CYTOGENETIC STUDIES OF NEUROSPORA CRASSA

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ABSTRACT

The literature pertinent to the nuclear cycle in the life history of *Neurospora* is reviewed. A description of methods used in the cytological studies is presented, and the most useful are summarized as schedules of treatment. The morphology of the mitotic and pachytene chromosomes of *N. crassa* is described and diagrammed. The chromosome cycle in the developing ascus is described in detail, and special points of interest are discussed. The cytological basis of spore abortion caused by the presence in the ascus, in the homozygous condition, of genes from the Abbott strains is discussed briefly. Five translocations, three previously found by McClintock (1945) and two others found by the writer, are described and tentatively identified as to the chromosomes involved. From a consideration of the consequences of crossing-over and disjunction in the translocation heterozygote, equations are derived by means of which the frequencies of the various types of exchange and disjunction can be calculated or estimated. Non-random segregation in T4637 following an effective exchange in a single interstitial segment is described, and a possible mechanism suggested.

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PART ONE. INTRODUCTION

In the twenty-one years since the establishment of the genus *Neurospora*, and the description of its life-history, this ascomycete has assumed a prominent position among organisms used for genetic and biochemical research. As a genetic organism it is distinguished by its suitability for tetrad (i.e., quartet) analysis, with the genetic position of the centromere sharply defined, and by the haploid state of the mycelium, which permits determination of genotypes by inspection. It is useful for biochemical studies because it can be grown in quantity on a chemically-defined medium of relatively simple composition. Its value as a subject for biochemical genetics lies in the many biochemically deficient mutants which can be obtained, their requirements determined, and inheritance followed.

The history of the development of *Neurospora* genetics can be roughly divided into four stages: first, the recognition of the perfect stages, the establishment of the life cycle, and the description of nuclear behavior in the ascus by Shear and Dodge (1927), Dodge (1927), Wilcox (1928), Colson (1934) and Backus (1939); second, the development of genetic techniques and concepts, and their application by Lindegren in a long series of papers; third, the recognition by Beadle and Tatum (1941) that *Neurospora* was an excellent organism with which to attack the problem of genetic control of biochemical reactions; finally, the demonstration by McClintock (1945) that *Neurospora* presented adequate material for cytological and cytogenetic study in detail hitherto undreamed of for the fungi.

The studies of *N. crassa* reported herein began in 1946 at the suggestion of Dr. Barbara McClintock. In their present state, they may be divided into five sections. First, a considerable amount of time has been devoted to the development of techniques by which particular phases of the cytology can best be studied. Many modifications of the general framework of the squash technique described by McClintock (1945) have been tested; some have proven useless, others unnecessary, while several are of utility for special points of study. The major problem to be solved, if *Neurospora* is to be of cytogenetic worth, is the securing of pachytene figures in some quantity. While this goal has not yet been consistently attained in practice, the general requirements which the technique must fulfill are now evident, and can be met.

Second, in the course of this and other phases of the work, it has been possible fully to corroborate the details of the chromosome cycle in the developing ascus, as outlined by McClintock; some additional information has been secured.

Third, special attention has been paid to the problem of establishing the morphology of the chromosomes at pachytene. As a result, it is now possible to present a map of the chromomere patterns mentioned by McClintock, by means of which the individual chromosomes are identifiable with a degree of ease. This is of importance if the techniques of cytogenetic analysis common in maize, for example, are to be applicable to *Neurospora*.

Fourth, in addition to the three translocations described by McClintock, two others have been found. In spite of the difficulties offered by lack of a truly adequate pachytene technique, some information has been obtained about the chromosomes involved in the translocations, and about the positions of the break-points.

Finally, using the technique of ascus analysis described by McClintock, with extensions to make it generally applicable to translocations other than the one with which she was concerned, a preliminary analysis of crossing-over in two of the translocations has been made.

Following a general review of previous literature on the life cycle and cytology of *Neurospora*, with special reference to *N. crassa*, these five aspects of the studies will be discussed in detail.

PART TWO. REVIEW OF PREVIOUS LITERATURE

Shear and Dodge (1927) erected the ascomycetous genus Neurospora to contain four species of the imperfect fungus Monilia of which the perfect stages had been found. These were N. sitophila, N. crassa, N. tetrasperma, and N. erythraea, of which the latter is known only from herbarium material. The first two are eight-spored, heterothallic species, while the latter two are four-spored, homothallic species (presumably homothallic, in the case of N. erythraea, since it has not been available for breeding tests). Since that time, two additional species have been described by Tai (1935): N. toroi, four-spored and homothallic, and N. intermedia, eight-spored and heterothallic.

Beadle (1945, 1946a) has presented reviews of the life-history of the mold. Figure 9 represents the life cycle in diagrammatic form, after Beadle (1946a). Vegetative cultures of the heterothallic species are of one or the other of two mating types, A or a, morphologically indistinguishable. Asexual reproduction of both mating types occurs by germination of conidia (multinucleate spores) or microconidia (presumably uninucleate), and by growth of fragments of mycelium. No sexual reproduction occurs when either mating type is cultured alone. Both mating types produce small bulbils (sclerotia or protoperithecia), which consist of a sheath of sterile cells surrounding a several-celled, multinucleate coil, the ascogonium, from which long processes, the trichogynes, protrude into the air. The development of the protoperithecium in N. tetrasperma has been described by Colson (1934) and by Dodge (1935), and in N. sitophila, by Backus (1939). In the absence of nuclei of the opposite mating type, development proceeds no further.

If nuclei of the other mating type are supplied, either by conidia, microconidia, or fragments of mycelium applied to the trichogynes, or by fusion of the two types of mycelium, then the protoperithecium develops into a perithecium in which ascogenous hyphae develop, nuclear fusion occurs, and asci with mature ascospores are formed as a result of meiosis and two subsequent mitoses. In the homothallic species, nuclear differences (i.e., mating types) exist as in the heterothallic species, but the sequence of events in the ascus is such that the ascospore normally contains nuclei of both types, and fertilization of the protoperithecium is unnecessary. However, heterothallic (or "unisexual") strains of the four-spored species can be obtained which behave essentially as do the eight-spored species, insofar as development of the protoperithecium is concerned.

The factor controlling the mating-type behaves as a single gene, (or chromosome segment), with a definite genetic locus. In _N. crassa_ this lies at a distance of about eight cross-over units from the centromere on one of the chromosomes (Lindegren, 1936a,b). No genes are known distal to it, which suggests that the factor may be a non-homologous chromosome segment. However, in _N. sitophila_, in which the mating-type locus is 28.7 units from the centromere, the gene pink (pk) lies 21.2 units distal to the mating-type locus (Whitehouse, 1942). No definite information is available as to the position of the mating-type locus in _N. tetrasperma_; the inference from published accounts is that the locus rarely crosses-over with the centromere. Otherwise a proportion of four-spored asci should yield heterothallic clones. However, Lindegren (1932) has described asci containing more than four spores, hence the small ones "unisexual", in which the arrangement was

such as to suggest that second division segregation (i.e., crossing-over between the centromere and the locus) had occurred. Apparently the mating-type locus has not been studied in detail in either _N. toroi_ or _N. intermedia_.

The custom has arisen, both in the literature (v. Lindegren, 1932, 1936a,b) and informally, of referring to the linkage group including the mating-type locus as the "sex-chromosome". While this may be a convenient designation, the implication is misleading. Actually, the "unisexual" mycelium is functionally hermaphroditic, in the same sense as any monoecious plant; the protoperithecia are essentially female organs, while the male function is exercised rather broadly by conidia, microconidia (spermatia) and mycelial fragments. But, as in monoecious flowering plants, in which self-fertilization is often prevented by the occurrence of self-sterility barriers, no sexual reaction occurs between strains of the same mating type. To the writer it seems preferable to call these "sexes" mating types, with the implication of incompatibility or self-sterility, and to refer to the linkage group involved as linkage group (or chromosome) 1. This is the attitude expressed or implied in the later papers of Dodge, and of Backus, as opposed to that of Lindegren.

The ascogonium, or coil of fertile cells, in the perithecium is, as was previously mentioned, multinucleate. As Backus (1939) has shown, several conidia, each of which is likewise multinucleate, may attach to the trichogyne system of the same protoperithecium. It therefore becomes of considerable interest, and in some cases of importance, to know how many of these nuclei in the ascogonium and conidium, and how many of the conidia actually function in the formation of the asci in a single perithecium.

This problem is difficult, though not impossible, to attack by direct cytological observation. However, several workers have studied it by genetic means, with fairly conclusive results. The most extensive data are those of Grant (1945).

She studied the question by using, in the one case, mixtures of conidia of different genetic constitutions, and in the other, by using protoperithecia derived from mycelia which were heterocaryotic (i.e., containing nuclei of more than one genetic constitution), and analyzing the products of single perithecia. In order to determine the number of conidia which function in fertilizing a single protoperithecium, she used mixtures of conidia of different genotypes, applied to protoperithecia of still different genetic constitution. Isolating spores at random from each perithecium, she found that in only two out of 79 perithecia tested were more than two genotypes recovered. In the two exceptions, three genotypes were recovered. It would seem that usually only one conidium functions on each protoperithecium.

Grant then attempted to determine how many nuclei functioned in the formation of a perithecium by hyphal fusion (i.e., when mycelia of both mating types are allowed to fuse, and produce perithecia after nuclear migrations). In her experiments, two heterocaryotic mycelia of opposite mating type, each containing two kinds of nuclei (thus four, in all), were grown together, and a number of spores taken at random from each perithecium tested. Five out of 79 such perithecia produced ascospores of more than two genotypes. While it might be concluded that usually only two nuclei enter into the make-up of perithecia produced by this method, Grant points out that nuclear ratios in the heterocaryons are often not

even. If one type of nucleus is in great excess, the random chance of both occurring in the same ascogonium is much reduced. This consideration tends to reduce the validity of the above conclusion, since nuclear ratios in the heterocaryons used were not well balanced.

To determine the number of ancestral nuclei in the ascogonium of the protoperithecium, she used protoperithecia developed on a heterocaryotic mycelium, fertilized by non-mutant conidia. Two out of 106 such perithecia produced more than two kinds of ascospores. Sansome (1947) attacked this same problem by the same method, and found that four out of eight perithecia analyzed produced ascospores of both mutant types occurring in the protoperithecial parent, in addition to the non-mutant type of the conidial parent. The discrepancy between these two results is more apparent than real. In the heterocaryon used by Grant, the ratio of the two types of nuclei was 1:112; thus tri-parental perithecia would be expected to occur in low frequency if at all. On the basis of all the data, it may be concluded that the ascogonium is probably populated by the descendants of more than one ancestral nucleus from the protoperithecial parent.

Coonradt (unpubl., cited by Grant, 1945) fertilized non-mutant protoperithecia by conidia developed on a heterocaryon of two mutant types, and found that usually only one nucleus per conidium was functional in fertilization.

The sequence of events within the ascus leading to production of ascospores was worked out cytologically by Dodge (1927) and Colson (1934) in _N. tetrasperma_, and by Wilcox (1928) in _N. sitophila_. Later, McClintock (1945) described the sequence in _N. crassa_. The three species are generally

alike, except for the special behavior of N. tetrasperma as regards spindle orientation and overlapping of spindles at the second division, and the cutting-out of spores.

From the multinucleate ascogonium, containing nuclei of both mating types, small ascogenous hyphae are protruded. These each contain two nuclei, one of each mating type. The hypha assumes a hook-shaped form, the crozier, with one nucleus in the tip, the other in the base. The two nuclei divide conjugately, with spindles oriented so that non-sister nuclei come to lie in the apex of the crozier. Walls are formed cutting off a tip cell and a basal cell, each uninucleate, with a binucleate apical cell between. The two nuclei in the apical cell fuse to form a diploid nucleus, the only such nucleus formed in the life cycle. As the apical cell grows, it takes on the form of the ascus, relatively long and slender. The fusion nucleus divides (Division I of meiosis) with the spindle oriented longitudinally in the ascus. The spindles of the second meiotic division are also longitudinal, and in tandem, not overlapping, in N. sitophila and N. crassa. In N. tetrasperma, they are more or less oblique, and overlap, so that adjacent telophase nuclei are non-sister. The third division is mitotic, and the spindles in all three species are oriented obliquely across the ascus, so that four telophase nuclei result on each side of the cell. In the eight-spored species, each nucleus is included in a separate spore, cut out through the activity of the centriole. The order of the spores in the mature ascus, with few exceptions, is the same as that of the nuclei. Thus the dividing line between the four spores at one end of the ascus and the four at the other is effectively that of the first division plane. It is this quality which renders ascus analysis (tetrad analysis) so profitable genetically. In N. tetrasperma, the four spores

include two nuclei each, at the time they are cut out. These are non-sister nuclei, products of two different third-division spindles. Because crossing-over of the mating-type locus with the centromere is apparently infrequent, the two nuclei are usually of opposite mating type; hence the spores are "bisexual", and the mycelia which develop from them, homothallic. Occasional irregularities result in more than four spores, some uninucleate, and "unisexual". A fourth division, mitotic in nature, occurs after the spores are cut out, so that mature ascospores of _N. sitophila_ and _N. crassa_ are binucleate, both nuclei identical, while those of _N. tetrasperma_ are tetranucleate, with two nuclei of each mating type.

Previous studies of nuclear behavior, with the exception of that of McClintock (1945), have been concerned chiefly with such gross relations. The chromosome cycle has not been followed in any of the species except _N. crassa_ (McClintock, 1945). The behavior of the centriole has been described, though sometimes the body was not specifically identified as such. Discussion of this topic will be deferred for a comparison with the observations on _N. crassa_. Chromosome numbers have been reported by several workers. Colson (1934) counted six chromosomes in _N. tetrasperma_, but McClintock (1945) reports seven. Lindegren and Rumann (1938) reported a number of six to nine, probably six, in _N. crassa_; the number in all strains thus far examined by McClintock and the writer has been seven. No counts have been reported for _N. sitophila_, _N. toroi_, or _N. intermedia_.

A detailed review of the 1945 paper of McClintock will not be presented here. Her observations of the chromosome cycle have been duplicated in the course of the present studies; the details will be presented en bloc in that section of the paper. Discussion of her work with the transloca-

tions will likewise be postponed until later.

Numerous reviews of ascomycete cytology in general are to be found in the literature. The most recent critical review is that of Martens (1946). The techniques of genetic analysis, and the detailed results of genetic studies carried out by the Lindegrens, and of the studies of biochemical genetics initiated by Beadle and Tatum are not of immediate concern in the present paper. Reviews are available by Lindegren (1942), Tatum and Beadle (1945), and Beadle (1945, 1946a,b).

PART THREE. METHODS AND MATERIALS

STOCKS

A number of "wild-type" and mutant strains of *N. crassa* have been examined cytologically. The pedigrees of these strains (except Emerson (Em) 5256A and Em 5297a), are given by Beadle and Tatum (1945), but are repeated below. All of the strains were obtained from the stocks of Dr. G. W. Beadle. Strain numbers correspond to those in use in his laboratory; mutant strain numbers are those of the original ascospore isolates, and are referable to the table of origins in Beadle and Tatum. None of the material used has been rederived by the writer.

