	0.66
(paba1-y)[3]	0.15 ± 0.10 ϕ	0.157 ± 0.010	0.00
(y-bi1)[4]	0.23 ± 0.12 ϕ	0.057 ± 0.004	2.08
(paba1-bi1)[3 + 4]	0.24 ± 0.073		

* Sources are as follows: -
(1) Käfer (1958) for *pro1* - *paba1* interval
(2) from Calef (1957) after correction of arithmetical error
(3) Käfer (1958)
(4) " "

** Chi square test of homogeneity between selective and standard recombination fractions. Those values having a probability of less than 0.01 are underscored.

ϕ Based on *W* recombinants only.

Table 60

Estimation of recombination between ad and paba1 in ad33 // paba1, ad32 // paba1 and ad15 // paba1 crosses

Cross A: $\dfrac{\text{pro1 AD33 paba1 y BI}}{\text{PRO ad33 PABA Y bi1}}$ $\dfrac{\text{PYRO}}{\text{pyro4}}$

Cross B: $\dfrac{\text{pro1 AD32 paba1 y BI}}{\text{PRO ad32 PABA Y bi1}}$ $\dfrac{\text{PYRO}}{\text{pyro4}}$

Cross C: $\dfrac{\text{pro1 AD15 paba1 y BI}}{\text{PRO ad15 PABA Y bi1}}$

	Selection for				
	PABA BI		AD PABA		
Plating	Spores plated	Colonies	Spores plated	Colonies*	Recombination fraction
			Cross A (ad33)		
i	7.43×10^4	1193	1.03×10^5	9	0.0012 ± 0.00040
ii	2.18×10^5	3330	6.26×10^6	662	0.0014 ± 0.000057
iii	2.59×10^5	1478	2.80×10^6	231	0.0015 ± 0.00010
Pooled and weighted estimate **					0.0014 ± 0.000049
			Cross B (ad32)		
	9.89×10^4	3649	4.95×10^6	2064	0.0025 ± 0.000068
			Cross C (ad15)		
	7.43×10^4	7263	1.36×10^6	1980	0.0033 ± 0.000082

See next page for footnotes

Table 60 (concluded)

Summary of X_1^2 values obtained in homogeneity tests of the recombination fraction estimates: - (X_1^2 values having probabilities less than 0.01 are underscored)

	A(i)	A(ii)	A(iii)	B	C
A(i)	--	0.24	0.53	<u>10.27</u>	<u>26.45</u>
A(ii)		--	0.75	<u>153.69</u>	<u>361.98</u>
A(iii)			--	<u>68.38</u>	<u>193.73</u>
B				--	<u>56.40</u>

---

* Diploids removed from the analysis.

** The three platings are homogeneous, see summary table on this page.

ϕ Homogeneity test with Calef's (1957 -- corrected) value gives $X_1^2 = 18.74, \quad P < 0.01$

Table 61

Classification of AD PABA recombinants from the ad // paba1 crosses in Table 60

Interval: a b c d

Crosses: $\dfrac{\text{pro1 \quad AD \quad paba1 \quad y \quad BI \quad PYRO}}{\text{PRO \quad ad* \quad PABA \quad Y \quad bi1 \quad pyro4**}}$

Phenotype	Cross-over type	No. colonies found in				
		Cross A		Cross B		Cross C
pro bi	b	180	363	159	306	313
pro bi pyro		183		147		
bi	ab	22	40	25	49	45
bi pyro		18		24		
pro y	bc	48	96	49	94	94
pro y pyro		48		45		
pro	bd	13	35	20	29	34
pro pyro		22		9		
y	abc	6	11	5	9	13
y pyro		5		4		
+	abd	2	5	4	7	6
+ pyro		3		3		
pro y bi	bcd	4	10	4	9	7
pro y bi pyro		6		5		
y bi	abcd		0		0	3
y bi pyro						
	Totals		560		503	515

* ad* stands for ad33 in Cross A, ad32 in Cross B and ad15 in cross C.

** Cross C is homozygous PYRO / PYRO.

Table 62

Comparison of recombination fractions derived from Table 61 with the standard map

Interval	Recombination fractions			
	Standard*	Cross A (*ad33*)	Cross B (*ad32*)	Cross C (*ad15*)
a (pro-adø)	0.079 ± 0.012	0.100 ± 0.013	0.129 ± 0.015	0.130 ± 0.014
c (paba-y)	0.157 ± 0.010	0.209 ± 0.017	0.223 ± 0.019	0.227 ± 0.018
d (y-bi)	0.057 ± 0.004	0.089 ± 0.012	0.089 ± 0.013	0.097 ± 0.013

Homogeneity tests of recombination fractions derived from the *ad* // *paba1* crosses with the standard values: -

(Chi square values corresponding to probabilities of less than 0.05 are underscored.)

Interval	X_1^2 for standard tested with		
	Cross A	Cross B	Cross C
a	1.41	*6.78*	*7.65*
c	*6.95*	*9.45*	*11.57*
d	*6.40*	*5.54*	*8.65*

---

* from Käfer (1958); interval *a* is for the *pro1* - *paba1* interval

ø for Cross A this interval is *pro1* - *ad33*; for Cross B, *pro1* - *ad32*; for Cross C, *pro* - *ad15* -- the selective values are thus being compared with the non-selective value for the larger *pro1* - *paba1* interval.

Table 63

Comparison between recombination fractions based on selective analysis and those based on non-selective analysis in crosses involving ad15 (or ad17) and paba1 (Data from Calef, 1957, and Elliott, 1960b)

pro1 --- ad15 (or 17) --- paba1 --- y --- bi1
Intervals: a b c d

	Recombination fractions in:							
	ad15 cross* (Cross 4)		ad17 crosses*					
			Cross 1		Cross 2		Cross 3	
Interval	Selective	Non-selective	Selective	Non-selective	Selective	Non-selective	Selective	Non-selective
a	0.257 ± 0.043	0.110 ± 0.0090 Y	0.1081 ± 0.0094	0.0876 ± 0.0085	0.0906 ± 0.0086	0.0712 ± 0.0080	0.124 ± 0.022	0.092 ± 0.019 ø
c	0.257 ± 0.043	0.127 ± 0.0089 Y	0.2247 ± 0.0179	0.1653 ± 0.0149 Z	0.2366 ± 0.0177	0.1157 ± 0.0136 Y	0.236 ± 0.028	0.179 ± 0.025
d	0.124 ± 0.033 ø	0.0362 ± 0.0054 Y	0.0626 ± 0.0104	0.0546 ± 0.0091	0.0518 ± 0.0092	0.0416 ± 0.0085	0.073 ± 0.017	0.058 ± 0.048

* Cross 1 (Elliott, 1960b): - pro1 ad17 y; Acr1 w3 // paba1 bi1
Cross 2 (Elliott, 1960b): - ad17 bi1; Acr1 w3 // pro1 paba1 y
Cross 3 (Calef, 1957) : - ad 17 bi1 // pro1 paba1 y
Cross 4 (Calef, 1957) : ad15 bi1 // pro1 paba1 y

ø arithmetical error of Calef (1957) corrected

Y Selective and non-selective estimates significantly different at 1% level.