"Wild-type" stocks used included the following:

Chilton a -- from material collected by Chilton in Louisiana.

Emerson (Em) 5256A -- segregate from the cross Abbott 4A x 25a.

Em 5297a -- segregate from the cross 1A x Abbott 12a.

(The preceding two "wild-types" are derived ones, designed to provide stocks free, on the one hand, of the spore-abortion genes present in the Abbott strains, and effective in the homozygous condition in the ascus, and, on the other hand, free from certain genes affecting the biochemical behavior which are present in the strains 1A and 25a (Emerson and Cushing, 1946).

Abbott 4A -- single ascospore strain from material collected by Abbott in Louisiana.

Other "wild-type" strains, not used in the present studies, but involved in the pedigrees of the strains used, are as follows:

LA and La -- strains from Lindegren.

1A and 25a -- single ascospore strains derived from crosses of the preceding strains.

Abbott 12a -- single ascospore strain from Abbott's Louisiana material.

Mutant strains investigated because of spore abortion or peculiar genetic behavior included the following:

4637A -- albino-1; from the cross LA x La; protoperithecia given an X-ray dose of 8250 r units.

5936A -- leucineless (unable to synthesize the amino-acid leucine); LA x La; protoperithecia rayed with 11,000 r.

36703a -- arginineless; 1A x 25a; conidia rayed with a dose of 9400 ergs/mm^2 of ultra-violet (source, a Westinghouse Sterilamp, with about 85% of its output at 2537 Angstroms).

44105a -- threonineless; Abbott 4A x 25a; radiation as for 36703a.

45502A -- pyrimidineless; same origin as 44105.

55701a -- temperature sensitive; Abbott 4A x Chilton a; conidia rayed with a dose of 27,500 r.

CULTURE MEDIA

For maintenance of stock cultures, a modified form of the complete medium described by Beadle and Tatum (1945) was used. Its composition is

as follows:

$K_2C_4H_4O_6 \cdot \frac{1}{2} H_2O$ -- 5.0 g.	Malt extract -- 5.0 g.
$NaNO_3$ -- 4.0 g.	Yeast extract -- 2.5 g.
KH_2PO_4 -- 1.0 g.	Vitamin mixture -- 10 ml.
$MgSO_4$ -- 0.5 g.	Hydrolyzed casein -- 1.2 ml.
NaCl -- 0.1 g.	Glycerine -- 20 ml.
$CaCl_2$ -- 0.1 g.	Agar -- 15 g.

Distilled water, to make -- 1.0 liter.

While this medium can be used for making crosses, it is unsatisfactory. Perithecial production is often low, and conidia are overabundant. In early stages of the work, crosses were made on commercial (Difco) cornmeal agar. While better than the complete medium, this often gave erratic results. The medium which has been routinely used for crosses since its development in the winter of 1946 is the nitrate-minimal medium of Westergaard and Mitchell (1947) (with trace elements omitted). The formula used is as follows:

KNO_3 -- 1.0 g.	Sucrose -- 20.0 g.
KH_2PO_4 -- 1.0 g.	Biotin -- 5.0 micrograms
$MgSO_4$ -- 0.5 g.	Agar -- 15.0 g.
NaCl -- 0.1 g.	Distilled water, to make -- 1.0 liter
$CaCl_2$ -- 0.1 g.	pH adjusted to ca. 6.5 with 1.25 ml. 2.5N NaOH

This medium has supported abundant production of perithecia in all crosses made in the course of these studies. By using as the protoperi-

thecial parent a strain which is not biochemically deficient, it has been possible to avoid the addition of vitamin or amino-acid supplements. This is desirable, since the staining behavior of asci produced on media thus supplemented has often been poor or erratic.

Strain 36703 grows very poorly on the complete medium. Stock cultures of it have been maintained on the nitrate-minimal medium, supplemented with 5.0 mg. of l-arginine per 20 ml. of medium.

CULTURE METHODS

Material for cytological study has been grown almost entirely in Petri plates, 9 cm. x 2 cm. A thin (ca. 5 mm.) layer of sterile medium was poured into a sterile plate, and allowed to cool before inoculation. Plates were usually inoculated with dry conidia of the strain to be used as a protoperithecial parent. If a number of plates of the same strain were desired, conidia were suspended in small tubes of sterile distilled water, and the plates inoculated with one or more drops of the suspension from a sterile pipette. In general, the _a_ mating type was used as the protoperithecial parent, unless the _a_ strain involved was biochemically deficient. In that case, an _A_ strain was used. Since almost all crosses were of "wild-type" by mutant, it was thus possible to avoid using supplemented media.

Plates after inoculation were kept either at room temperature (20-27° C.) or in a 25° C. constant temperature room. At the end of five days, the plates would usually have produced an abundance of protoperithecia. In general, the _a_ mating type produced more protoperithecia

than the A.

Inoculation of protoperithecial plates with the other mating type was usually accomplished by dusting the plate with dry conidia, taken from stock tubes with a platinum transfer lance. Occasionally, when several plates were to be inoculated with the same strain, suspensions of conidia were made in sterile distilled water, the mouth of the small tube flamed, and the suspension poured over the plate, which was then swirled to spread the suspension. Dry conidia usually gave both more perithecia and more uniform distribution of perithecia than did suspended conidia. Also, the excess water added via the suspension seemed to affect productivity adversely.

There was a pronounced effect of the thickness of the layer of medium on the amount of perithecia produced. Thin layers consistently resulted in many more perithecia than did thick ones. Plates which had been poured for several days, and consequently were somewhat dried out, often produced a much denser population than fresh plates. This effect of thickness of medium, and drying, was also noted in test-tube crosses. Here, the most abundant production of perithecia usually occurred in the upper one-third of the agar slant, where the layer of medium was thinner, and drying first began.

No effort has been made to establish a close timing of developmental stages. As a result of repeated observations, however, the time sequence can be roughly stated. In plates which were five days old at the time of inoculation with the conidial parent, and kept at 25° C. throughout development, protoperithecia began to enlarge and blacken by the end of the

first day after fertilization. By the end of the second day, ascogenous hyphae and croziers, and a few young asci in fusion and post-fusion stages were found. At the end of the third day, many asci in middle and late pachytene were present. On the fourth day, some of the asci contained uninucleate ascospores, and others were present in all stages previous to this. By the eighth day, some of the spores were fully pigmented. The speed of development was noticeably affected by temperature. At 20° C., asci required nearly twice as long to reach pachytene as at 25°.

It might be objected that the use of Petri plates, rather than test tubes, offers increased chance of contamination of material by spores from strains other than the intended parents. Contamination during the process of inoculation with the protoperithecial and conidial parents can be held to a minimum through the use of ordinary precautions. Plates were not disturbed thereafter, except for securing material for study. The taking of samples for fixing certainly must result in contamination, if spores are present in the laboratory air in any quantity. However, samples were never taken from the same plate more than three days after the first sample had been removed from it, and in most cases, not later than two days subsequent to the first sample. Under these conditions, contaminants would scarcely have an opportunity to reach a stage of development at which they might be confused with the original inoculants.

CYTOLOGICAL METHODS

Problems involved

A description of the general technique of the squash method may profitably precede discussion of specific problems, and their attempted solutions.

Work previous to that of McClintock (1945) had been carried out almost exclusively by the study of sectioned material. While such material is excellent for the general anatomy of the ascus, published figures would indicate its inadequacy for study of finer details of chromosome behavior. McClintock's introduction of the squash technique in the study of the ascus enabled her to study intact, whole nuclei, and to flatten them at will. This is of the utmost importance.

The writer's technique is in general that described by McClintock. Several (4-6) perithecia, fresh or fixed, are placed in a drop of stain (aceto-orcein, in most cases). All stages of manipulation are carried out under a binocular dissecting microscope. Three needles are routinely used; two of them have the tip 1 mm. bent at an angle of 45°, and honed so that the bottom side is flat, the edges sharp. The third is a straight needle with sharpened tip. Pressure is exerted on the side of the perithecium with the flat side of a bent needle; usually the asci are extruded from the ostiole in small clumps of from three to twenty. The perithecial wall and all fragments of mycelium are removed, and the clumps of asci teased apart as much as possible with the two bent needles. If one wishes to tear the asci, a clump is held down with a bent needle, and the straight needle used to break and tear away portions of the clump. The cover slip is then placed on, and the preparation allowed to stand for a time (10-20 minutes). This permits penetration of the stain. When staining is sufficiently intense, the slide is inverted on blotting

paper, and the desired amount of pressure applied to the back of the slide. The preparation is then sealed with a mixture of gum mastic and paraffin (1:1), applied with a hot wire.

This general procedure must be variously modified, depending upon the stage of development which it is desired to study. A number of specific problems arose for which solutions were sought. These will be outlined here, and later discussed individually.

Various types of fixation result in varying behavior as to staining and other qualities of preparations. Cytoplasmic staining, both with orcein and carmine, is often quite intense in fresh material, less so in material which has been fixed. Ideally one would wish a completely unstained cytoplasm, with intensely stained chromosomes. The cytoplasm of material fixed directly in the stain is usually of good quality, finely granular, with few large vacuoles or bubbles. Material fixed in glacial acetic-absolute ethyl alcohol (1:3) suffers from extensive vacuolation, which often hinders observation of the nucleus.

Especially for measurements, and for accurate determination of interchromosomal relationships, well-flattened preparations are necessary. Asci in clumps do not flatten well, nor do they stain as well as single asci. It is desirable to have as few clumps, and as many single asci as possible. Mechanical separation is inefficient, tedious and time-consuming. The latter is a factor because of extensive stain precipitates in preparations exposed to air for any considerable time. Flattening of clumps by mechanical pressure from the fingers is difficult to control accurately; a little too much pressure ruins the slide. Too little

renders a large proportion of division figures unusable because the chromosomes are not sufficiently spread and flattened. A flattening process subject to control is needed.

The problem of securing adequate numbers of usable pachytene figures has been especially acute. While the raw material for such preparations is abundant (three-day-old perithecia contain only pachytenes, and many of them), only occasional good figures are ordinarily encountered. Much effort has been expended to remedy this situation.

A different problem exists with regard to the occurrence of early diakinesis and metaphase of the third division. Such stages, when found, are not difficult to study, but they occur at the rate of one to a few per preparation. Apparently these stages are completed very rapidly in the ascus, so that only a few asci are at this stage at any given time. It would be desirable to increase the frequency with which these two stages were found.

Methods of Fixation

Fresh material, fixed directly in the stain-fixative (orcein, in 45% or 60% acetic acid) is adequate for many stages. It is particularly good for stages from the crozier through synapsis, and for metaphases, where the chromosomes are condensed. Such material is usually easy to flatten, and the cytoplasmic fixation image is good. Disadvantages are (a) that a constant supply of fresh material involves considerable work and attention in keeping fresh crosses going, and (b) that pachytene and interphase stages are not good. Particularly, the pachytene chromosomes, while staining intensely, are not well separated and usually show little or no

chromomere detail.

Preservation of material is accomplished by cutting out agar blocks (circa 1 cm. x 2 cm.) bearing perithecia, trimming off as much agar as feasible, and placing the block in 10-15 cc. of fixative. A considerable excess of fixative is necessary because of the large amount of water carried in by the agar; fixation of large amounts of material in a small amount of fixative usually results in poor fixation. The fixative most routinely used has been glacial acetic acid-absolute ethyl alcohol, 1:3. The cytoplasm is usually very vacuolate; this often causes difficulties, especially where the vacuoles overlie the nucleus, obscuring chromosome detail. However, material may be kept in fixative, in the refrigerator (10° C.) for at least two weeks. This is convenient if one happens to find an abundance of a particular desired stage. Further, the pachytene chromosomes are well fixed; the homologues are rather widely separated, and chromomere detail is usually well shown. Asci flatten as well after fixation as they do fresh. Stainability of the chromosomes with orcein gradually decreases after two or three weeks.

A number of other acid-alcohol fixatives have been used. Lactic acid-absolute alcohol, 1:3, and lactic acid-acetic acid-absolute alcohol, 1:1:6, give a more evenly granular cytoplasm. The pachytene homologues are widely separated, but not much more so than in acetic-alcohol. These two fixatives have not been extensively used, because the refractive qualities of the cytoplasm are so altered as to produce a disagreeably glassy appearance which is extremely annoying. Staining is often weaker after these fixatives. Propionic acid-absolute alcohol, 1:3, has somewhat the same effect as the lactic acid mixtures; aside from a brief

trial, it hasnot been much used.

Carnoy's, as chloroform-acetic acid-absolute alcohol, 1:3:6, and in a number of other proportions, seems to have no advantage over acetic-alcohol alone. The clumps of asci fixed in Carnoy's are difficult to separate, and the ascus wall seems toughened, so that flattening is poor. Orcein staining seems impaired. Thomas's (1940) method of premordanting material in acetic-alcohol to which ferric acetate has been added has been tried with aceto-carmine as a stain. Cytoplasmic staining is intensified, and chromosomal staining is no better than that with acetic-alcohol fixation alone.

Bouin's and LaCour's 2BE are useless. The perithecial wall is so soft that it crumbles on the slide, and is impossible to get off. Consequently, proper flattening of the preparation is impossible. Further, the asci remain in large clumps, and cannot be well separated.

Recently 60% acetic acid alone has been used as a fixative, in an attempt to avoid the vacuolation of acetic-alcohol, yet permit securing of quantities of favorable material. The cytoplasm is fixed in the same manner as by the stain-fixative. After a few days in fixative, the asci separate easily from one another, so that flattening and stain penetration are optimal. Material keeps at least as long, in the refrigerator, as that fixed in acetic-alcohol.

Since the perithecia in the fresh condition contain a bubble of air, penetration of fixatives, especially aqueous ones, is sometimes unsatisfactory. Fixation in a partial vacuum, produced by an aspirator fastened to a water faucet, overcomes this difficulty. Even with full water flow, there

seems to be no distortion of the asci by the forces thus produced.

Stains

The dyes principally used have been orcein (Eastman Synthetic, Certification No. FOr-2) and carmine (National Aniline, Certification No. NCa-13). Lacmoid, hematoxylin, crystal violet, Feulgen, brilliant cresyl blue and a mixture of the latter with toluidin blue have been tried with little or no success.

Aceto-orcein was prepared by boiling gently for two hours, or by refluxing for three hours, two grams of dye in 100 ml. of acetic acid of the desired concentration (45% or 60%). The resulting solution was filtered, after cooling to room temperature, to remove undissolved dye particles. At the concentrations of acetic acid used, the solutions were saturated, and a considerable amount of the dye undissolved.

Aceto-orcein is by all odds the most useful stain encountered, for all purposes. With proper preparation of the slide, the chromosomes are well differentiated; cytoplasmic staining, which can be annoying with this stain, can be controlled by previous hydrolysis of perithecia in 1N HCl, as noted later. Slides which have been kept for some days in the refrigerator usually show well-differentiated spindles, and the centrioles stain in asci undergoing the third division and in those in which spores are being formed. Nucleoli stain well in prophase and metaphase stages, less well in interphases. The nucleolar stain is light and transparent, as opposed to the dense opaque stain obtained with carmine.

Maximum differentiation is usually not attained until the second or third day after the preparation is made, if it is kept in the refrigerator at 10° C. At room temperature, most of the single asci will become much overstained within one day; asci in clumps stain more slowly, and seldom attain the degree of differentiation of the single asci. Such slides can often be profitably destained with lactic acid-orcein, as noted below. Refrigerated slides have been kept for periods up to four months, with progressive improvement, especially of pachytene stages. Slides with a good deal of material, and well flattened, keep better than those with less, and those not so well flattened. There is a pronounced influence of the amount of surface of the ascus directly exposed to the stain. Relatively unflattened asci, with a good deal of both sides exposed to the staining solution, usually overstain rapidly, even in the refrigerator. Well-flattened asci, with most of the surface pressed against cover slip or slide, seldom overstain.

Orcein in 60% acetic has in general given better results than that in 45%, probably because of the greater amount of dye dissolved in the former. Saturated solutions give better results than those diluted by one part of acetic acid of the same concentration. There is no noticeable effect of the method of preparation of the stain, except that the lots prepared by boiling were so dark that manipulation of perithecia and asci in making slides was difficult to observe. Usually the dissections were made in refluxed stain, and, if desired, a drop of the boiled stain added before the cover slip was placed on.

A saturated solution of orcein in 45% lactic acid has been used, but for most purposes is unsatisfactory. Cytoplasmic staining is much less

than with aceto-orcein, but differentiation of the chromosomes is much slower. In addition, the lactic acid produces the same glassiness as mentioned above for the lactic fixatives. Following a technique which Dr. McClintock (unpubl.) had found useful, a small drop (as much as could be picked up on the end of a bent needle) of lacto-orcein was sometimes added to an aceto-orcein preparation before placing on the cover slip. Results have tended to be erratic. At its best, this method gives maximum differentiation of the chromosomes, with little cytoplasmic staining. However, the addition of a little too much lactic may result in complete destaining. If the asci have not become fairly intensely stained before addition of the lactic, staining is often very slow. Control of the proportions of lactic and acetic in a given preparation is difficult; this is probably the reason for the erratic results. In Dr. McClintock's hands, this method has given beautiful results. The writer's failure to achieve those same results is probably due as much to ineptness as to the fact that his synthetic orcein differs markedly in staining qualities from the natural product used by McClintock.

A saturated solution of orcein in 45% propionic acid results in poor staining, and very slow differentiation of chromosomes. Zirkle's (1947) lacto-gelatin-orcein is not good if used as a staining medium. However, material may be stained in a drop of aceto-orcein, and a drop of the Zirkle medium added before placing on the cover slip. Such preparations, unsealed, last for at least two weeks at room temperature, gradually becoming well differentiated. Additional lacto-gelatin may be added at the side of the cover slip if the preparation dries to the extent that air starts moving under the slip.

Heating freshly-made orcein preparations usually does not improve differentiation. Slight over-heating usually results in complete destaining of the preparation. Heating of two- or three-day-old preparations, with or without addition of fresh stain, has sometimes, but not always, resulted in improved differentiation of the chromosomes.

Carmine has been used as 45% aceto-carmine. Lots prepared by the writer were made by bringing 45% acetic acid to a boil, adding a large excess of dye, boiling for two or three minutes longer, cooling and filtering. Lots prepared by Dr. Barbara McClintock and by Dr. C. R. Burnham were also used.

For studies of the chromosomes themselves, carmine is inferior to orcein. However, for studies of the nucleolus, especially in the interphase nuclei, and for studies of the centriole, carmine has decided advantages. Even the tiny mucleoli of the spore nuclei are intensely stained; orcein seldom differentiates them to this degree. Orcein is erratic in staining the centriole; the conditions required for staining are not under control. Usually the centriole can be differentiated at the third division, and during spore formation, but not always. Carmine consistently stains the centriole at all stages.

Iron has been added to the carmine preparations in a number of ways. First, iron needles were used, and the drop of stain stirred for three or four minutes. Also, the Thomas (1940) premordanting at the time of fixation with a dilute solution of carmine and ferric acetate in glacial acetic acid used as the acetic component of 1:3 acetic-alcohol has been tried. Aceto-carmine to which various amounts of glacial acetic acid saturated

with ferric acetate was added, has also been used. There was no appreciable effect on chromosomal staining of the amount or method of addition of iron to the staining solution; large amounts of iron increased cytoplasmic staining.

Heating the carmine preparations by several passes through a small alcohol flame improves the quality. In addition, it has been noted that pachytene stages are often caused to be extruded completely from the ascus wall, if the ascus has been previously broken. However, such figures are usually not well spread. The chromosomes tend to cluster around the nucleolus. This effect of "shucking" the wall is not under control; its occurrence suggests that heating is at least in part responsible. Similar behavior with orcein is rare.

Mixtures of equal parts of aceto-carmine and aceto-orcein in 45% acetic acid, and also aceto-orcein in 45% acetic to which an excess of carmine has been added, boiled, and filtered, have been tried in an effort to combine the good qualities of the two stains. Results have in general been inferior, for the chromosomes, to orcein used alone.

Lacmoid (supplied through the courtesy of Dr. Mogens Westergaard), used after the method of LaCour (Darlington and LaCour, 1942), has resulted in very poor chromosomal staining. It was necessary to carry out the preliminary hydrolysis in intact perithecia, then press the asci out into fresh stain. This may account for failure of the stain, since staining within the perithecium is slight with all stains used.

Squash preparations made in Delafield's hematoxylin and in the iodine-

ripened hematoxylin of Cole (1943, p. 132), following acetic-alcohol fixation, gave poor chromosomal staining. However, nucleoli and centrioles were very well stained. Preparations did not overstain easily. These stains might be excellent for sectioned material in which it was desired to study nuclear orientations and migrations subsequent to the third division.

Feulgen, applied to asci on the slide, or to intact perithecia, has been uniformly poor. While proper adjustment of the hydrolysis time might remedy the situation, to date no nucleus has been seen in which the chromosomes could be made out.

While crystal violet would not be expected to be too successful after alcoholic fixation, its use on squashes at pachytene was tried. Asci were squashed in water on an albumen-smeared slide, the cover slip removed, and the slides treated as sections. Material tended to float off the slide, in spite of the albumen fixative. Those cells which remained were uniformly poorly stained.

Brilliant cresyl blue in propionic acid, and a mixture of brilliant cresyl blue and toluidin blue in 45% acetic acid (Mittler and Bartha, 1948) have given almost no chromosomal staining in squashes of fixed material.

Bulk staining, by placing intact fresh or fixed perithecia in the staining solution for varying periods, up to 24 hours, has been attempted with most of the stains used. Asci pressed from such perithecia are uniformly lightly stained, if at all. The perithecial wall, the masses of paraphyses and clumps of asci seem to present a formidable barrier to the

penetration of the stain.

Permanents

No serious attempt has been made to develop a technique for making preparations permanent. Refrigeration will usually keep a slide until it can be studied, drawn, and photographed. The relative impermanence of such preparations has a salutary effect; the tendency to rest on previous laurels is curtailed.

Addition of Zirkle's medium may offer at least a semi-permanent method of preparation. Some permanents have been made by the cedarwood-oil method of LaCour (Darlington and LaCour, 1942). Results are in general rather unsatisfactory; asci tend to become loosened from the slide in places and to twist and curl. Desirable figures are often ruined by distortion due to this behavior. Staining of condensed stages usually remains fair, but pachytenes tend to become faint. Use of albumen-smeared slides does not eliminate loosening of asci, and often lessens contrast. Permanents made by partial dehydration with 95% ethyl alcohol, followed by clearing and mounting in diaphane, have been unsatisfactory. Orcein-stained material tends to darken excessively in alcohol, and clearing in diaphane has not been efficient. Permanents made by the McClintock (1929) method suffer from distortion due to loosening of asci from the slide, and from destaining in the acetic mixtures.

A successful permanent method will probably involve the following: (a) all stages of infiltration carried out with the cover slip in place; (b) avoidance of acetic acid; and (c) rapid passage through alcohols.

(Since the above was first written in draft, an technique has been described by Bradley (1948) which seems to meet these requirements. It has not yet been tried with *Neurospora*.)

Special Methods

A number of special treatments have been used, primarily in an attempt to improve the frequency and quality of pachytene figures. Since one barrier to proper spreading and flattening of the chromosomes seemed to be the confining presence of the ascus wall, most of these treatments were tried because they gave promise of removing or softening the wall.

Several of the common cellulose solvents were used. Fixed asci were pressed into a drop of zinc chloride solution (6.0 g. $ZnCl_2$ in 10 ml. water), and heated. The reagent was pipetted off, and the asci rinsed by several changes of water, added and removed dropwise. Then stain was applied. The asci flattened excellently, but nuclear staining was very faint. Fixed asci treated in the same manner with ammoniacal cupric oxide (Schweitzer's reagent), without heating, for varying periods of time, gave flattening in proportion to the duration of the treatment, but staining was very faint. Tetra-sodium pyrophosphate, suggested to Dr. McClintock by Dr. Wanda Farr, was also tried. Fixed asci were treated on the slide for varying periods of time at room temperature with a drop of the following solution: 0.1% $Na_4P_2O_7 \cdot 10\ H_2O$ in 0.1% Na_2CO_3. Flattening was satisfactory, but staining progressively fainter with increasing time of treatment. All three of these reagents dissolve or soften the ascus wall adequately, but simultaneously affect the staining capacity of the chromosomes to such an extent that they are of no utility for the problem at hand.

Fresh asci were dissected into a drop of 5% citric acid, and torn as much as possible. This reagent should prevent "clotting" of the cytoplasm in such broken cells, which might then discharge their contents into the solution. There were indications that this discharge took place more freely than usual, but the ideal of isolated nuclei was not attained, and staining was inferior.

Hydrolysis with 1N hydrochloric acid has proven a very useful tool, though not so much so for pachytenes as had been hoped. Fixed perithecia were transferred to 1N HCl at room temperature for 10-15 minutes, then placed in a vial of the same reagent heated to 50-60° C., and held there for varying periods. Hydrolysis for 5 minutes proved to be optimal. In squashes made from such perithecia, the asci separate from each other on emergency from the ostiole, so that large numbers of single asci result with little or no necessity for mechanical separation. The wall is not noticeably softer, but lack of clumps facilitates flattening. The most useful feature of such hydrolysis is the almost complete absence of cytoplasmic staining which follows it. Also, spindle structure is sharply differentiated in hydrolyzed asci (v. Figs. 10e, 17f, 18d, 23a). The centrioles of Division III and stages in spore formation stain quite regularly in such material (v. Figs. 19g, 21a-c). Chromosomal staining is quite sharp with five minutes hydrolysis at 50-60° C. With higher temperature or longer treatment time, staining may be appreciably reduced.

Hydrolysis for two minutes at 60° C. in 1:3 acetic-alcohol gives excellently flattened asci, but almost no nuclear staining. Hydrolysis for two minutes at 60° C. in 1:3 lactic-alcohol results in little flattening. Pachytene staining is fainter than in untreated material, and the

cytoplasm seems to stain more deeply.

Enzymatic digestion was also attempted. Fixed asci placed in a drop of 1% takadiastase in distilled water, and allowed to stand for periods up to two hours, are progressively better flattened with increasing duration of treatment, but staining also becomes progressively poorer. Snail stomach cytase (Faberge, 1945), as crude gastric juice of a garden snail common in the vicinity, gives excellent preparations, though not of pachytene. (v. Figs. 10e, 11c, 11l, 20b, 21 e, 23b). The wall of the ascus can be completely digested away, and the ascus then flattened to any desired degree. Staining is quite good, if concentration and time are properly adjusted. The material used was kindly extracted from snail stomachs by Dr. H. J. Teas. Used undiluted, treatment times are difficult to control, as the action is quite rapid. Best results were obtained with stomach contents diluted with three parts of tap water. Fixed asci were placed in a drop of reagent, and allowed to stand in a Petri dish over water for 30 minutes to 1½ hours. Then as much of the reagent as possible was pipetted off, and stain added. Addition of the stain seems effectively to stop further action of traces of the reagent which may be left in the preparation. This technique promises to be particularly useful with stages of the very young asci, in which it is ordinarily difficult to get all 14 chromosomes flattened sufficiently for accurate observation or photography. It should also be useful for prometaphases and metaphases of the third and fourth divisions. For example, one can flatten the ascus sufficiently to get all sixteen nuclei of an ascus with spores into one focal plane for photography (v. Fig. 23b). Overtreatment reduces chromosomal staining, but the time limit within which staining is satisfactory is quite broad. A disadvantage is the slimy mess involved in securing the juices from the

stomach. Once obtained, the material can be kept for several months under toluene in a cold room (3° C.).

Several miscellaneous observations seem worth recording. It was found, by accident, that if fixed asci were allowed to stand in a drop of 60% acetic acid until the drop had almost completely evaporated, then a drop of stain added and the preparation stirred, a considerable number of pachytene nuclei, and large numbers of somatic (paraphysis) nuclei would become completely free of cytoplasm. They would float free in the stain as a nucleolus and associated group of chromosomes. Unfortunately, the chromosomes usually coat the nucleolus, and are not workable in that condition. Staining is excellent, and chromomere morphology seems almost as clear as that in the better figures otherwise obtained. If spreading of the chromosomes could be induced in such nuclei, this material might be very satisfactory for pachytene analysis.

In flattening preparations, pressure must be carefully regulated. Too much pressure forces the layer of cytoplasm above and below the nuclei into the same focal plane as the chromosomes. Observation of such figures is difficult because of the vacuolate nature of the cytoplasm. The inter-vacuolar cytoplasm accumulates stain and usually obscures pertinent regions of pachytene configurations. Further, excessive pressure has an effect, the mechanism of which is not clear, of causing the chromosomes at all stages to lose their staining capacity, becoming clumped and diffuse, even though the pressure be later released by addition of more stain under the cover slip before sealing.

The effect of heating the preparation (orcein-stained) is variable.

McClintock (unpubl.), using a natural orcein, consistently got better differentiation in material which was heated by several passes through a flame. With the synthetic orcein, heat does not seem to have this effect. If fresh aceto-orcein or lacto-orcein is added, and the slide heated, destaining occurs. This can be turned to advantage with slides which have become overstained. Fresh stain is added at the edge of the cover slip, and allowed to diffuse across the preparation. The slide is then heated by passage through a small flame, with observation of the degree of differentiation after each passage. Since some further destaining will occur during the period following the heating, care must be taken not to overheat, or destaining will progress too far. Excellent preparations, particularly of condensed stages, have been obtained by deliberate overstaining before applying the cover slip, followed by destaining as above. Such preparations usually have a very clear, non-vacuolar cytoplasm against which the nuclei and chromosomes stand out sharply.