Z Selective and non-selective estimates significantly different at 2% level.

Table 64

Half-tetrad analysis of a diploid heterozygous for ad32 and ad15

Trans Diploid: $\frac{\text{pro1 ad32 AD15 PABA Y bi1}}{\text{PRO AD32 ad15 paba1 y BI}} \; \frac{\text{W ACR}}{\text{w3 acr2}}$

Genotype of wild type recombinant *	Probable type of recombination	No. obtained
PRO AD PABA Y bi1 pro1 ad paba1 y BI	Single (the selected) crossover	2
PRO AD paba1 y BI pro1 ad PABA Y bi1	Double:- unselected cross-over in pro - ad interval	8
pro1 AD PABA Y bi1 PRO ad paba1 y BI	Double:- unselected cross-over in ad - paba interval	2
PRO AD paba1 ? bi1 pro1 ad PABA ? BI	Triple:- unselected cross-overs in pro - ad and paba - bi intervals	1

* All recombinants still heterozygous acr2 w3 / ACR W . The genotypes of these recombinants with respect to ad were not determined.

Table 65

The relationship between the recombination fraction for the selected interval and the recombination fraction in the interval between *pro1* and the proximal *ad* allele

Cross involving selection between	Recombination fraction (x 10^5)	Recombination fraction between *pro* and proximal *ad* allele (x 10^2)
ad33 & *ad15*	370	7.2 ± 1.8
ad33 & *paba1*	140	10.0 ± 1.3
ad33 & *ad17*	(6.1 (4.7	37 ± 1.8
n-ad13 ad9 & *ad32*	1.0	26 ± 5.5
ad9 & *ad15*	9.3	21 ± 5.6
ad9 & *ad32*	0.46	21 ± 3.4
	0.41	48 ± 9.3
	0.84	23 ± 6.3
ad32 & *paba1*	250	12.9 ± 1.3
ad32 & *ad15*	1.5	28 ± 5.3
i-ad13 & *ad9*	5.1 ø	(59 ± 5.7
		(10 ± 4.6
" "	1.4 ø	(67 ±17
		(32 ± 9.3
i-ad13 & *ad17*	1.7	35 ± 8.2
ad17 & *paba1*	540 *	9.9 ± 0.6 *
ad17 & *ad15*	1.4	(8.2 ± 3.3
		(15.7 ± 1.8 **
		(29.6 ± 3.7 ***

(continued next page)

Table 65 (concluded)

Cross involving selection between	Recombination fraction (x 10^5)	Recombination fraction between *pro* and proximal *ad* allele (x 10^2)
ad15 & *paba1*	330	13.0 ± 1.4
	760 *	25.7 ± 4.3 *

ø Frequency with which two-strand double recombinations within the inversion (giving rise to adenine-independent progeny) are estimated to occur, see pp. 101-103.

* Data from Calef (1957).

** Pooled data from Crosses A and B of Calef (1957).

*** Data from Cross A" of Calef (1957).

Table 66

The relationship between the recombination fraction for the selected interval and the recombination fraction in the interval between the distal ad allele and paba1

Cross involving selection between	Recombination fraction (x 10^5)	Recombination fraction between distal ad allele and paba (x 10^3)	Control value (x 10^3)	Difference
ad33 & ad15	370	19 ± 9.5	3.3	15.7
ad33 & ad17	(6.1 (4.7	6.3 ± 4.4) 40 ± 7.1)	5.4 **	0.9 34.6
i-ad13 & ad9	(5.1 ø (1.4 ø	27 ±14	3.4 *	23.6
i-ad13 & ad17	1.7	29 ±29	5.4 **	23.6
ad32 & ad15	1.5	320 ±55	3.3	316.7
ad17 & ad15	1.4	(280 ± 57 (355 ± 16 **	3.3 7.6 **	276.7 347.4
ad9 & ad32 ***	(.45 *** (.93 ***	150 ± 21	2.5	147.5

ø Frequency with which two-strand double recombinations within the inversion (giving rise to adenine-independent progeny) are estimated to occur, see pp. 101-103.

* Data from Pritchard (1956).

** Data from Calef (1957).

***Pooled and weighted data from ad9 // ad32 and n-ad13 ad9 // ad32 crosses. The two estimates of recombination in the selected interval were obtained from pooled and weighted data of crosses giving homogeneous estimates.

Table 67

Negative interference in mitotic recombination:- the effect on recombination in the pro1 - ad13 and ad32 -paba1 intervals of a displacement of the selective interval

			S ↓		S ↓						
Diploid A:	pro1	n-ad13		AD9		ad32	PABA	Y	bi1	w3	acr2
	PRO	AD13		ad9		AD32	paba1	y	BI	W	ACR
			X ↓		S ↓						
Diploid B:	pro1	n-ad13		ad9		AD32	PABA	Y	bi1	w3	acr2
	PRO	AD13		AD9		ad32	paba1	y	BI	W	ACR

LEGEND: S = selected interval

X = interval selected against (i.e. the mean effective pairing segment midpoint is displaced to the right in diploid B as compared to diploid A.

Interval	Recombination fractions based on half-tetrads: Diploid A	Recombination fractions based on half-tetrads: Diploid B
centromere-pro1	0.04	0
pro - ad13	0.46	0.22
ad32 - paba1	0.20	0.48
paba1 - y	0.18	0.12
y - bi1	0	0
(Total half-tetrads)	(50)	(50)

APPENDIX B.

PROPOSED TEST OF THE HYPOTHESIS THAT AN ADENINE-INDEPENDENT HAPLOID POSSESSES THE ad13 INVERSION.

(including Table B-1)

It should be possible to determine genetically whether the *ad13* inversion has been introduced into an otherwise wild-type *ad9* cistron. The crucial test would be to compare the results from half-tetrad analysis of the *trans* recombinants from the following two *cis* diploids: -

(1) a diploid composed of an adenine-independent strain suspected to have the inversion, and also of an *i-ad32 ad13* (or *i-ad9 ad13*) strain, ?

(2) a diploid consisting of an adenine-independent strain suspected to have the inversion, and also of a *n-ad13 ad32* (or *n-ad13 ad9*) strain.

If diploid (1) indicates that a single crossing-over in the *ad32* - *ad13* (or *ad9* - *ad13* for the alternative diploid given in 1) interval can result in viable recombinants, but not diploid (2), then it will be shown that the *i?-AD32 AD13* (or *i?-AD9 AD13*) strain does in fact have the rearrangement. Conversely, should single crossovers be found among recombinants of diploid (2), but not diploid (1), then the *AD* strand would necessarily possess the normal sequence of *ad9* cistron sites.