A drop of orcein precipitates rapidly when exposed to air. When it is desired to allow the preparation to stand, without a cover slip, it is placed on fragments of glass in a Petri plate, the bottom of which is covered with 60% acetic acid. Evaporation of the drop is slowed down, and precipitation, while not completely avoided, is considerably lessened. By this means, preparations can be allowed to overstain badly without the asci becoming coated by precipitated stain. The precipitate usually does not redissolve to any extent when the preparation is destained, and all too often obscures the nuclei in which one is interested. There seems to be a definite effect, perhaps connected with oxidation of the dye, of the amount of stain surface exposed; asci in drops which have not spread over the surface of the slide often do not stain well, while material in drops which

have spread out into a thin film stains rapidly and differentiates well.

In material grown at ordinary temperatures, diakinesis stages are usually very late, almost metaphase, and not frequent. Figures of prometaphase and metaphase of Divisions III and IV are also relatively infrequent. This is probably a reflection of the rapidity with which these stages are completed in the ascus. It has been found that if Petri plate cultures are kept in the refrigerator (10° C.) for 24 hours, beginning at a time when the oldest asci are just reaching third and fourth division stages, or cutting out spores, that the frequency of such ordinarily scarce stages can be greatly increased. When such plates are brought out to room temperature, most nuclei are in interphase stages. Within an hour there is a burst of divisions, so that third and fourth divisions will be found in abundance. Further, very early diakinesis figures are frequent. The exact timing of this burst of activity has not been followed. Usually material is checked by fresh preparations at ten or fifteen minute intervals from the time when the material is removed from the refrigerator. When considerable numbers of division figures begin to appear, perithecia are fixed to provide further material for study.

An interesting result of such cold treatment has recently been observed on plates which were again sampled 24 hours after removal from the refrigerator. Several asci were found which contained only two nuclei; the general appearance was that of a second-division ascus. However, at opposite sides of each nucleus there was a group of interphase, or, in some cases, prophase chromosomes. The idea that these might represent asci in which the second division of the chromosomes had occurred normally, but nuclear division had been blocked, was rendered very probable

by the finding of one such nucleus in which 14 prometaphase chromosomes could be counted. These chromosomes were typical of third prometaphase in size and morphology, and were not yet oriented on the metaphase plate. Asci containing only four spores were also observed; no fourth divisions were found, but the nuclear and spore size in such asci was definitely greater than normal. What the course of division of such an abnormal diploid nucleus would be is an interesting subject for speculation. No further observations are available at present to afford a basis for worthwhile conjecture.

Pachytenes

The requirements of an adequate pachytene technique are (a) that the chromosomes should be sufficiently differentiated to be followed without difficulty, and that as much chromomere detail as possible should be presented; (b) that the seven pairs should be as well spread as possible, particularly so that synaptic relations may be accurately determined; and (c) that the nucleus should be as much flattened as is consistent with (a) and (b), so that observation is not too much impeded, and so that measurements (from drawings) are not rendered too inaccurate because of vertical curvature of the threads.

In nuclei which are homozygous for any given arrangement, (c) above is not so important. Many of the pachytene figures seen in the standard, or "wild-type", strains are workable, even though the chromosomes are not flattened and spread in a single plane. Staining of such figures is usually not difficult. Unflattened nuclei heterozygous for an aberration are quite difficult to decipher. But flattening of an intact nucleus in

an intact ascus is usually not successful. The ascus wall and nuclear membrane constitute barriers to the free expansion of the chromosomes, so that little or no spreading occurs. Excessive flattening usually destroys the stainability of the threads. It was at first supposed that removal of the ascus wall would permit breakage by pressure of the nuclear membrane, and consequent chromosomal spreading and flattening, comparable to that obtainable in maize. However, when the ascus wall is completely removed, as with snail cytase, the nuclear membrane does not break with pressure to any considerable degree, and pachytenes are still unsatisfactory.

After a considerable amount of time had been devoted to such technique, it was realized that all of the really good pachytene figures observed had one thing in common -- in all, the ascus had been broken at or very near the nucleus, and the ascus contents had started to ooze out through the broken region. The break should preferably be completely <u>across</u> the ascus. Two breaks which completely cut off the nucleus-bearing segment of the ascus would be ideal. If such asci are present in a small drop of stain, the pressure of the cover slip alone is sufficient to flatten and spread the chromosomes. A drop which is just or not quite sufficient to reach the edge of the cover slip is about the right size.

The chief problem is then reduced to that of producing such fractures in reasonable quantity. To date, no special tools or techniques have been developed for the job. The usual method is to press out asci from three or four perithecia into a drop of aceto-orcein. Then the bent needle previously described is used to hold down a clump of asci, while a straight, sharp-pointed needle is scraped along the edge of the bent needle, breaking a few asci at each scrape. Not all of the asci thus broken will be

at the proper stage (middle to late pachytene); further, not all of the breaks will be in the proper position. But the frequency of usable pachytene figures has increased from one per hundred preparations to one in ten or better.

Suggested Schedules

The most useful of the above-described techniques will be summarized in the form of schedules of treatment:

(1) Fresh material.

(a) Press asci into a drop of 60% aceto-orcein, and separate clumps as well as possible.

(b) Place on cover slip, and allow to stand until asci have taken up considerable stain (10-20 minutes).

(c) Place slide inverted on blotting paper, and apply pressure with fingers to the back of the slide. The amount of pressure is regulated by experience; it must be sufficient to flatten, but not so much as to crush.

(d) Seal preparation. Lightly-stained slides should improve on keeping in the refrigerator. Heavily-stained ones may be destained by the addition of fresh aceto-orcein or lacto-orcein at the edge of the cover slip, followed by judicious heating.

Fresh material is good for all stages where chromosomes are condensed (i.e., prometaphase and metaphase of all divisions, middle to late diakinesis, croziers and pre-pachytene asci).

(2) Fixed material.

(a) Fix blocks of agar bearing perithecia in a large volume of 1:3 acetic alcohol or in 60% acetic acid. The former is better for pachytenes; the latter seems adequate for most other stages. Allow material to stand in fixative for 12 hours or longer. Material in acetic-alcohol may lose staining capacity in two weeks or longer. Accumulation of metaphases and early diakinesis may be obtained by placing Petri plate cultures in a 10° C. refrigerator for 24 hours, and fixing at the proper time after removal to room temperature, as determined by test preparations.

(b) Material in 60% acetic acid usually separates well without hydrolysis. If well-separated asci from acetic-alcohol fixed material are desired, hydrolyze perithecia in bulk in 1N HCl for five minutes at $50-60^{\circ}$ C. This will also render the cytoplasm relatively non-staining, and gives excellent spindle and centriole differentiation. If very well-flattened material is desired, as for young asci and metaphases, omit acid hydrolysis. Instead, press asci into a drop of snail cytase (diluted with three parts of water), and allow to stand in a moist chamber for 30 minutes to $1\frac{1}{2}$ hours. Then pipette off cytase mixture and replace with stain.

(c) Place on cover slip, and treat as in 1(c) and (d).

Material for all stages except pachytene may be treated in this fashion.

(3) Pachytenes.

(a) Use material fixed in 1:3 acetic alcohol.

(b) Omit any treatment designed to separate asci, as single asci are almost impossible to handle.

(c) Press asci into drop of aceto-orcein. With needles, tear

as many asci as possible. Breaks should ideally be at the nucleus; usually such precision cannot be attained freehand.

(d) Add sufficient stain so that when the cover slip is placed on, the stain will just or not quite reach to the edge. Place on cover slip.

(e) Seal the preparation without applying any additional pressure.

Of the asci torn as above, a goodly proportion will be broken too far from the nucleus. Of those properly broken, many will be protected by clumps from the pressure of the cover slip. Of those properly torn and flattened, a few should be pachytenes of the proper stage for study. These will often stain slowly. Preparations kept in the refrigerator will attain the desired degree of differentiation in a few days, and will keep for weeks.

MICROSCOPY

During most of the period covered by this report, a Spencer binocular microscope, with interchangeable monocular tube, fitted with Zeiss apochromat objectives (90x, N. A. 1.30; 120x, N. A. 1.30) and Zeiss Mobini compensating oculars (10x, 15x, 20x), and a Spencer achromatic condenser (N. A. 1.40), has been used. The light source has been a Bausch and Lomb lamp, with ribbon filament bulb, used with a Wratten green filter, No. 61.

Photomicrographs were taken with the Leitz 1X Makam, using its set of Periplan oculars (10x, 15x), with the monocular tube of the microscope set at 160 mm. Some photographs were taken with the 10x ocular

and 120x objective. In general, better resolution and considerably greater depth of focus was obtained with the 15x ocular used in combination with the 90x objective. Water immersion of the substage condenser was used for critical observation, and routinely for photography. The film used was Eastman Contrast Process Panchromatic, developed at 20-25° C. for five minutes with developer Eastman D-11.

PART FOUR. CHROMOSOME MORPHOLOGY

Relative lengths and centromere positions in the seven chromosomes of *N. crassa* are of such a nature as to facilitate identification of individual chromosomes. In the present study, considerable effort has been expended to get a maximum of information about the morphology of the chromosomes, as a basis for future work on the cytogenetics of the organism.

McClintock (1945) presented data on the relative lengths, absolute sizes, and centromere positions of the haploid complement. The relative lengths of the seven chromosomes were the same at all divisions. She stated that the longest chromosome attained a length of approximately 15 microns at pachytene, of 2.5 microns at third metaphase, and of 1.5 microns at the fourth division (spore mitosis). She noted that the pachytene chromosomes showed definite and distinctive chromomere patterns.

Dr. McClintock and the writer have adopted a system of numbering the cytological chromosomes like that in maize. The chromosomes are numbered in order of length, from chromosome 1, the longest, to chromosome 7, the shortest. When two chromosomes are equal, or nearly equal, in length, the one with the longest long arm is given the lower number (e.g., chromosomes 4 and 5, 6 and 7). This system bears no relation to the numbers previously given to the linkage groups; with one possible exception, no correlation between the two sets of numbers can be established at the present time.

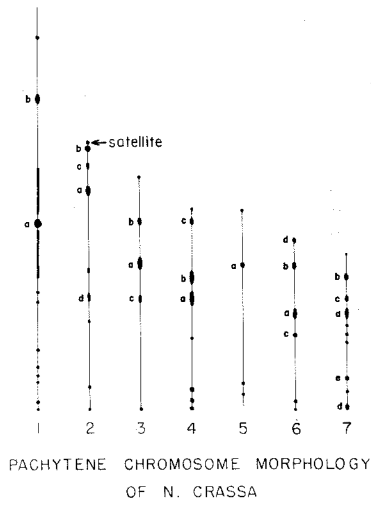
Most of the measurements reported in the present paper are of chromosomes at late pachytene. However, sufficient figures of other stages have

been measured to permit the following statements. The lengths of the chromosomes within the haploid complement do, barring stretching in squashing, maintain a fairly constant relationship to each other. These relative lengths are given in Figure 2. The longest chromosome is about 2.6-2.7 times as long as the shortest. At prometaphase of the conjugate division in the crozier, chromosome 1 is about 2.5 microns in length; at metaphase it has shortened to 1.5 microns (i.e., about the size of the same chromosome at fourth metaphase). At late pachytene, it has an average length of 18.9 microns (maximum - 22.4 microns, minimum - 15.8 microns) in the figures measured. At first metaphase, chromosome 1 forms a bivalent which, in side view, measures about 1.2 microns wide by 1.7 microns long. At second metaphase, the longest dyad is between 1.0 and 1.5 microns in length. (Relative lengths at this division have not been determined; they are probably roughly the same as at other divisions, but the chromosomes are so short as to make accurate measurement difficult.) The lengths of chromosome 1 at third metaphase (2.5 microns) and fourth metaphase (1.5 microns) found by the writer are the same as those given by McClintock.

MITOTIC CHROMOSOME MORPHOLOGY

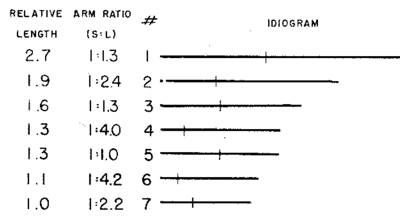
The morphology of the mitotic (i.e., third and fourth metaphase) chromosomes is shown diagramatically in Figure 2. Centromere positions are indicated by cross-lines. (V. also Figs. 19c-f, 22b-c.)

These data are essentially the same as those diagrammed by McClintock. She stated that the centromere positions were adequately determined only for chromosomes 1 and 2, and tentatively for the rest. The few third and



PACHYTENE CHROMOSOME MORPHOLOGY
OF N. CRASSA

FIGURE 1



MITOTIC CHROMOSOME MORPHOLOGY
OF N. CRASSA
FIGURE 2

fourth divisions studied by the writer were in essential agreement with her placement of the centromeres. However, no extensive study was made of critical third prophase figures, at which stage centromere placement can be most accurately determined.

Chromosome 1 has, about half-way out on the long arm, a slight constriction, at which point the arm often bends or curves. Chromosome 2 has a small satellite on the end of the short arm, distal to a constriction at which the nucleolus is attached.

PACHYTENE CHROMOSOME MORPHOLOGY

The morphology of the chromosomes at pachytene is shown diagramatically in Figure 1. (V. also Figs. 12-15, 16a-c.) Almost all of the figures on which this is based are from the cross Em5256A by Em5297a. However, the chromosomes of strain Chilton-a also have the same morphology.

This arrangement is regarded by the writer as the standard morphology in the species. Other wild collections may be expected to differ from it to a greater or lesser degree. Some of the mutant strains certainly differ from it by translocations and possibly by inversions. Since, without extensive collecting, there is no possible way of telling what arrangement is most prevalent in the species, this arrangement has been adopted as standard (not "wild-type"), and others will be described as variants from the standard.

Relative lengths of the chromosomes at pachytene are given in Table 1.

TABLE 1

PACHYTENE CHROMOSOME LENGTHS

	Relative Length (single Nuclei)			Absolute Length (microns)			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Chromosome	Maximum	Minimum	Average	Maximum	Minimum	Average	Relative Length (from column 7)
1	3.6	2.1	2.6	22.4	15.8	18.9	2.6
2*	2.0	1.3	1.7	14.9	9.5	12.0	1.7
3	1.9	1.3	1.5+	14.0	8.0	11.2	1.5+
4**) 5**)	1.6	1.0	1.3	12.3	6.0	9.2	1.2
6	1.5	0.9	1.1	10.4	5.3	7.9	1.1
7	1.0	1.0	1.0	9.0	5.5	7.2	1.0

* Satellite not included in these measurements.

** Since chromosomes 4 and 5 are the same, or nearly the same length, and could not always be distinguished by morphology, they are grouped together in this table.

They were arrived at in the following manner. Twelve excellent figures in which all or nearly all chromosomes could be identified and followed throughout their length were drawn by camera lucida. The chromosomes of each nucleus were measured, and the relative length of each chromosome determined, using the length of chromosome 7 as 1.0. The maximum and minimum figures for the relative length of each chromosome thus obtained are given in Table 1, columns 2 and 3. The average of the relative lengths is given in column 4. The absolute lengths, in microns, are given in columns 5 and 6. The average absolute lengths were determined, and are presented in column 7. Relative lengths calculated from these averages are given in column 8. The relative lengths arrived at by both methods are in agreement, except for a discrepancy of 0.1 unit for chromosomes 4 and 5. This agreement would indicate a consistent relation in lengths of the various chromosomes between nuclei. All of the nuclei were at about the same degree of extension (late pachytene) when measured. The differences between these relative lengths and those found at third metaphase are probably not significant, due rather to greater opportunity for differential stretching in the pachytene nuclei than to differential condensation at the two stages.

The chromomere patterns shown in Figure 1 represent the most recent compilation. An earlier map had been constructed in the winter of 1946-1947, by means of which chromosomes 1, 2, 6, and 7 were readily identifiable. The map, as presented, is still subject to correction with accumulation of further workable figures. The word "chromomere" is used here in its broadest sense -- a local accumulation of stainable substance on the chromosome. Some of the larger chromomeres, especially those which are characteristically ovoid or elongate, rather than

spherical, certainly suggest heterochromatic segments. No attempt has been made to demonstrate heterochromatin as such (i.e., by differential staining or "nucleic acid starvation") in the pachytene chromosomes.

Not all of the chromomeres which have been seen are included in the present map. Within any given nucleus, one usually finds the major chromomeres present, but the smaller ones may not be visible, or may be condensed into a single larger chromomere. Even within one chromosome, it often happens that one homologue will show two small chromomeres where the other has a single larger chromomere. This phenomenon is probably due to different degrees of stretching in the two homologues, rather than to actual structural differences.

The positions given for the chromomeres shown in the diagram are average ones. In arriving at these positions, for chromosome 1, for example, all the figures of chromosome 1 were plotted on a single card, with chromomeres transferred from the camera-lucida drawing to the plot by dividers. It was then clear (a) that many smaller chromomeres appeared in one or two figures only, and (b) that those chromomeres which were present in all or most of the figures varied considerably in their relative distance from a fixed point on the chromosome (the closest end of the chromosome usually being the point of reference). The small chromomeres referred to in (a) were omitted from further consideration, since the prime function of a map at this point is to facilitate identification of the chromosomes. A chromomere which is more likely to be invisible than not is a greater hindrance than a help.

The relative distance of a given chromomere from the closest end of

the chromosome was then measured for all the separate plots, and the average determined. The chromomere was then placed on the final map at this average position. While this is a somewhat rough method, it does tend to equalize differences due to varied amounts of stretching in different nuclei. The map, as shown, then represents the average positions of the more constant chromomeres, on chromosomes whose lengths likewise are averages.

To facilitate reference to individual chromomeres, the major ones on each chromosome have been designated by letter in order of decreasing size -- a for the largest, b, next largest, etc. The smaller chromomeres have not been individually designated.

Several points should be mentioned concerning individual chromosomes. The b chromomere ends of the homologues characteristically adhere to each other in chromosomes 1 and 7, giving rise to a spear-shaped end for each of these bivalents. This is very marked, especially when fixation and preparation have been such that the rest of the homologues are rather widely separated. In addition, the region of chromosome 7 between the a and c chromomeres is frequently more closely approximated than the rest of the chromosome, except the end.

On either side of the a chromomere in chromosome 1, a rather thick, dark-staining region occurs, which is probably heterochromatic. It seems probable that these two regions account for most of the heterochromatin which is seen around the aggregated centromeres of the interphase nucleus. The very large chromomeres in general may be heterochromatic; the a chromomere of chromosome 1 is particularly striking.

It can be seen without difficulty in almost every pachytene nucleus, whether the rest of the pattern is visible or not.

After the pattern shown in Figure 1 had been compiled, a striking superficial correspondence between the position of the heavier chromomeres and the mitotic centromere positions was noticed. The centromeres themselves have not been observed in pachytene chromosomes. A comparison of the arm ratios in the mitotic chromosomes, and of the ratios of chromosome segments on either side of the heavy chromomeres is presented in Table 2.

The correspondence between these figures is close enough to suggest strongly that the centromeres of the pachytene chromosomes are associated with (in, at, or near) one of the heavier chromomeres on each chromosome. For chromosomes 1, 3, 6 and 7 the agreement is quite good. Part of the discrepancy in chromosome 2 may be due to the fact that the satellite, which makes up a considerable portion of the short arm in the mitotic chromosome, was not included in the pachytene measurements. (The satellite at pachytene is minute, and often not seen.) The greatest deviation is in chromosomes 4 and 5. But the numbering of these two chromosomes at pachytene, where they are the same length, and the centromeres are not visible, is arbitrary; it has not yet been established whether the pachytene numbers for these two correspond to the mitotic numbers. So the pachytene chromosome 4 may well be the mitotic chromosome 5, and vice-versa. If this is the case, the correspondence of centromere position to heavy chromomeres is considerably closer.

It is fully realized that an absolute correspondence of this nature

TABLE 2

COMPARISON OF MITOTIC ARM RATIOS AND

POSITIONS OF HEAVY CHROMOMERES

Chromosome	Mitotic Arm Ratio	Chromomere	Ratios of Pachytene Chromosome Segments on Either Side of Chromomere
1	1:1.3	a	1:1.6
2	1:2.4	a	1:4
3	1:1.3	a	1:1.6
4	1:4.0	a	1:1.17
		b	1:2
5	1:1.0	a	1:2.7
6	1:4.2	a	1:1.5
		b	1:4.8
7	1:2.2	a	1:1.5
		c	1:2.3

is, on the basis of present data, not established. The structure of the chromosomes involved in the rearrangements available must be worked out before reliable data on this point can be obtained.

The data presented in this part represent an essential base for further work in the cytogenetics of _Neurospora crassa_. It is now possible to identify individual chromosomes with a fair degree of certainty, even though all the chromosomes of the nucleus cannot be measured, nor followed throughout their length. Chromosomes 1, 2 and 7 can almost always be recognized; 3 and 4 are usually recognizable. Chromosomes 5 and 6 offer more difficulty.

PART FIVE. THE CHROMOSOME CYCLE IN THE DEVELOPING ASCUS

The description of chromosome and nuclear behavior presented in the following pages includes the observations of McClintock (1945) and subsequent observations of Dr. McClintock (unpubl.) and the writer. The major part of the cycle is documented by photomicrographs from the writer's files, presented in Figures 10-23. The writer assumes responsibility for conclusions and interpretations presented.

The nuclear and chromosomal cycle has been followed from the last mitosis previous to nuclear fusion (i.e., the conjugate mitosis in the crozier) to the interphase following the fourth division in the ascus (i.e., the spore mitosis). Only the most cursory attention has been paid to mitoses of hyphal nuclei, and slightly more to the structure of the nuclei of the paraphyses in the perithecium.

OBSERVATIONS

The Interphase ("Resting") Nucleus

All non-dividing nuclei in which any detail can be made out, as well as those interphase nuclei between the divisions in the ascus, have certain structural similarities. A general description of their appearance will be presented here, and in later discussion, only the variations from this structure at particular interphases will be noted.

A typical interphase nucleus, such as that found between divisions in the ascus, or the nuclei of the paraphysis cells, consists of a limit-

ing membrane (by inference, since only a line of demarcation between cytoplasm and nucleoplasm is actually observed), a clear, non-staining nucleoplasm, and chromatin, which is usually visible as distinct chromosome arms radiating out into the nucleus from a heterochromatic region at one side of the nucleus. This polarized arrangement of the chromosomes results from their poleward movement at anaphase, at the end of which the centromere regions are gathered together, with the arms trailing back toward the former equatorial plate. In interphases between the divisions in the ascus, it can be established that this anaphase orientation persists through the interphase, because the individual chromosome arms can be seen as such throughout the interphase stage. The block of heterochromatin lies around the aggregated centromere regions of the chromosomes. That this block is bipartite, either as a segment on either side of one centromere, or as a segment near the centromere on two different chromosomes, is quite clear in preparations which have been considerably flattened. In these, instead of the single heterochromatic mass usually seen, a bilobate body, or two separate masses can be made out. From the analysis of pachytene morphology previously presented, it appears probable that the two heterochromatic segments seen at interphase correspond to the heterochromatic segments on either side of the _a_ chromomere of chromosome 1.

The same type of nucleus is found in the cells of the paraphyses, in which, however, no divisions have been observed to occur. The chromosome arms, which are usually rather condensed and often compactly massed together, extend out from the heterochromatic region at one side of the nucleus. This heterochromatin can also be identified in hyphal nuclei; in these, however, the chromatin is so compact that the chromosomes them-

selves have not been made out.

The polarized anaphase orientation of the chromosomes is maintained throughout the interphase in the ascus. Sister-nuclei characteristically show an orientation with relation to each other, such that the centromere-heterochromatin regions lie at the farthest sides of the two nuclei, and the chromosome arms of each nucleus point toward the other nucleus. This is the orientation to be expected if telophase reorganization occurs without a shift either in the positions of the two nuclei or in the relative positions of the chromosomes from their previous anaphase orientation. Within the ascus, at least, such a shift seems to be rare, except during the interphase following the third division, and the nuclei show at prophase the relic orientation of the previous anaphase.

Nucleoli of the resting nuclei are quite small, and often not visible in orcein preparations. In carmine preparations they stand out clearly. Very frequently the nucleoli in sister-nuclei are in mirror-image positions with reference to each other. This behavior, when found as late as early prophase, would indicate little shifting in the relative positions of the chromosome arms within the nucleus during the interphase.

The Conjugate Mitosis in the Crozier

Soon after the ascogenous hypha has bent into the form of the crozier, the two nuclei are seen in prophase (Fig. 10a). The chromosomes become distinct, and at prometaphase can be individually identi-

fied by size and position of centric regions (Fig. 10b). The metaphase plate is usually crowded; only occasionally can the chromosomes be spread sufficiently for identification. At prometaphase, chromosome 1 is about 2.5 microns long; at metaphase, about 1.5 microns. This is approximately its size at the fourth division in the ascospore.

The metaphase plates in the two nuclei are at about the same level in the crozier, the one in the tip region being usually midway between the tip of the crozier and the apex of the bend (Fig. 10c). The spindles are oriented longitudinally, one pole of each toward the apex of the crozier, the others toward the tip and base respectively. Anaphase separation and telophase clumping of the chromosomes occurs, accompanied apparently by considerable elongation of the entirely intranuclear spindle (Fig. 10e). Four nuclei are formed; one in the tip, one in the base, and two, non-sister, in the apex. Walls are laid down cutting off the two apical nuclei from the tip and basal ones, and isolating the apical cell as the prospective ascus (or primary ascogenous cell) (Fig. 10d).

Nuclear behavior in the basal region of the crozier has not been closely followed. In other ascomycetes (v. Smith, 1938, p. 419), the tip cell usually refuses with the basal cell, and its nucleus migrates into the basal cell. A new crozier is then formed from the basal cell, and the entire process repeated a number of times, so that numerous asci are descendants of a single initial ascogenous hypha. Indications of nuclear migrations have been seen in Neurospora (Fig. 10d); it is quite clear that several asci are joined to a common base, and that the tip cell does re-fuse with the basal cell, but the details have not been followed.

The apical nuclei become interphasic in organization. The individual chromosome arms usually become indistinct, so that the nucleus shows a nucleolus, a mass of chromatin, and, at the upperside, a small block of heterochromatin marking the position of the aggregated centromere regions. The two nuclei, which at first were some distance apart, and near the base of the cell, move apically and centrally until they are in contact along the upper one-half of their adjacent sides. During this period the heterochromatic region of the nucleus is drawn out into a fine "beak", so that the nucleus is pear-shaped in side view. The "beak" is ordinarily oriented toward the point at which the two nuclei will meet. The inference is drawn that the nucleus is being towed or pulled by some agency acting primarily on the centromere regions of the chromosomes, or on that portion of the nucleus in which they lie.

Nuclear Fusion and Synapsis

At about this time, the apical region of the apical cell protrudes in a bulge which gradually takes on a club-shaped form, the definitive shape of the ascus. At first the bulge is short and broad; as growth progresses, it becomes longer and relatively more slender. Actually there is growth in diameter as well as in length. At about the time at which the bulge forms (either before, during, or after its formation), the two nuclei of the ascus fuse. There are some indications that the time of fusion, relative to the other changes occurring, is different in different strains. At the time of fusion, the chromosomes are still in the interphase condition. The nucleoli may fuse soon after nuclear fusion, or may remain separate until synapsis is well advanced. When the nucleoli fuse early, the resultant single nucleolus is usually central in

the nucleus, between two masses of interphase chromosomes. The short arms of the two chromosomes 2 (attached to the nucleolus) are often stretched considerably out from this mass, especially in flattened preparations.

Soon the chromosomes undergo contraction, and there appear in the nucleus two groups of seven chromosomes each (Fig. 11h). At this time, in the wild-type strains most examined, the individual chromosomes are about the size of the prometaphase chromosomes of the conjugate crozier division. The same relative sizes are observed within the complements as are later seen at the third and fourth mitoses. In asci in which the fusion nucleus has rounded up (i.e., the parental nuclear outlines having been lost), the chromosomes are usually more or less scattered around the nucleus, and the parental groupings are obscured (Fig. 11a-g, 11i).

Now, either by random or directed movement, the chromosomes become assorted so that chromosomes of similar size are adjacent, and synapsis begins. (Fig. 11j-k). In a given nucleus some chromosomes may be seen which are completely synapsed, others synapsed along only part of their length, and still others adjacent, but not touching each other. Either slightly before, during, or after synapsis, depending in part at least upon the strains involved, the chromosomes begin to elongate. In no case does this elongation exceed more than three times the original length of the condensed chromosomes before synapsis is complete; in most cases the pre-synaptic elongation is less than this (Fig. 11, l-n). Post-synaptic elongation keeps pace with growth in size of the ascus to a remarkable extent; one can usually tell from the size and shape of the ascus what the state of extension of the chromosomes will be.

Pachytene through Diakinesis

Elongation of the synapsed chromosomes continues until a stage of maximum elongation is reached at late pachytene (Figs. 12-16h). At this time chromosome 1 has an average length of 18.9 microns (maximum - 22.4 microns, minimum - 15.8 microns, in the nuclei measured). The chromosomes of the complement have the same relative lengths as at the mitotic divisions. The detailed morphology of the pachytene chromosomes has already been presented.