It is not possible to isolate auxotrophic recombinants from prototrophic parental types by the techniques employed in the present studies (i.e. it is not possible to select, by these methods, *from* the *cis* *for* the *trans* arrangement of *ad* alleles). However, a scheme involving selection for p.a.b.a.-independent segregants from *paba1* / *paba6* diploids should afford an indirect method of doing so, provided the following conditions are met: -

(a) That the two diploids to be compared are of the constitution indicated below (see Table B-1 for derivation and authentication of strains),

(i): $$\frac{\text{PRO i?-AD32 AD13 paba1 PABA y BI}}{\text{pro1 i-ad32 ad13 PABA paba6 Y BI}}\quad\frac{\text{w3 acr2}}{\text{W ACR}}$$

(ii): $$\frac{\text{PRO i?-AD32 AD13 paba1 PABA y BI}}{\text{pro1 n-ad13 ad32 PABA paba6 Y BI}}\quad\frac{\text{w3 acr2}}{\text{W ACR}}\ ,$$

(b) That selection for recombination in the *paba1* - *paba6* interval is accompanied by a high negative interference extending proximally into the *ad13* - *ad32* interval.

Under this scheme, one is interested in only those of the p.a.b.a.-independent segregants showing an adenine-requirement which have a single *ad* allele in coupling with *PABA1 PABA6*. That the proportion of such recombinants, in relation to the total number of adenine-requirers, will probably be high enough to make this test technically feasible is suggested by the half-tetrad analyses of

Table B-1

Origin and authentication of strains involved in a proposed test for the presence of a rearrangement within the *ad9* cistron of an adenine-independent haploid

a) *Suspected rearrangement in i?-AD32 AD13 strain*.

Strain: *PRO i-AD32 AD13 paba1 y BI*

Source: Class iii, Table 17.

b) *i-ad32 ad13 strain*.

Strain: *i-ad32 ad13 paba1 y*; *acr2 w3*

Source: Class i, Table 17

Crosses necessary to introduce markers:-

(1) outcross to obtain *pro1 (i-ad32 ad13) y*

(2) outcross (1) to obtain *pro1 (i-ad32 ad13) paba6 Y BI*

c) *Confirmation that pro1 (i-ad32 ad13) paba6 Y BI carries i-ad32 ad13*:-

(1) Presence of *ad* mutants determined by genotype elucidation (as in Chapter I)

(2) Presence of inverted sequence determined by mitotic recombination experiment involving *pro1 (i-ad32 ad13) paba6 Y BI* / *PRO ad9 PABA y bi1*. This should give results similar to *i-ad32 ad13* / *ad9* (Tables 28 and 29).

d) *n-ad13 ad32 strain*.

Strain: *pro1 n-ad13 ad32 PABA Y bi1*; *w3 acr2*

Source: Class iii, Table 17.

Crosses necessary to introduce markers:

(1) Outcross to obtain *pro1 (n-ad13 ad32) paba6 Y BI*

Table B-1 (concluded)

e) Confirmation that pro1 (n-ad13 ad32) paba6 Y BI carries n-ad13 ad32.

(1) Presence of ad mutants determined by genotype elucidation (as in Chapter I)

(2) Presence of normal sequence determined by mitotic recombination experiment involving pro1 (n-ad13 ad32) paba6 Y BI / PRO ad9 PABA y bi1. This should give results similar to n-ad13 ad32 / ad9 (Tables 28 and 29).

n-ad13 ad32 / *ad9* (Table 31) and *n-ad13 ad9* / *ad32* (Table 67).

In the case of the first diploid, 29/50 segregants had at least one recombination outside the *ad13* - *ad32* interval. Of these, five had a crossing-over in the *paba* - *y* region and five, in both the *pro* - *ad* and *paba* - *y* intervals. This suggests that about a third of the segregants showing additional recombination exhibited negative interference over at least the distance from the *n-ad13* - *ad9* interval to distal of the *paba1* site.

Similar results were obtained with the second diploid. Five of the 33 segregants showing additional recombination had a crossing-over in the *paba* - *y* interval and one, in both the *pro* - *ad* and *paba* - *y* intervals. The finding of fewer recombinations in the *pro* - *ad* interval for diploid *n-ad13 ad9* / *ad32* was expected in the light of the effective pairing hypothesis (Pritchard, 1955, 1960a -- see discussion in section III, Chapter III of this thesis).

According to Siddiqi (personal communication), *paba1* and *paba6* recombine in meiosis with a frequency of *ca*. 10^{-6}, suggesting the same order of magnitude for the *paba1* -

paba6 interval as for the ad9 - ad32 interval. This would lead one to expect a similar intensity of negative interference (cf. Pritchard, 1960a) in the proposed analysis of paba1 / paba6 diploids as has been found in the half-tetrad analyses just described.

LITERATURE CITED

Ahmad, M. and Catcheside, D.G. (1960). Physiological diversity amongst tryptophan mutants in *Neurospora crassa*. *Heredity*, *15*, 55-64.

Anfinsen, C.B. (1959). *The Molecular Basis of Evolution*. New York: John Wiley and Sons, Inc.

Avery, O.T., MacLeod, C.M. and McCarty, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid isolated from *Pneumococcus* type III. *J. exp. Med.*, *79*, 137-158.

Balbinder, E. (1960). Mapping, complementation and mutation studies with two tryptophan loci of *Salmonella typhimurium* (abst.). *Genetics*, *45*, 973.

Beadle, G.W. (1945). Biochemical genetics. *Chem. Rev.*, *37*, 15-96.

Beadle, G.W. (1957). The role of the nucleus in heredity. *The Chemical Basis of Heredity*, pp. 3-22, Baltimore: The Johns Hopkins Press.

Beadle, G.W. (1960). Physiological aspects of genetics. *Ann. Rev. Physiol.*, *22*, 45-74.

Beadle, G. W. and Coonradt, V.L. (1944). Heterokaryosis in *Neurospora crassa*. *Genetics*, *29*, 291-308.

Belling, J. (1931). Chromomeres in liliaceous plants. *Univ. Calif. Publ. Bot.*, *16*, 153-170.

Belozersky, A.N. and Spirin, A.S. (1958). A correlation between the compositions of deoxyribonucleic and ribonucleic acids. *Nature*, *182*, 111-112.

Benzer, S. (1955). Fine structure of a genetic region in bacteriophage. *Proc. Natl. Acad. Sci. (U.S.)*, *41*, 344-354.

Benzer, S. (1957). The elementary units of heredity. *The Chemical Basis of Heredity*, pp. 70-93, Baltimore: The Johns Hopkins Press.

Benzer, S. (1959). On the topology of the genetic fine structure. *Proc. Natl. Acad. Sci. (U.S.)*, *45*, 1607-1620.

Benzer, S., Ingram, V.M. and Lehmann, H. (1958). Three varieties of human haemoglobin D. *Nature*, *182*, 852-854.

Bonner, D.M. (1956). The genetic unit. *Cold Spring Harb. Symp. quant. Biol.*, *21*, 163-170.