Elongation of the chromosomes is accompanied by increase in size of the nucleus and of the nucleolus. At late pachytene, the latter may have a diameter of 5-6 microns, whereas in the nuclei at the time of synapsis it measures about 2 microns in diameter (with a volume, in the latter case of about 4.2 cubic microns, in the former, of 65-113 cubic microns).

Synapsis is very close during early stages. But, in fixed material which has not been heated, synapsis at middle and late pachytene is quite loose; the homologues lie side by side, but at a distance of about one-half micron from each other. There is little relational coiling; many pairs will extend parallel throughout their length. In others, the number of relational twists may vary from one to four per bivalent. To what extent this looseness of association is artefactual is difficult to determine. It is consistently found after alcoholic fixatives, especially if the preparation is not heated. In material fixed in stain-fixative, association may be closer. Heating the preparation, especially in material fixed for a short time only (1-4 days), often results in appression of the homologues to form a single thick strand.

Insofar as the writer has been able to determine, the pachytene homologues are optically single. Diplotene separation, with formation of typical chiasmata (Fig. 16d), sets in after the state of maximum elongation is reached. The chromosomes stain rather poorly at this stage; coupled with the diffuseness of the threads and the loops formed by the separating homologues, this renders observation difficult. Contraction begins, (Fig. 16e-h), resulting eventually (probably after a considerable time, to judge by the proportions of diplotene asci found) in typical diakinesis bivalents, with interstitial and terminal chiasmata (Fig. 16j-17c). The nucleolus decreases in size during the period of contraction; at diakinesis and metaphase it is about the same size as in early synapsis (circa 2 microns in diameter).

The chiasma frequency has not been precisely determined, largely owing to a scarcity of suitable figures. However, it may be stated that, in general, chromosome 1 has three (occasionally only two) chiasmata, chromosomes 2 and 3 either two or three, while the remainder of the complement usually have two chiasmata each. Insufficient data are available for any conclusions to be drawn as to possible localization of chiasmata, or as to the degree of terminalization which occurs.

<u>First Metaphase to First Interphase</u>

At metaphase of the first division, the seven bivalents become arranged on the equatorial plate (Fig. 17d). They are now at a stage of maximum condensation; the bivalent formed by chromosome 1 is about 1.2 microns wide by 1.7 microns long, in side view. The nucleolus is still attached to chromosome 2, and is often pressed against the nuclear mem-

brane. The spindle, which can now be differentiated with proper treatment, is entirely intranuclear, and oriented longitudinally in the ascus.

Anaphase separation of the chromosomes occurs in a typical manner (Fig. 17e-g). The nucleolus remains attached to the chromatids of chromosome 2, and at anaphase may behave in one of several ways: it may be torn in two by the separating chromatids, and a part pass to each pole; or it may be torn loose from one of the dyads, and go entirely to one pole; or again, it maybe completely torn loose from the chromosomes, and float free in the nucleus, subsequently passing into the cytoplasm, where it may persist at least as late as metaphase of the second division (Fig. 18c).

The nucleus becomes much stretched by the elongating spindle, and assumes an almost cylindrical shape. The exact mechanism by which the nucleus is divided into two parts has not been determined; it seems probable that the membrane is broken under the tension of the elongated spindle, and two new membranes are reconstituted. (Colson (1934) describes a disappearance of the membrane at late anaphase.) The chromosomes of the telophase nucleus (Fig. 17h) are clumped together, with the centromere regions collected at the pole. Uncoiling occurs and interphase nuclei are formed. The state of extension of the chromosomes during first interphase is less than that at the second or third; the longest chromosome arm reaches a length of about 5 microns. A tiny nucleolus (circa one-half micron in diameter), forms near the end of one of the chromosome arms and remains attached to it throughout the cycle. The fate of the old nucleolus, when it is included in one or both of the daughter nuclei, has not been determined.

The region of the interphase nucleus containing the aggregated centromere regions of the chromosomes is frequently drawn out into a beak-like protrusion which points toward the nearer end of the ascus; thus the beaks of the sister-nuclei point in opposite directions. Since nuclei at the second division seem to lie relatively farther apart than the first telophase chromosome groups, it is possible that there is further movement apart of the interphase nuclei, subsequent to telophase, and that the beak-like appearance is a result of a pulling force applied to the aggregated centromere regions or associated structures during this movement.

The Second Division

Prophase of the second division is initiated by a shortening of the chromosome arms; the orientation of the chromosomes within the nucleus is maintained until late prophase. At metaphase, the chromosomes are oriented on the equatorial plate, with the spindles of both nuclei longitudinal in the ascus, and in tandem, not overlapping. Occasional aberrant orientations are found (Fig. 18a), in which one or both spindles lie obliquely across the ascus. The metaphase chromosomes are typical dyads, H- or X-shaped (Fig. 18b). The longest is about 1.0 to 1.5 microns in length. Centromere regions are conspicuous, with the arms diverging widely from them. Anaphase separation occurs normally; the nucleolus may be torn in two, or pass to one pole, or be torn free from both chromosomes 2, as in the first division. It is sometimes found on the plate between the two anaphase or telophase groups of chromosomes (Fig. 18e). The spindles elongate considerably during anaphase and telophase (Fig. 18d-e), pushing the sister chromosome groups still further apart, and

probably rupturing the nuclear membranes. However, non-sister nuclei are seldom, if ever, pushed past one another. Interphase proceeds as before, but the chromosomes undergo a considerably greater extension, the longest arm reaching a length of about 10 microns (i.e., an extension of the same magnitude as at late pachytene). The heterochromatic region around the centromeres is always conspicuous at this time (Fig. 18f, 19a). The nucleolus of the interphase nucleus is about the same size as at first interphase, and is formed near the end of one of the shorter chromosome arms (undoubtedly the short arm of chromosome 2).

The Third Division

Prophase contraction results in sharply-stained chromosomes in which the centromere region is well defined by reason of the relic orientation of these regions at one side of the nucleus. The chromosomes shorten still further (Fig. 19c-e), and congress upon the equatorial plate. The spindles are oriented obliquely across the ascus (Figs. 19g-h, 20a). Chromosome 1 at this time has a length of 2.5 microns. A small nucleolus is still attached to chromosome 2 (Fig. 19c-d).

Anaphase separation of the chromosomes, and telophase breakage of the nuclear membranes result in eight nuclei (Fig. 20b), usually arranged four in a row on either side of the ascus. Sister nuclei are at opposite sides, and adjacent nuclei within a row are non-sister. The nucleus undergoes the usual interphasic changes; centromere-heterochromatin regions are toward the ascus wall, while the chromosome arms project toward the sister nucleus on the opposite side of the ascus. A

small nucleolus is again seen, usually in mirror-image position with reference to that in the sister-nucleus.

To what extent this two-ranked arrangement of the nuclei is the usual arrangement in the living ascus is not known. Shearing forces exerted in squashing the preparation may distort such relationships to a considerable degree. Occasional asci show one or more pairs of nuclei out of the usual two-rowed arrangement. This may be a result of different spindle orientation, or may be artefactual.

A stage of rather confused orientation now follows, in which the nuclei diverge from the two-ranked order, and are oriented in various directions, with the heterochromatic regions of the nuclei pulled out into beaks. This is succeeded by an orderly arrangement, in which all eight nuclei are in one row along one side of the ascus (Fig. 20c). It is inferred that during this time the nuclei migrate about the ascus, either all progressing to some single line, or the four on one side moving across to the other. The exact sequence of events, and the relationships involved, cannot well be established in squash preparations. Sectioned material would be more adequate for this purpose. The genetic behavior would indicate that the movements are accomplished with a high degree of regularity and precision.

The centriole, which is not visible in orcein preparations during the first and second divisions, can be seen from early prophase of the third division. At this time, two rod-like bodies are seen close together at the side of the nucleus nearest the aggregated centromeres. During prophase, they move apart and take up positions on opposite

sides of the nucleus, at the site of the future spindle poles. The spindle, when formed, projects across the nucleus between the centrioles. At metaphase, the centriole usually appears as a heavy, dark-staining rod, often curved (Fig. 19g). However, its length varies as one focusses up and down, in such a fashion that a triangular shape was inferred. This inference was verified in numerous asci in which the centriole had been forced into a plane at right angles to that it normally occupied. Then it appeared as an equilateral triangle, with sides approximately 2.5 microns long. The thickness of the body is of the order of one-quarter micron. The centriole attaches to the nucleus by one of the points of the triangle. It is rather flexible, often curving over the nucleus, and cupping around the heterochromatic region. When the nuclei are in the state which has been referred to as migration, the centriole is always at the point which is most pulled out. It seems to precede the nucleus in its movements.

At the stage when all eight nuclei are aligned in a row, the centriole lies between the nucleus and the ascus wall. The cytoplasm around it takes on a fibrous appearance, with the focus of the fibers at the centriole. This fibrous pattern extends out and around a region of cytoplasm, enclosing the nucleus and cutting out the cytoplasm of the spore (Fig. 21a-d). Soon after, the spore cytoplasm becomes more vacuolate, while the cytoplasm not included in the spore, the epiplasm or periplasm, retains its granular appearance.

The orientation of nucleus, centriole, and spore is such that the fusiform spore usually lies with its long axis diagonally across the ascus, the centriole at the lower tip, and the nucleus in the lower

quarter of the spore. In a few critical figures it has seemed that the major portion of the triangular centriole was pressed against the outside of the spore wall.

The Fourth Division

The nucleus of the spore now takes up a more central position, and the chromosomes condense (Fig. 21e, 22a) to a metaphase condition (Fig. 22b-e), congressing upon the equatorial plate. At this time chromosome 1 is about 1.5 microns long. There is still a small nucleolus on chromosome 2 (Fig. 22b-c). The spindle is generally oriented along the short axis of the spore; two telophase nuclei result (Fig. 23a-b), one on either side of the spore. They become interphasic in organization (Fig. 23c), with centromere heterochromatin, chromosome arms extending toward the sister nucleus, very small nucleoli, and possibly small heterochromatic segments on the distal portion of some chromosome arms.

The spore wall becomes thicker, develops a system of hyaline ribs, and becomes pigmented in the area between the ribs. Lindegren and Scott (1937) have described the mechanism of formation of the rib system. No observations in the present study either support or negate their description.

DISCUSSION

Several phases of the cycle described above merit further consideration. These are (a) the synaptic phenomena, (b) the implications of observed chiasma frequencies, (c) the behavior of the nucleolus and (d)

centriole behavior.

Synapsis

The synapsis of condensed (presumably much-coiled) chromosomes, followed by elongation (presumably uncoiling) of the synapsed homologues, is difficult to reconcile with current theories of chromosome pairing. This difficulty may have prompted the suggestion of one worker that the bodies actually seen to synapse are the heterochromatic segments of the chromosomes; the euchromatic segments are presumably unstained, uncoiled, and synapsed, but cannot be seen (with orcein staining). (This interpretation has been given verbally by K. W. Cooper, and reported to the writer by numerous persons.)

In view of somewhat analogous behavior in other organisms (e.g., the euchromatic "ghost-ends" of the pachytene chromosomes of tomato), such an interpretation may be plausible a priori. However convenient it may be, first-hand observation of the process reveals several considerations which contradict the assumption.

First, there is a close correspondence of the relative lengths of the unsynapsed chromosomes to those of other divisions in the ascus. This would not be expected, a priori, if these "chromosomes" are actually "prochromosomes", or heterochromatic segments.

Second, a continuous series of nuclei can be found in any preparation, extending from those with unsynapsed, much-condensed chromosomes, through stages of synapsis, to completely synapsed chromosomes which are

little longer than the unsynapsed chromosomes at the beginning. Further nuclei will be seen in which the synapsed chromosomes are more elongate, slenderer, and progressively less stainable. This series is accompanied by simultaneous increase in size of ascus, nucleus and nucleolus, so that confidence may be placed in the correctness of the seriation. On the basis of synapsis of heterochromatic segments, with euchromatic segments also synapsed, but unstained, it must be also assumed that the euchromatic segments gradually acquire the capacity to stain, and that this gain proceeds from the heterochromatic segment distally toward the ends of the chromosomes, on all chromosomes simultaneously.

Third, after the pachytene chromosomes have become sufficiently elongate so that chromomere morphology can be made out, and some of the chromosomes individually identified, there is still a further increase of 50-100% in length before the state of maximum extension is reached.

It should be possible to test the correctness of the two alternatives. With translocation stocks in which the interchange is such as to result in vastly different lengths than those of the standard strains (e.g., translocation 45502), it should be possible on the interpretation proposed by McClintock to identify the interchanged chromosomes in the fusion nucleus, before synapsis, and to show that again the length relations were the same as in later divisions. This has not yet been attempted.

The looseness of synaptic association in fixed material of middle to late pachytene offers further opportunity for speculation. If this

is a true picture of in vivo conditions, then the mechanism involved in crossing-over is a puzzle. It seems definite that chiasmata are not present at late pachytene. The relational twists seen may resemble chiasmata superficially, but many bivalents do not show any such twists. Yet, to date, no nuclei in crosses of standard strains have shown univalents at diakinesis or metaphase I. To assume that the relational twists are chiasmata is to assume also that they bear no relationship to metaphase association of bivalents. However, it does seem probable that crossing-over and chiasma formation occur in the period immediately following late pachytene. The whole problem may be more apparent than real, in view of the possible artefactual nature of the loose synapsis.

Chiasma Frequency

The available data on chiasma frequency are scanty. In themselves, however, they suggest an average frequency of 14-18 chiasmata per nucleus. Their distribution, and the expected genetic chromosome lengths are as follows:

Chromosome 1 -- 3-4 (?) chiasmata -- 150-200 (?) units
" 2 -- 2-3 chiasmata -- 100-150 units
" 3 -- 2-3 (?) chiasmata -- 100-150 units
" 4-7 -- 2 chiasmata -- 100 units

A total genetic length of from 700 to 900 cross-over units would then be expected.

Nucleolar Behavior

There is a consistent correlation between size of the nucleolus and

the state of extension of the chromosomes throughout the nuclear cycle; maximum size of the nucleolus is attained when the chromosomes are in a state of maximum extension, minimum size corresponds to maximum condensation of the chromosomes. The retention of the nucleolus during metaphase, particularly at metaphase I, where it is as large as in the presynaptic nucleus, is unusual. This suggests a lack of complete synchrony of the synthesis of the substances which are involved; more seems to accumulate in the nucleolus than is used by the chromosomes during condensation. The escape of the nucleolus into the cytoplasm at anaphase I, a frequent occurrence, suggests that the membrane is broken at this time. This behavior of the nucleolus is also figured in *N. tetrasperma* by Colson (1934), who states that the nuclear membrane actually disappears at late anaphase.

Centriole Behavior

The behavior of the *Neurospora* centriole has been figured by Dodge (1927), Wilcox (1928) and Colson (1934), and described by McClintock (1945). None of the first three workers identified the body as the centriole; Dodge speaks of it as the "spindle-end" or "appendage", and Colson follows his usage, while Wilcox speaks of the "antler-like" structure or "hooked-end" of the nucleus.