Brenner, S. (1957a). On the impossibility of all overlapping triplet codes in information transfer from nucleic acid to proteins. *Proc. Natl. Acad. Sci. (U.S.)*, *43*, 687-694.

Brenner, S. (1957b). Genetic control and phenotypic mixing of the adsorption cofactor requirement in bacteriophages T2 and T4. *Virology*, *3*, 560-574.

Calef, E. (1956). Functional relationships between three adenineless mutants in *Aspergillus nidulans* (abst.). *Heredity*, *10*, 279.

Calef, E. (1957). Effect on linkage maps of selection of crossovers between closely linked markers. *Heredity*, *11*, 265-279.

Carlson, E. A. (1959a). Allelism, complementation and pseudoallelism at the dumpy locus in *Drosophila melanogaster*. *Genetics*, *44*, 347-373.

Carlson, E. A. (1959b). Comparative genetics of complex loci. *Quart. Rev. Biol.*, *34*, 33-67.

Case, M.E. and Giles, N.H. (1958a). Evidence from tetrad analysis for both normal and aberrant recombination between allelic mutants in *Neurospora crassa*. *Proc. Natl. Acad. Sci (U.S.)*, *44*, 378-390.

Case, M.E. and Giles, N.H. (1958b). Recombination mechanisms at the *pan-2* locus in *Neurospora crassa*. *Cold Spring Harb. Symp. quant. Biol.*, *23*, 119-135.

Case, M.E. and Giles, N.H. (1960). Comparative complementation and genetic maps of the *pan-2* locus in *Neurospora crassa*. *Proc. Natl. Acad. Sci. (U.S.)*, *46*, 659-676.

Catcheside, D.G. (1960a). Relation of genotype to enzyme content. *Symp. Soc. gen. Microbiol.*, *10*, 181-207.

Catcheside, D.G. (1960b). Complementation among histidine mutants of Neurospora crassa. Proc. Roy. Soc., B, 153, 179-194.

Catcheside, D.G. and Overton, A. (1958). Complementation between alleles in heterocaryons. Cold Spring Harb. Symp. quant. Biol., 23, 137-140.

Cavalli-Sforza and Jinks, J.L. (1956). Studies on the genetic system of Escherichia coli K-12. J. Genet., 54, 87-112.

Chase, M. and Doermann, A.H. (1958). High negative interference over short segments of the genetic structure of bacteriophage T4. Genetics, 43, 332-353.

Chovnick, A. (1958). Aberrant segregation and pseudoallelism at the garnet locus in Drosophila melanogaster. Proc. Natl. Acad. Sci. (U.S.), 44, 333-337.

Chovnick, A. R., Lefkowitz, J. and Fox, A.S. (1956). A phenogenetic study of the lozenge pseudoalleles in Drosophila melanogaster. II. Effects on the development of tarsal claws in heterozygotes. Genetics, 41, 589-604.

Crawford, I. and Yanofsky, C. (1958). On the separation of tryptophan synthetase of Escherichia coli into two protein components. Proc. Natl. Acad. Sci. (U.S.), 44, 1161-1170.

Crick, F.H.C. (1958). On protein synthesis. Symp. Soc. Exptl. Biol., 12, 138-163.

Crick, F.H.C. (1959). The present position of the coding problem. Brookhaven Symp. Biol., 12, 35-39.

Crick, F.H.C., Griffith, J.S. and Orgel, L.E. (1957). Codes without commas. Proc. Natl. Acad. Sci. (U.S.), 43, 416-421.

Dauben, W.C. and Pitzer, K.S. (1956). Conformational analysis. Steric Effects in Organic Chemistry (M.S. Newman, Ed.), pp. 3-60, New York: John Wiley and Sons, Inc.

Demerec, M. and Hartman, P. (1959). Complex loci in microorganisms. Ann. Rev. Microbiol., 13, 377-406.

Demerec, M. and Ozeki, H. (1959). Tests for allelism among auxotrophs of Salmonella typhimurium. Genetics, 44, 269-278.

DeSerres, F.J. (1956). Studies with purple adenine mutants in *Neurospora crassa*. I. Structural and functional complexity of the *ad-3* region. *Genetics*, *41*, 668-676.

DeSerres, F.J. (1958). Recombination and interference in the *ad-3* region of *Neurospora crassa*. *Cold Spring Harb. Symp. quant. Biol.*, *23*, 111-118.

DeSerres, F.J. (1960). Studies with purple adenine mutants in *Neurospora crassa*. IV. Lack of complementation between different *ad-3A* mutants in heterokaryons and pseudowild types. *Genetics*, *45*, 555-566.

DeMoss, J.A. and Bonner, D.M. (1959). Studies on normal and genetically altered tryptophan synthetase from *Neurospora crassa*. *Proc. Natl. Acad. Sci. (U.S.)*, *45*, 1405-1412.

Dobzhansky, T. (1931). The decrease of crossing-over observed in translocations, and its probable explanation. *Amer. Nat.*, *65*, 214-232.

Dobzhansky, T. (1934). Studies on chromosome configuration. III. Behaviour of duplicating fragments. *Z. indukt. Abstamm. -u. VererbLehre*, *68*, 137-162.

Dobzhansky, T. and Epling, C. (1948). The suppression of crossing over in inversion heterozygotes of *Drosophila pseudoobscura*. *Proc. Natl. Acad. Sci. (U.S.)*, *34*, 137-141.

Doty, P., Marmur, J. and Sueoka, N. (1959). The heterogeneity in properties and functioning of deoxyribonucleic acids. *Brookhaven Symp. Biol.*, *12*, 1-16.

Edgar, R.S. (1958a). Phenotypic properties of heterozygotes in the bacteriophage T4. *Genetics*, *43*, 235-248.

Edgar, R.S. (1958b). Mapping experiments with *rII* and *h* mutants of bacteriophage T4D. *Virology*, *6*, 215-225.

Edgar, R.S. (1959). Genetic structure of host range mutants of coliphage λ. *Virology*, *7*, 347-348.

Elliott, C.G. (1960a). The cytology of *Aspergillus nidulans*. *Genet. Res.*, *1*, 462-476.

Elliott, C.G. (1960b). Non-localised negative interference in *Aspergillus nidulans*. *Heredity*, *15*, 247-262.

Fahmy, O.G. and Fahmy, M.J. (1959). Complementation among the sub-genic mutants in the *r*-locus of *Drosophila melanogaster*. *Nature*, *184*, 1927-1929.

Fincham, J.R.S. (1957). Modified glutamic acid dehydrogenase as a result of gene mutation in *Neurospora crassa*. *Biochem. J.*, *65*, 721-728.

Fincham, J.R.S. (1958). The role of chromosomal loci in enzyme function. *Proc. Xth int. Congr. Genet.*, *1*, 355-363.

Fincham, J.R.S. (1959a). The biochemistry of genetic factors. *Ann. Rev. Biochem.*, *28*, 343-364.

Fincham, J.R.S. (1959b). On the nature of the glutamic dehydrogenase produced by inter-allele complementation at the *am* locus of *Neurospora crassa*. *J. gen. Microbiol.*, *21*, 600-611.

Fincham, J.R.S. and Pateman, J.A. (1957a). A new allele at the *am* locus of *Neurospora crassa*. *J. Genetics*, *55*, 456-466.

Fincham, J.R.S. and Pateman, J.A. (1957b). Formation of an enzyme through complementary action of mutant 'alleles' in separate nuclei in a heterokaryon. *Nature*, *179*, 741.

Fratello, B., Morpurgo, G. and Sermonti, G. (1960). Induced somatic segregation in *Aspergillus nidulans*. *Genetics*, *45*, 785-800.

Freese, E. (1957a). The correlation effect for a histidine locus of *Neurospora crassa*. *Genetics*, *42*, 671-684.

Freese, E. (1957b). Über die Feinstruktur des genoms im Bereich eines *pab* Locus von *Neurospora crassa*. *Z. indukt. Abstamm.-u. VererbLehre*, *88*, 338-406.

Freese, E. (1958) . The arrangement of DNA in the chromosome. *Cold Spring Harb. Symp. quant. Biol.*, *23*, 13-18.

Fresco, J.R., Alberts, B.M. and Doty, P. (1960). Some molecular details of the secondary structure of ribonucleic acid. *Nature*, *188*, 98-101.

Freudenthal, H. (1958). Ein kombinatorisches Problem von biochemischer Herkunft. *K. Nederlandse Akad. Proc. A*, *61*, 253-258.

Gamow, G., Rich, A. and Yčas, M. (1956). The problem of information transfer from the nucleic acids to proteins. *Adv. in Biol. and Med. Phys.*, *4*, 23-68.

Garen, A. (1960). Genetic control of the specificity of the bacterial enzyme, alkaline phosphatase. *Symp. Soc. gen. Microbiol.*, *10*, 239-247.

Gierer, A. (1960). Ribonucleic acid as genetic material of viruses. *Symp. Soc. gen. Microbiol.*, *10*, 248-271.

Giles, N.H. (1958). Mutations at specific loci in *Neurospora*. *Proc. Xth. int. Congr. Genet.*, *1*, 261-279.

Giles, N.H., Partridge, C.W.H. and Wilson, N.J. (1957). The genetic control of adenylosuccinase in *Neurospora crassa*. *Proc. Natl. Acad. Sci. (U.S.)*, *43*, 305-317.

Glass, B. (1957). A summary of the symposium on the chemical basis of heredity. *The Chemical Basis of Heredity*, pp. 757-834, Baltimore: The Johns Hopkins Press.

Glassman, E. (1960). Genetic complementation between mutants of *Drosophila melanogaster* lacking xanthine dehydrogenase (abst.). *Genetics*, *45*, 989.

Golomb, S.W., Welch, L.R. and Delbrück, M. (1958). Construction and properties of comma-free codes. *K. Danske Videnskab. Selskab. Biol. Medd.*, *23*, 1-34.

Gorini, L. (1960). Antagonism between substrate and repressor in controlling the formation of a biosynthetic enzyme. *Proc. Natl. Acad. Sci. (U.S.)*, *46*, 682-690.

Green, M.M. (1960). Double crossing over or gene conversion at the white loci in *Drosophila melanogaster*? *Genetics*, *45*, 15-18.

Green, M.M. and Green, K.C. (1949). Crossing-over between alleles at the lozenge locus in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. (U.S.)*, *35*, 586-591.

Green, M.M. and Green, K.C. (1956). A cytogenetic analysis of the lozenge pseudoalleles in *Drosophila*. *Z. induk. Abst. Vererbs.*, *87*, 708-721.

Grigg, G.W. (1952). Back mutation assay method in microorganisms. Nature, 169, 98-100.

Grigg, G.W. (1958). Competitive suppression and the detection of mutations in microbial populations. Australian J. Biol. Sci., 11, 69-84.

Gross, J. and Englesberg, E. (1959). Determination of the order of mutational sites governing L-arabinose utilization in Escherichia coli B/r by transduction with phage P1bt. Virology, 9, 314-331.

Gross, S.R. and Fein, A. (1960). Linkage and function in Neurospora. Genetics, 45, 885-904.

Grüneberg, H. (1935). A new inversion of the X-chromosome in Drosophila melanogaster. J. Genetics, 31, 163-184.

Hartman, P.E., Hartman, Z. and Šerman, D. (1960b). Complementation mapping by abortive transduction of histidine-requiring Salmonella mutants. J. gen. Microbiol., 22, 354-368.

Hartman, P.E., Loper, J.C. and Šerman, D. (1960a). Fine structure mapping by complete transduction between histidine-requiring Salmonella mutants. J. gen. Microbiol., 22, 323-353.

Hershey, A.D. and Chase, M. (1952). Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. gen. Physiol., 36,39-56.

Hirs, C.H.W. (1960). The structure of ribonuclease. Ann. New York Acad. Sci., 88, 611-641.

Horowitz, N.H. (1948). The one gene - one enzyme hypothesis. Genetics, 33, 612-613.

Horowitz, N.H. and Fling, M. (1953). Genetic determination of tyrosinase thermostability in Neurospora. Genetics, 38, 360-374.

Horowitz, N.H. and Fling, M. (1956). The role of the genes in the synthesis of enzymes. Enzymes: Units of Biological Structure and Function, pp. 139-145, New York: Academic Press.

Horowitz, N.H., Fling, M. MacLeod, H.L. and Sueoka, N. (1959). Multigenic control of tyrosinase synthesis in Neurospora (abst.). Genetics, 44, 516.

Horowitz, N.H. and Leupold, U. (1951). Some recent studies bearing on the one gene - one enzyme hypothesis. Cold Spring Harb. Symp. quant. Biol., 16, 65-74.

Hunt, J. A. (1959). Identity of the α-chains of adult and foetal human haemoglobins. Nature, 183, 1373-1375.

Hunt, J.A. and Ingram, V.M. (1958). Allelomorphism and the chemical differences of the human haemoglobins A, S and C. Nature, 181, 1062-1063.

Ikeda, Y., Ishitani, C. and Nakamura, K. (1957). A high frequency of heterozygous diploids and somatic recombination induced in imperfecti fungi by ultra-violet light. J. gen. appl. Microbiol., 3, 1-11.

Ingram, V.M. (1957). Gene mutations in human haemoglobin: The chemical difference between normal and sickle-cell haemoglobin. Nature, 180, 326-328.

Ingram, V.M. (1959). Constituents of human haemoglobin: Separation of the peptide chains of human globin. Nature, 183, 1795-1798.

Ingram, V.M. and Stretton, A.O.W. (1959). Genetic basis of the thalassaemia diseases. Nature, 184, 1903-1909.

Ishikawa, T. (1960). Complementation and genetic maps of ad-8 locus in Neurospora crassa (abst.). Genetics, 45, 993.

Itano, H.A. (1957). Human haemoglobins: Their properties and genetic control. Adv. in Protein Chem., 12, 216-268.