The behavior of the centriole is comparable to that in ascomycetes in general; it is present at the ends of the spindle during division, and initiates cytokinesis at the time of spore formation by the production of fibers which extend around and delimit the cytoplasm of the spore.

The complete history of the centriole was not followed in the present work. However, the gaps can be filled from the figures and descriptions of the previous workers. The centriole is characterized, among other things, by increasing size as the ascus develops. In _N. tetrasperma_ (Colson, 1934), at metaphase I the centriole is a short, thick, heavily-staining rod (insection, at least) about 0.6 micron in length; at metaphase II it is slightly longer, and at metaphase III is still thicker and about 1.0 micron long. There seems to be further growth, for at anaphase III the centriole is 1.3 microns long, and heavier than at metaphase. (Unfortunately, neither Dodge nor Wilcox indicate magnifications in their figures; the values given are from measurements made on the drawings of Colson.) The centriole of _N. crassa_, at metaphase or anaphase III is about 2.0 microns long, when viewed as a rod; the sides of the triangular face are about 2.5 microns long.

The single centrioles of daughter nuclei divide during late anaphase, telophase or interphase (the exact time is uncertain) to give a typically "antler-shaped" figure (i.e., the two resultant rods diverge from a common point on the nuclear membrane). Apparently the centriole does not project into the nucleus, although a few of the published drawings give that impression.

During spore delimitation, the centriole is still attached to the nucleus. The substance of the centriole is apparently not consumed by the process of fiber- and wall-formation; it can be seen in its original size and shape, and stains as heavily as previously, in spores whose walls are completely laid down and have started to thicken.

Dodge and Wilcox figure the centriole as included within the spore wall. The writer's impression, from a few clear figures, is that it may actually be outside the wall. In any case, it seems to lose its connection with the nucleus. The latter takes up a central position in the spore, while the centriole lies against the wall; no visible connection between centriole and nucleus is present. Dodge does not figure the centriole as present at the fourth division in the spore; Wilcox shows a small rod about the size of the centriole at metaphase I. In the writer's material, no centriole has been seen at division IV.

While the centriole in this genus presents no functional peculiarities not found in ascomycetes in general, its continued growth, its close connection with the centromere regions of the chromosomes, its division at approximately the same time as has been postulated for splitting of the chromosomes, and its activities in fiber formation are of interest. The previously known intimate functional and possibly structural correlations between centriole and centromere, as exemplified in the work of Pollister (1939) and Pollister and Pollister (1943), invite one to speculate upon the nature of the growth process here.

The fate of the major portion, at least, of the centriole which is left against the spore wall needs further study, as does the problem of the transmission of the centriole to the spore nuclei. Whether the two gametic nuclei also contribute centrioles to the fusion nucleus, how many, and which of these function at the first division, are also factors which deserve further attention.

SUMMARY

The sequence of events in the ascus described in the present paper is essentially the same as that reported by McClintock (1945). Further details concerning particular phases of the processes have been observed. A pictorial record is presented.

Points of special interest in the cytology of the ascus include (a) the synapsis of condensed chromosomes, followed by elongation; (b) the synchrony of nucleolus size and state of chromosomal condensation, especially in view of the unorthodox cycle of the latter in the diploid nucleus, and (c) the behavior and functions of the centriole.

PART SIX. CHROMOSOME ABNORMALITIES

Description and analysis of the normal behavior of an organism in its normal environment seldom yields sufficient data for complete understanding of structures and processes. Study of the organism in abnormal environmental conditions, or of abnormal forms of the organism frequently casts light upon the normal structure and functions. In the field of nuclear cytology, the utilization of altered environmental conditions leads into the domain of experimental cytology; the study of abnormal forms, into that of cytogenetics. In the present study, the latter approach is adopted.

There are three general ways in which abnormal chromosome behavior may be detected. First, such behavior may be fortuitously discovered in material already under study for other reasons. Second, since many types of chromsome abnormalities lead to the production of inviable spores or gametes, one may search for signs of partial or total sterility. This is a particularly useful index of the presence of structural changes in chromosomes. Finally, peculiar genetic results (e.g., changed linkage relations, "pseudo-linkage", etc.) are often a result of malfunction or change in the chromosome apparatus, and may be used as an index of such changes. The aberrant types of behavior described in the present section were discovered by use of the second and third indices.

McClintock (1945) reported three translocations in _N. crassa_, occurring in mutant strains produced by irradiation. These were strains 4637 (albino -1), 45502 (pyrimidineless), and 44105 (threonineless). In the course of the present work, two additional translocations in mutant stocks

were found: 36703 (arginineless) and 5936 (leucineless). A further case of abnormal behavior, apparently not a simple aberration, in crosses of strains Abbott 4A and 55701a, called to my attention by Dr. M. Westergaard, was studied briefly. Undoubtedly many other chromosome aberrations exist in the many irradiation-produced mutant stocks accumulated by Dr. G. W. Beadle; no attempt has been made to search the stocks for such strains. Those studied were selected because of known sterility or peculiar genetic behavior reported to the writer by others who had worked with the strains.

The aberrant behavior of the 55701a x Abbott 4A cross will be discussed briefly; relatively little has been done toward a detailed analysis of the situation, but certain practical considerations make mention of the problem advisable. The available genetic and cytological data relevant to the identification of the chromosomes involved in the five translocations will be presented here, but discussion of their genetic effects will be postponed until a later section of the paper.

SPORE ABORTION IN THE ABBOTT STRAINS

At the request of Dr. M. Westergaard, the writer examined asci in various stages from the cross 55701a x Abbott 4A. This cross, as well as that of Abbott 12a x Abbott 4A, is characterized by a very high degree of sterility, due to production of many inviable spores.

Genetic Basis of Abortion

Emerson and Cushing (1946) state that whenever a certain gene, pre-

sent in both of the Abbott strains, is present in the homozygous condition in the ascus, aborted spores are produced. The abortion is not 100% however; a certain number of viable spores result. Strain 55701a, studied in the present work, is the result of a cross between Abbott 4A and Chilton a. It has probably received the Abbott abortion gene from its Abbott parent. The gene is apparently effective only when homozygous (i.e., it is recessive to the normal allele); it should be noted that the ascus is the only structure in the life cycle in which such a dominance relation will normally be found. If a strain not related to the Abbotts is crossed to one of them, no abortion occurs.

Cytological Basis of Abortion

Pachytenes of the cross 55701a x Abbott 4A are characterized by extreme clumping of the chromosomes, so that spreading is impossible. The chromosome mass resembles a tightly-interwoven tangle of threads. At metaphase I, seven bivalents were normally observed, but anaphase disjuntion was highly irregular. In most cases seven dyads apparently went to each pole, but bridges connecting the separating homologues were frequent. No count of such bridges was made; in general, the frequency was at least one, or more often two or three, bridges per nucleus. Telophase nuclei were frequently connected by long slender chromatic strands which persisted into interphase.

Bridges were also observed at the other divisions in the ascus. Only a few countable metaphase figures were found. In some metaphase IV figures, it was clear that nuclei occurred which contained hypo- or hyperploid complements; metaphases with as few as three or four chromo-

somes, and others with as many as ten or more were seen.

No counts were made to determine the frequency of spore abortion. Few asci contained eight normally-matured spores; many contained only two or one normal spores, the rest being abortive to various degrees. Spores which were normal in wall development and pigmentation were frequently much larger than the normal ascospore of N. crassa, and falcate rather than fusiform in shape.

Discussion

The cytological picture of the process leading to formation of aborted spores bears a marked resemblance to Beadle's (1932) description of the effects of the "sticky-chromosome" gene in maize. Whether the two are actually comparable is uncertain. The problem is worthy of further study. It would, for example, be desirable to know if the mutation rate in viable spores from the cross were the same, or higher, than that in normal material. Also, it might be supposed that, if the gene concerned behaves as a simple hypomorph or amorph, the same type of behavior might occur in somatic divisions. Further, one may speculate as to the possibility of rendering the cross normal in behavior by altered environment or nutrition.

The condition has a certain practical importance in genetic work. Both of the Abbott strains have been extensively used as parents of the mutant strains obtained by Beadle and Tatum (v. table of origins of the mutants, Beadle and Tatum, 1945); approximately half of the mutants with one Abbott parent may be expected to carry the gene. This is quite pos-

sibly one cause of the not infrequent sterility observed in some mutant-by-mutant crosses. Further, if the Abbott strains are used as wild-type partners in crosses to maintain ascospore stocks of the mutants, a still wider spread of the gene throughout the mutant stocks may be expected. The obvious remedy is to cease using the Abbott strains, except where they are absolutely required for some reason. Mutant stocks carrying the gene can be "cleaned" by outcrossing to non-Abbott wild-types, and testing the segregates by back-crossing to an Abbott stock; on a large scale the labor of such a procedure would be prohibitive.

Teas (1947) reported a suppression of crossing-over in certain crosses of a mutant having one Abbott parent. The suppression occurs when the mutant is crossed to a non-Abbott wild-type; crossing-over is much reduced as compared to the cross mutant x Abbott. He suggests the presence of a chromosome aberration, either inversion or translocation. The mechanism of suppression would seem to involve failure of synapsis, rather than elimination of cross-over chromatids, since there is no appreciable sterility accompanying the suppression. An inversion would seem the more probable of the two types of aberration, since even without crossing-over, a translocation would be expected to give a certain amount of aborted spores (v. Part Seven).

IDENTIFICATION OF CHROMOSOMES INVOLVED IN THE TRANSLOCATIONS

Reciprocal translocation between chromosomes of the complement may or may not result in chromosomes of significantly different morphology, depending upon the relative size of the interchanged segments. If lengths are markedly altered, identification of the chromosomes in-

volved should be possible at the metaphase of division III. If such is not the case, then identifications must be made, for the most part, from pachytene morphology and configuration. Unfortunately for rapid identification of chromosome changes, the pachytene technique in *Neurospora* is not yet the equivalent of that in maize. As a consequence, the identification of the chromosomes involved in the five translocations thus far found is in most cases tentative at best, and has been achieved by devious methods.

Dr. Barbara McClintock has most kindly permitted the citation of her unpublished data on three of the translocations (4637, 45502, 44105). The conclusions reached from these data are, except where otherwise expressly noted, those of the writer.

Nomenclature of Translocations and Translocated Chromosomes

For the present, a given translocation will be identified by the strain number of the mutant with which it was associated when found, prefixed by a T (e.g., T4637, T45502, etc.). If further work leads to the accumulation of many aberrations, it will probably be desirable to adopt the nomenclature currently used in maize, whereby a translocation is identified by the numbers of the two chromosomes involved, prefixed by T, and followed by a lower-case letter denoting the particular translocation among the many involving the same two chromosomes. Thus T4637 would become T 1-7a, since it involves chromosomes 1 and 7, and is the first such translocation found. Such a nomenclature would eliminate confusion due to the same numbers being used to designate both mutant genes and translocations.

Within a translocation heterozygote, the normal chromosomes have been identified by their normal numbers, where known, and the translocated chromosomes by the number of the centromere followed by a T (thus a nucleus heterozygous for T4637 contains chromosomes 1, 7, 1T and 7T). Again, the maize terminology may be more appropriate; the translocation chromosomes carry the number of the centromere, with a superscript of the number of the translocated segment (thus T 1-7a would consist of chromosomes 1^7 and 7^1). In the present state of knowledge, it is not always possible to identify the segments of the translocated chromosomes which carry the centromere, so that in some cases the numbering of the translocated chromosomes may be the reverse of the truth.

<u>Translocation T4637</u>

In the mutant strain 4637 (albino-1) McClintock (1945) found a translocation involving chromosome 1 and (unpubl.) one of the short chromosomes. In pachytenes, pairing was variously as two "bivalents", partially unsynapsed, or as quadrivalents with frequent asynapsis of some regions. At diakinesis and metaphase I either a ring or a chain of four chromosomes or two "bivalents" was observed.

From her unpublished drawings and from the writer's observations, chromosome 1 is definitely involved. From Dr. McClintock's drawings, it appears that the short chromosome involved is chromosome 7; the "Spear-shaped end" and heavy chromomeres of this chromosome are remarked in her drawings and notes. The break-point in chromosome 1 is nearest the non-spear-shaped end of that chromosome. The position of the

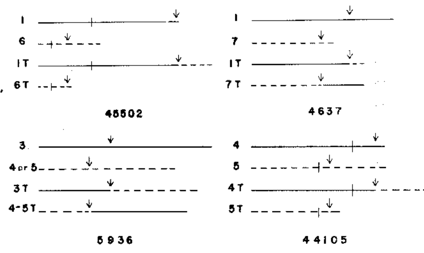
break-point in chromosome 7 is not certain, but appears likewise to be nearest the non-spear-shaped end.

In addition to these data, the writer has observed two clear pachytenes (one shown in Fig. 24b) in which the quadrivalent could be followed throughout. The rest of the complement was not workable, but lengths of the normal and translocated chromosomes were obtained. No chromomere detail was visible in these preparations. The morphology of the normal and interchanged chromosomes is shown in Fig. 3. This is based upon the considerations presented above, and the average measurements which follow. If the considerations pertaining to the positions of the centromeres at pachytene are valid, then it is probable that the break-point in chromosome 1 is in the short arm, and that in 7 in the long arm. No observations are available as to mitotic morphology.

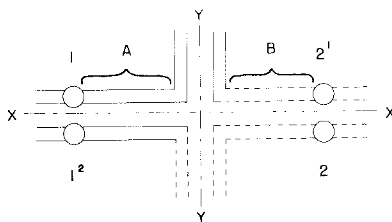
The relative lengths of the various chromosomes (average of three pachytene figures) is as follows: chromosome 1 - 1.7; chromosome 7 - 1.0; chromosome 1T - 1.3+; chromosome 7T - 1.2+. The lengths of the interchanged segments (average) are such that chromosome 1 has lost 0.42 of its total length to 7T and chromosome 7 has transferred 0.23 of its length to 1T.

The mutant albino-1 is linked with the mating-type gene and also linked with the translocation (Hungate, 1945). This would indicate that genetic linkage-group 1 is carried either on chromosome 1 or chromosome 7.

Translocation T45502



NORMAL & TRANSLOCATED CHROMOSOMES
FIGURE 3



SYNAPTIC ASSOCIATION OF NORMAL AND TRANSLOCATED CHROMOSOMES
FIGURE 4

CONSTITUTION	SPORE TYPE	CHARACTER
1,2 (normal) 1^2, 2^1 (transloc.)	+	normal
1, 2^1 (terminal 1^2, 2 dupl / def)	A & B	more or less defective
1, 1^2 (centric 2, 2^1 dupl / def)	D	very defective

CHROMOSOME CONSTITUTION OF SPORE TYPES
FIGURE 5

McClintock (1945) identified T4-5502 as involving a break very near the end of the long arm of chromosome 1 and very near the centromere in the long arm of one of the chromosomes with a sub-terminal centromere. Her drawings (unpubl.) show metaphase III chromosomes in which the long arm of the translocated chromosome 1T is much longer than that of chromosome 1, while the short translocated chromosome is reduced almost to a small sphere. From one of Dr. McClintock's pachytene drawings (unpubl.) and one of the writer's it appears that the chromomere morphology of the short chromosome involved is similar to that of chromosome 6. The writer has observed only chromosome 1T at metaphase III, in nuclei in which it was not possible to determine the other chromosome involved by elimination.