Itano, H.A. and Robinson, E. (1959). Constituents of human haemoglobin: Formation of normal and doubly abnormal haemoglobins by recombination of haemoglobin I with S and C. Nature, 183, 1799-1800.

Itano, H.A. and Robinson, E. (1960a). Specific recombination of the subunits of hemoglobin. Ann. New York Acad. Sci., 88, 642-654.

Itano, H.A. and Robinson, E. (1960b). Identification of the recombinant products of canine and human haemoglobins. *Nature*, *188*, 798-800.

Itano, H.A. and Robinson, E. (1960c). Genetic control of the α- and β- chains of hemoglobin. *Proc. Natl. Acad. Sci. (U.S.)*, *46*, 1492-1501.

Iwatsubo, M., Ishibasi, M., Takashina, T., Ibaraki, K. and Kashida, H. (1954). Mode of combination of the substrate and diphosphopyridine nucleotide in the glutamic acid dehydrogenase system. *Symp. on Enzyme Chem.* (Japan), *10*, 135-141, in *Chem. Abst.*, *49*, 14833 (1955).

Iwatsubo, M., Watari, H., Soyama, T., Ito, K., Nichimiki, T. and Hiroaka, K. (1957). Crystallization and the active group of glutamic dehydrogenase. *Kôso Kagaku Shinkojiumu*, *12*, 36-41, in *Chem. Abstr.*, *52*, 1314 (1958).

Käfer, E. (1958). An 8-chromosome map of *Aspergillus nidulans*. *Adv. in Genetics*, *9*, 105-145.

Käfer, E. (1960). High frequency of spontaneous and induced somatic segregation in *Aspergillus nidulans*. *Nature*, *186*, 619-620.

Kaiser, A.D. (1957). Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*. *Virology*, *3*, 42-61.

Kendrew, J.C., Dickerson, R.E., Strandberg, B.E., Hart, R.G. and Davies, D.R. (1960). Structure of myoglobin: A three-dimensional fourier analysis at 2 Å resolution. *Nature*, *185*, 422-427.

Lacy, A.M. and Bonner, D.M. (1958). Complementarity between alleles at the *td* locus in *Neurospora crassa* (abst.). *Proc. Xth. int. Congr. Genet.*, *2*, 157.

Lederberg, E. (1952). Allelic relationships and reverse mutation in *Escherichia coli K-12*. *Genetics*, *37*, 469-483.

Lederberg, E. (1960). Genetic and functional aspects of galactose metabolism in *Escherichia coli K-12*. *Symp. Soc. gen. Microbiol.*, *10*, 115-131.

Lederberg, J. (1955). Recombination mechanisms in bacteria. *J. cell. comp. Physiol.*, *45* (suppl. 2), 75-107.

Leupold, U. (1958). Studies on recombination in *Schizosaccharomyces pombe*. *Cold Spring Harb. Symp. quant. Biol.*, *23*, 161-170.

Levinthal, C. (1954). Recombination in phage T2; its relationship to heterozygosis and growth. *Genetics*, *39*, 169-184.

Levinthal, C. (1959a). Genetic and chemical studies with alkaline phosphatase of *E. coli*. *Brookhaven Symp. in Biol.*, *12*, 76-85.

Levinthal, C. (1959b). Coding aspects of protein synthesis. *Revs. Mod. Phys.*, *31*, 249-255.

Lewis, D. (1958). Gene control of specificity and activity; loss by mutation and restoration by complementation. *Nature*, *182*, 1620-1621.

Lewis, D. (1960). Genetic control of specificity and activity of the *S* antigen in plants. *Proc. Roy. Soc.*, *B*, *151*, 468-477.

Lewis, E.B. (1951). Pseudoallelism and gene evolution. *Cold Spring Harb. Symp. quant. Biol.*, *16*, 159-174.

Lindegren, C.C. (1955). Non-Mendelian segregation in a single tetrad of *Saccharomyces* ascribed to gene conversion. *Science*, *121*, 605-607.

McIlwain, H. (1941). A nutritional investigation of the antibacterial action of acriflavine. *Biochem. J.*, *35*, 1311-1319.

Maguire, M.P. (1960). The relation of replication, recombination, synapsis and chromosome coiling. *Genet. Res.*, *1*, 487-488.

Marmur, J. and Doty, P. (1959). Heterogeneity in deoxyribonucleic acids. I. Dependence on composition of the configurational stability of deoxyribonucleic acids. *Nature*, *183*, 1427-1429.

Martin-Smith, C.A. (1957). The *ad9* series in *Aspergillus nidulans*. *Microb. Genet. Bull.*, *15*, 20-21.

Megnet, R. (1959). Complementation between allelic adenylosuccinaseless mutants at the *Ad-8* locus of *Schizosaccharomyces pombe* (abst.). *Genetics*, *44*, 526.

Mitani, S. (1957a). Sulfhydryl groups in glutamic dehydrogenase. I. Spectrophotometric studies. *Ōsaka Daigaku Igaku Zasshi*, *9*, 829-834, in *Chem. Abst.*, *52*, 4725 (1958).

Mitani, S. (1957b). Sulfhydryl groups in glutamic dehydrogenase. II. Aperometric studies. *Ōsaka Daigaku Igaku Zasshi*, *9*, 1069-1075, in *Chem. Abst.*, *52*, 4725 (1958).

Mitchell, H.K. (1957). Crossing over and gene conversion in *Neurospora*. *The Chemical Basis of Heredity*, pp. 94-113, Baltimore: The Johns Hopkins Press.

Mitchell, M.B. (1955a). Aberrant recombination of pyridoxine mutants in *Neurospora*. *Proc. Natl. Acad. Sci. (U.S.)*, *41*, 215-220.

Mitchell, M.B. (1955b). Further evidence of aberrant recombination in *Neurospora*. *Proc. Natl. Acad. Sci. (U.S.)*, *41*, 935-937.

Mitchell, M.B. (1956). A consideration of aberrant recombination in *Neurospora*. *Compt. Rend. Lab. Carlsberg Ser. Physiol.*, *26*, 285-298.

Morgan, D.T. (1950). A cytogenetic study of inversions in *Zea mays*. *Genetics*, *35*, 153-174.

Morpurgo, G. and Sermonti, G. (1959). Chemically induced instabilities in heterozygous diploids of *Penicillium chrysogenum*. *Genetics*, *44*, 137-152.

Morse, M.L. (1959). Recombination and segregation in *Gal* heterogenotes showing position effect. *Rec. Genetics Soc. Amer.*, *28*, 88.

Murayama, M. and Ingram, V.M. (1959). Constituents of human haemoglobin: Comparison of normal adult human haemoglobin with haemoglobin I by 'fingerprinting.' *Nature*, *183*, 1798-1799.

Murray, N.E. (1960a). The distribution of methionine loci in *Neurospora crassa* . *Heredity*, *15*, 199-206.

Murray, N.E. (1960b). Complementation and recombination between methionine-2 alleles in Neurospora crassa. Heredity, 15, 207-217.

Novitski, E. and Braver, G. (1954). An analysis of the crossing over within a heterozygous inversion in Drosophila melanogaster. Genetics, 39, 197-209.

Pardee, A.B., Jacob, F. and Monod, J. (1959). The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by E. coli. J. Mol. Biol., 1, 165-178.

Pardee, A.B. and Prestidge, L.S. (1959). On the nature of the suppressor of β-galactosidase synthesis in Escherichia coli. Biochim. et Biophys. Acta, 36, 545-547.

Pateman, J.A. (1957). Back-mutation studies at the am locus in Neurospora crassa. J. Genet., 55, 444-455.

Pateman, J.A. (1960a). Inter-relationships of alleles at the am locus in Neurospora crassa. J. gen. Microbiol., 23, 393-399.

Pateman, J.A. (1960b). High negative interference at the am locus in Neurospora crassa. Genetics, 45, 839-846.

Perutz, M.F., Rossmann, M.G., Cullis, A.F., Muirhead, H., Will, G. and North, A.C.T. (1960). Structure of haemoglobin: A three-dimensional fourier synthesis at 5.5 - Å resolution, obtained by X-ray analysis. Nature, 185, 416-422.

Pontecorvo, G. (1950). New fields in the biochemical genetics of micro-organisms. Biochem. Soc. Symp., 4, 40-50.

Pontecorvo, G. (1952). Genetical analysis of cell organisation. Symp. Soc. exp. Biol., 6, 218-229.

Pontecorvo, G. (1955). Gene structure and action in relation to heterosis. Proc. Roy. Soc., B, 144, 171-177.

Pontecorvo, G. (1956). Allelism. Cold Spring Harb. Symp. quant. Biol., 21, 171-174.

Pontecorvo, G. (1958a). Trends in Genetic Analysis. New York: Columbia University Press.

Pontecorvo, G. (1958b). Self-reproduction and all that. Symp. Soc. Exptl. Biol., 12, 1-5.

Pontecorvo, G. and Käfer, E. (1956). Mapping the chromosomes by means of mitotic recombination. Proc. Roy. Phys. Soc., 25, 16-20.

Pontecorvo, G. and Käfer, E. (1958). Genetic analysis based on mitotic recombination. Adv. in Genetics, 9, 71-104.

Pontecorvo, G., Roper, J.A., Hemmons, L.M., MacDonald, K.D. and Bufton, A.W.J. (1953). The genetics of Aspergillus nidulans. Adv. in Genetics, 5, 141-348.

Pontecorvo, G., Tarr Gloor, E. and Forbes, E. (1954). Analysis of mitotic recombination in Aspergillus nidulans. J. Genet., 52, 226-237.

Pritchard, R.H. (1953). Ascospores with diploid nuclei in Aspergillus nidulans (abst.). Proc. 9th int. Congr. Genet., Caryologia, 6 (supple.), 1117.

Pritchard, R.H. (1955). The linear arrangement of a series of alleles of Aspergillus nidulans. Heredity, 9, 343-371.

Pritchard, R.H. (1956). A genetic investigation of some adenine-requiring mutants of Aspergillus nidulans. Ph.D. Thesis, University of Glasgow.

Pritchard, R.H. (1960a). Localized negative interference and its bearing on models of gene recombination. Genet. Res., 1, 1-24.

Pritchard, R.H. (1960b). The bearing of recombination analysis at high resolution on genetic fine structure in Aspergillus nidulans and the mechanism of recombination in higher organisms. Symp. Soc. gen. Microbiol., 10, 155-180.

Raffel, D. and Muller, H.J. (1940). Position effect and gene divisibility considered in connection with three strikingly similar scute mutations. Genetics, 25, 541-583.

Rhinesmith, H.S., Schroeder, W.A. and Martin, N. (1958). The N-terminal sequence of the β chains of normal adult human hemoglobin. *J. Amer. Chem. Soc.*, *80*, 3358-3361.

Rhinesmith, H.S., Schroeder, W.A. and Pauling, L. (1957). A quantitative study of the hydrolysis of human dinitrophenyl (DNP) globin: The number and kind of polypeptide chains in normal adult human hemoglobin. *J. Amer. Chem. Soc.*, *79*, 4682-4686.

Rhoades, M.M. and Dempsey, E. (1953). Cytogenetic studies of deficient-duplicate chromosomes derived from inversion heterozygotes in maize. *Amer. J. Bot.*, *40*, 405-424.

Richards, F. M. (1958). On the enzymic activity of subtilisin-modified ribonuclease. *Proc. Natl. Acad. Sci. (U.S.)*, *44*, 162-166.

Ris, H. (1957). Chromosome structure. *The Chemical Basis of Heredity*, pp. 23-69, Baltimore: The Johns Hopkins Press.

Roman, H. (1956). Studies of gene mutation in *Saccharomyces*. *Cold Spring Harb. Symp. quant. Biol.*, *21*, 175-183.

Roman, H. (1958). Sur les recombinaisons nonréciproques chez *Saccharomyces cerevisiae* et sur les problèmes posés par ces phénomènes. *Ann. Génétique*, *1*, 11-17.

Roman, H. and Jacob, F. (1958). A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the recombination of outside markers. *Cold Spring Harb. Symp. quant. Biol.*, *23*, 155-160.

Roper, J.A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia*, *8*, 14-15.

Roper, J.A. and Käfer, E. (1957). Acriflavine-resistant mutants of *Aspergillus nidulans*. *J. gen. Microbiol.*, *16*, 660-667.

Roper, J.A. and Pritchard, R.H. (1955). The recovery of the complementary products of a mitotic crossing over. *Nature*, *175*, 639.

Rothfels, K.H. (1952). Gene linearity and negative interference in crosses of *Escherichia coli*. *Genetics*, *37*, 297-311.

Russell, W.A. and Burnham, C.R. (1950). Cytogenetic studies of an inversion in maize. Sci. Agric., 30, 93-111.

St. Lawrence, P. (1956). The q locus of Neurospora crassa. Proc. Natl. Acad. Sci. (U.S.), 42, 189-194.

St. Lawrence, P. and Bonner, D.M. (1957). Gene conversion and problems of allelism. The Chemical Basis of Heredity, pp. 114-122, Baltimore: The Johns Hopkins Press.

Schroeder, W.A. and Matsuda, G. (1958). N-terminal residues of human fetal hemoglobin. J. Amer. Chem. Soc., 80, 1521.

Schwartz, D. (1955). Studies on crossing-over in maize and Drosophila. J. cell. comp. Physiol., 45 (supple. 2), 171-188.

Schwartz, D. (1958). Deoxyribonucleic acid side-chain model of the chromosomes. Nature, 181, 1149-1150.

Schwartz, H.C., Spaet, T.H., Zuelzer, W.W., Neel, J.V., Robinson, A.R. and Kaufman, S.F. (1957). Combinations of hemoglobin G, hemoglobin S and thalassemia occuring in one family. Blood, 12, 238-250.

Sermonti, G. and Morpurgo, G. (1959). Action of manganous chloride on induced somatic segregation in Penicillium chrysogenum diploids. Genetics, 44, 437-447.

Singer, S.J. and Itano, H.A. (1959). On the asymmetrical dissociation of human hemoglobin. Proc. Natl. Acad. Sci. (U.S.), 45, 174-184.

Sinsheimer, R. L. (1959). Is the nucleic acid message in a two-symbol code? J. Mol. Biol., 1, 218-220.

Soyama, T. (1958). Molecular size and polymerization of crystalline glutamic dehydrogenase. Ôsaka Daiaku Igaku Zasshi, 10, 131-139, in Chem. Abst., 52, 9266 (1958).

Spurway, H. and Philip, U. (1952). Genetics and cytology of Drosophila subobscura. VII. Abnormal gene arrangements in element A. J. Genet., 51, 198-215.

Stadler, J. and Yanofsky, C. (1959). Studies on a series of tryptophan-independent strains derived from a tryptophan-requiring mutant of Escherichia coli. Genetics, 44, 105-123.

Streisinger, G. and Franklin, N.C. (1956). Mutation and recombination at the host range genetic region of phage T2. Cold Spring Harb. Symp. quant. Biol., 21, 103-111.

Strickland, W.N. (1957). Tetrad analysis in Aspergillus nidulans (Eidam) Winter. Ph.D. Thesis, University of Glasgow.

Strickland, W.N. (1958a). An analysis of interference in Aspergillus nidulans. Proc. Roy. Soc., B, 149, 82-101.

Strickland, W.N. (1958b). Abnormal tetrads in Aspergillus nidulans. Proc. Roy. Soc., B., 148, 533-542.

Sturtevant, A.H. (1955). Evaluation of recombination theory. J. cell. comp. Physiol., 45 (supple. 2), 237-242.

Sturtevant, A.H. and Beadle, G.W. (1936). The relations of inversions in the X chromosome of Drosophila melanogaster to crossing over and disjunction. Genetics, 21, 554-604.

Sueoka, N., Marmur, J. and Doty, P. (1959). Heterogeneity in deoxyribonucleic acids. II. Dependence of the density of deoxyribonucleic acids on guanine-cytosine content. Nature, 183, 1429-1431.

Suskind, S.R. (1957). Properties of a protein antigenically related to tryptophan synthetase in Neurospora crassa. J. Bact., 74, 308-318.

Suskind, S.R. (1958). The role of suppressor genes in controlling tryptophan synthetase activity in Neurospora crassa (abst.). Proc. Xth int. Congr. Genet., 2, 284.

Suskind, S.R. and Jordan, E. (1959). Enzymatic activity of a genetically altered tryptophan synthetase in Neurospora crassa. Science, 129, 1614-1615.

Suskind, S.R. and Kurek, L.I. (1957). Enzyme-inhibitor complex in a tryptophan-requiring mutant of Neurospora crassa. Science, 126, 1068-1069.

Suskind, S.R. and Kurek, L.I. (1959). Mechanism of suppressor gene regulation of tryptophan synthetase activity in Neurospora crassa. Proc. Natl. Acad. Sci. (U.S.), 45, 193-196.

Suyama, Y., Munkres, K.D. and Woodward, V.W. (1959). Genetic analyses of the pyr-3 locus of Neurospora crassa: The bearing of recombination and gene conversion upon intraallelic linearity. Genetica, 30, 293-311.

Taylor, J. H. (1957). The time and mode of duplication of chromosomes. Amer. Nat., 91, 209-221.

Taylor, J.H. (1958a). The organization and duplication of the genetic material. Proc. Xth int. Congr. Genet., 1, 63-78.

Taylor, J. H. (1958b). The duplication of chromosomes. Scientific Amer., 198, 36-42.

Thom, C. and Raper, K.B. (1945). A Manual of the Aspergilli, London: Bailliere, Tindall and Cox.

Wagner, R.P., Somers, C.E. and Bergquist, A. (1960). Gene structure and function in Neurospora. Proc. Natl. Acad. Sci. (U.S.), 46, 708-717.

Watson, J.D. and Crick, F.H.C. (1953a). A structure for desoxyribose nucleic acids. Nature, 171, 737-738.

Watson, J.D. and Crick, F.H.C. (1953b). Genetical implications of the structure of desoxyribose nucleic acids. Nature, 171, 964.

Watson, J.D. and Crick, F.H.C. (1953c). The structure of DNA. Cold Spring Harb. Symp. quant. Biol., 18, 123-131.

Webber, B.B. (1959). Comparative complementation and genetic maps of the hist-3 locus in Neurospora crassa (abst.). Genetics, 44, 543-544.

Welshons, W.J. and VonHalle, E.S. (1960). Further studies of pseudoallelism at the Notch locus of Drosophila (abst.). Genetics, 45, 1017.

Woodward, D. O. (1959). Enzyme complementation *in vitro* between adenylosuccinaseless mutants of *Neurospora crassa*. *Proc. Natl. Acad. Sci. (U.S.)*, *45*, 846-850.

Woodward, D.O., Partridge, C.W.H. and Giles, N.H. (1958). Complementation at the *ad-4* locus in *Neurospora crassa*. *Proc. Natl. Acad. Sci.(U.S.)*, *44*, 1237-1244.

Woodward, D.O., Partridge, C.W.H. and Giles, N.H. (1960). Studies of adenylosuccinase in mutants and revertants of *Neurospora crassa*. *Genetics*, *45*, 535-554.

Woodward, V.W. and Cook, W. (1960). Genetic vs. complementation mapping of the *pyr-3* region in *Neurospora* (abst.). *Genetics*, *45*, 1017-1018.

Yanofsky, C. (1956). Gene interactions in enzyme synthesis. *Enzymes: Units of Biological Structure and Function*, pp. 147-160, New York: Academic Press.

Yanofsky, C. and Crawford, I.P. (1959). The effects of deletions, point mutations, reversions and suppressor mutations on the two components of the tryptophan synthetase of *Escherichia coli*. *Proc. Natl. Acad. Sci. (U.S.)*, *45*, 1016-1026.

Yanofsky, C. and St. Lawrence, P. (1960). Gene action. *Ann. Rev. Microbiol.*, *14*, 311-340.

Yanofsky, C. and Stadler, J. (1958). The enzymatic activity associated with the protein immunologically related to tryptophan synthetase. *Proc. Natl. Acad. Sci. (U.S.)*, *44*, 245-253.

Yčas, M. (1960). Correlation of viral ribonucleic acid and protein composition. *Nature*, *188*, 209-212.

Yuill, E. (1939). Two new *Aspergillus* mutants. *J. Bot.*, *77*, 174-175.

Yuill, E. (1950). The numbers of nuclei in conidia of *Aspergilli*. *Trans. Brit. mycol. Soc.*, *33*, 324-331.