The arm ratios (average of three metaphase III's) of chromosome 1 (1:1.55) and chromosome 1T (1:2.00) indicate that chromosome 1T has gained a segment equivalent to at least 0.45 of the short arm of chromosome 1. The short translocation chromosome (chromosome "X"T) shown in Dr. McClintock's drawings has a length equal to about 0.22 of the total length of chromosome 1. The relative lengths of the three chromosomes are then: chromosome 1 - 4.55; chromosome 1T - 5.36; and chromosome "X"T - 1.00.

There are three chromosomes with subterminal centromeres which could lose from their long arms an amount equal to 0.45 of the short arm of chromosome 1: chromosomes 4, 6 and 7. With the relative length for chromosome 1 of 4.55, 4T would then have a relative length of 1.36, 6T of 1.03, and 7T of 0.80. Thus it is probable that chromosome 6 is the short chromosome involved, since chromosome 4 is considerably too

long to fit the length relationships required, and, as noted below, chromosome 7 can be eliminated on other grounds. The normal and translocation chromosomes are diagrammed in Fig. 3.

The mutant gene 45502 (pyrimidineless) shows pseudo-linkage with both cholineless-34486 (linkage-group 4) and adenineless-3254 (linkage-group 2) (Mrs. Mary B. Houlahan, personal communication). Chromosome 1 is plainly involved in both T45502 and T4637. The latter is linked with the mating-type locus; the former is not. Therefore, chromosome 1 must carry the genes of either linkage-group 2 or 4. Chromosome 7 must carry the genes of linkage-group 1 since T45502 does not involve genes of this linkage-group, and chromosome 7 is not involved in T45502.

Translocation T44105

McClintock (1945) described rings of four chromosomes, figures with four univalents, and other figures with one or more univalents at diakinesis and metaphase I in this translocation. The only data as to the chromosomes involved are given in notes to two of her drawings (unpubl.), in which she makes a tentative identification of the chromosomes present in the ring or as univalents as chromosomes 4 and 5, 4T and 5T, with break-points and centromere positions as shown in Fig. 3. Relative lengths (calculated by the writer from her drawings) are: chromosome 4 - 1.5; 5 - 1.5; 4T - 2.0; 5T - 1.0. 0.25 of the short arm of 4 is transferred to 5T; 0.83 of one arm of 5 is transferred to 4T.

The writer has not studied this translocation to any great extent. Metaphase III morphology should be sufficiently altered to permit confir-

mation or change of the identification given. Pachytenes of this strain are extremely unsatisfactory; they much resemble the pachytenes of the Abbott strains, one of which is a parent of mutant 44105. The chromosomes are tangled, squeezed together, and spread and stain poorly. For pachytene work it may be necessary to outcross this translocation stock to the Emerson strains, which have good pachytenes, and attempt to derive a translocation stock with workable figures. Nothing is known as to the genetic relations of the translocation.

Translocation T36703

Dr. Adrian Srb (personal communication) had found pseudo-linkage of mutant gene 36703 (arginineless) to the mating-type locus (linkage-group 1) and to iso-leucine-valineless (16117, linkage-group 5). The strain was investigated for the presence of a translocation. Rings of four chromosomes were observed in many diakinesis and metaphase I figures; occasionally two "bivalents" were present instead. No additional data are available as to the chromosomes involved, except that chromosome 2, the nucleolus chromosome, is not involved. On the basis of data previously presented, it would be expected that chromosome 7 is involved, since the translocation involves linkage-group 1.

Translocation T5936

Dr. David Regnery (personal communication) found a considerable frequency of aborted spores in crosses involving mutant strain 5936 (leucineless). The wild-type allele tended to be included in the normal spores in a non-random frequency.

At diakinesis, rings or chains of four chromosomes are frequent; they are not associated with the nucleolus, hence chromosome 2 is not involved. In some figures, two "bivalents" were found, in addition to the five bivalents present in all figures. Photomicrographs of diakinesis configurations are shown in Fig. 16j-l and Fig. 17a-b. No alteration in mitotic morphology could be established in a number of metaphase III figures studied. Pachytene quadrivalent configurations are shown in Fig. 12b-c and Fig. 24c-f. Asynapsis of one or more regions in the quadrivalent was common.

In none of the pachytenes was it possible to identify the chromosomes involved by their chromomere morphology. Nor was it possible to measure the lengths of all the chromosomes of the complement in any nucleus in which the quadrivalent association could be followed. The relative lengths of the four chromosomes involved in the quadrivalents (average of five figures) were 1.0, 1.0, 1.17 and 1.21. From the configurations found, it was possible to determine that the normal chromosomes must be those with lengths of 1.0 and 1.21, the translocation chromosomes, those with lengths of 1.0 and 1.17, or the reverse. No data are available to decide which of these is the correct order. The diagram in Fig. 3 is arbitrarily constructed on the former order. However, if identifications given below are correct, this is probably the proper order. The change in lengths is so slight that mitotic morphology would not be expected to differ significantly from normal; no such difference was observed.

An attempt can be made to identify the chromosomes involved indirectly. First, chromosomes 1, 2, and 7 can be eliminated from

further consideration. The rings at diakinesis are not associated with the nucleolus. Both chromosome 1 and chromosome 7 have been clearly identified, and were not involved, in nuclei in which the quadrivalent could be followed. Thus chromosomes 3 through 6 remain.

The ratio of the lengths of the chromosomes involved (shorter: longer) is approximately 1:1.2. The ratio of the lengths of chromosomes 4 or 5 to chromosome 3 is 1:1.15; of chromosome 6 to chromosome 3, 1:1.4. The ratio of chromosome 6 to chromosomes 4 or 5 is 1:1.18. Thus a translocation involving chromosomes 3 and 4, 3 and 5, 4 and 6 or 5 and 6 is not excluded on the basis of relative lengths. Apparently chromosomes 4 and 5 are not both involved. The diagram shown in Fig. 3 identifies the chromosomes as 3 and either 4 or 5; chromosome 3 was tentatively selected because the length of the longest chromosome involved in the quadrivalent configurations in general seemed greater than that of chromosomes 4 and 5 in the average pachytene.

<u>Summary</u>

The data concerning the relative lengths of normal and translocated chromosomes and the size of the interchanged segments is summarized in Table 3. The available data concerning the cytological and genetic relations of the translocations is summarized in Table 4. The cytological identification of the chromosomes involved in the translocations and the determinations of the break-points are in no case completely satisfactory. A further search for analyzable pachytene figures, especially those in which chromomere detail is workable, and a more intensive study of metaphase III figures are necessary.

TABLE 3

RELATIVE LENGTHS OF NORMAL AND TRANSLOCATED CHROMOSOMES

AND OF INTERCHANGED SEGMENTS IN THE TRANSLOCATIONS

TRANSLOCATION	CHROMOSOME RELATIVE LENGTHS	LENGTH OF INTERCHANGED SEGMENTS
T4637	Chr. 1 - 1.7 7 - 1.0 1T - 1.3+ 7T - 1.2+	0.42 of total length of chr. 1 transferred to 7T; 0.23 of total length of chr. 7 transferred to 1T.
T45502	Chr. 1 - 4.55 6 - 1.85 1T - 5.36 6T - 1.0	Very short terminal segment of chr. 1 long arm transferred to 6T; 0.57 of long arm of 6 transferred to 1T.
T44105	Chr. 4 - 1.5 5 - 1.5 4T - 2.0 5T - 1.0	0.25 of short arm of chr. 4 transferred to 5T; 0.83 of arm of chr. 5 transferred to 4T.
T36703	No data	No data, except that chr. 2 is not involved.
T5936	*Chr. 3 - 1.21 4 or 5 - 1.0 3T - 1.17 4T or 5T - 1.0	0.41 of total length of chr. 3 transferred to 4T or 5T; 0.39 of total length of 4 or 5 transferred to 3T.

* Alternative possibilities noted in text.

TABLE 4

CYTOLOGICAL AND GENETIC RELATIONS OF THE TRANSLOCATIONS

TRANSLOCATION	CYTOLOGICAL CHROMOSOMES INVOLVED	LINKAGE GROUPS INVOLVED
T4637	1 and 7	1 and (?)*
T 45502	1 and 6 (?)**	2 and 4
T44105	4? and 5?***	unknown
T36703	unknown (not 2)	1 and 5
T5936	3?? and 4?? or 5??****	unknown

* (?) - expected to be either linkage-group 2 or 4.

** (?) - identification of chromosome 6 not firmly established.

***? - identification tentative.

****?? - alternative possibilities suggested in text.

The gaps in the genetic data are obvious; when both sets of data are complete, it should be possible to establish a one-to-one relationship between the chromosomes and the linkage-groups. This, no less than a thorough knowledge of chromosome morphology, is a requisite for the future of *Neurospora* cytogenetics.

PART SEVEN. CROSSING-OVER AND DISJUNCTION IN TRANSLOCATION HETEROZYGOTES

McClintock (1945) described four types of asci formed in a cross of T45502 with a standard strain, and offered an interpretation of their origin in terms of crossing-over in the interstitial segments and disjunction. In T45502 one interstitial segment is so short that little or no crossing-over would be expected to occur in it. Under these special circumstances she was able to conclude, from the frequencies of the various types of asci produced, that (1) alternate and adjacent-I disjunction occurred equally frequently when no crossing-over occurred in the long interstitial segment, (2) adjacent-II disjunction occurred relatively infrequently, and (3) a cross-over occurred in the long interstitial segment in about one-half of the ascus nuclei. (Adjacent-I disjunction involves disjunction of homologous centromeres at Division I; adjacent-II disjunction, non-disjunction of homologous centromeres.)

Such a translocation is a special case; usually both interstitial segments are of such a length that crossing-over may occur in both. Under those circumstances, analysis of crossing-over and disjunction is not so simple. In this section of the paper, the development of tools for such an analysis, and the application of the method to data on ascus type frequencies in T45502 and T4637 will be described.

DERIVATION OF THE ASCUS TYPES

Synapsis in the Translocation Heterozygote

The expected synaptic configuration in the pachytene nucleus of a

translocation heterozygote is shown in Figure 4. The normal chromosomes are designated as 1 (solid lines) and 2 (dashed lines); the translocated chromosomes, as 1^2 and 2^1. Centromeres are shown as open circles. The interstitial segment in chromosomes 1-1^2 is Region A; that in chromosomes 2-2^1, Region B. Plane X---X is that of adjacent-I disjunction (disjunction of homologous centromeres); plane Y---Y, that of adjacent-II disjunction (non-disjunction of homologous centromeres). Alternate disjunction would result, in the absence of crossing-over in the interstitial segments, in chromosomes 1 and 2 going to one pole, chromosomes 1^2 and 2^1, to the other pole at Division I.

Incomplete synapsis, including the formation of "bivalents" in which non-homologous parts did not synapse, does not materially affect this picture, nor the considerations which follow. The net result of "bivalent" formation will be to increase the randomness of disjunction. If the "bivalents" are composed of homologous centric regions, crossing-over in the interstitial segments will not be affected, except as asynapsis of the segment occurs. If the "bivalent" is composed of non-homologous centric regions, crossing-over in the interstitial segment will be non-homologous or none.

Chromosome Complements Produced by the Heterozygote

Several possible products result from meiosis in the heterozygote (Figure 5). Complements composed of chromosomes 1 and 2 are complete and standard; those of chromosomes 1^2 and 2^1 are complete, but translocated. Complements of $1,2^1$ or $1^2,2$ are duplicated for one of the translocated segments, deficient for the other. The two types are

complementary, in that the segment duplicated in one is deficient in the other. These may be referred to as terminal duplication/deficiency complements. Complements composed of chromosomes $1,1^2$ or $2,2^1$ possess duplications of the centric region of the arm involved in the translocation and of the whole un-involved arm of one chromosome, and a corresponding deficiency of those regions of the other chromosome. They may be termed centric duplication/deficiency complements.

Meiotic products containing the complements 1,2 or $1^2,2^1$ are normal in appearance and fully viable. Those carrying $1,2^1$ or $1^2,2$ may be expected to be more or less abnormal in appearance, and more or less inviable, the degree of abnormality and inviability depending upon the length and content of the duplicated and deficient segments. The two types may differ in appearance from each other if the duplication/deficiency involves the proper genes. Products carrying chromosomes $1,1^2$ or $2,2^1$, since they are duplication/deficiency types involving at least a whole chromosome arm, may be expected to be very abnormal in appearance, and inviable. The two may be distinguishable, but more probably will not be because of the extreme abnormality expected.

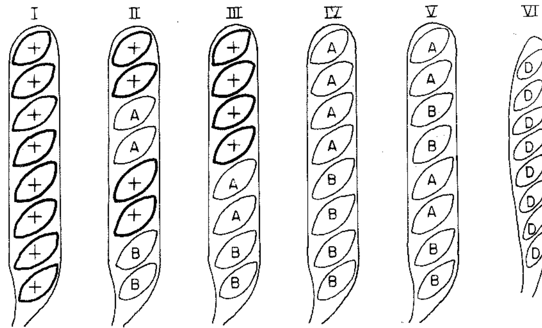
Ascus Types Produced in the Heterozygote

In *N. crassa* the four immediate products of meiosis each give rise to two ascospores. Since the normal ascospore has a definite and rather complex morphology, it might be expected that the deficient types mentioned above would be recognizable. In general, four types of spores can be recognized in the five available translocations. The normal spores have a fairly thick wall, heavily black-pigmented except

for a pattern of hyaline ribs. The spores are two-nucleate, with a rather dense cytoplasm having a characteristic fixation image after acetic-alcohol. In addition, three abnormal types can be seen in all of the translocations. One of these, which has been designated the D-type spore, is extremely abnormal in all of the translocations, with a thin wall and cytoplasm which has usually partially degenerated. No pigment or wall thickenings are developed. The other two deficient types lie between the D-type and the normal. Two such types are usually distinguishable, one more nearly normal than the other. In T45502, for example, one of these deficient types is not separable from normal until maturity, at which time it is slightly less pigmented; the other is distinguishable from normal, and from the D-type, at an earlier stage. The two types of semi-deficient spores have been called A- and B-types, the more nearly normal being the A-type spore.

If, for purposes of development, the spores with the complements of $1,2$ and $1^2,2^1$ are designated by the symbol $\underline{+}$, $1,2^1$ and $1^2,2$ by $\underline{A}$ and $\underline{B}$, respectively, and $1,1^2$ and $2,2^1$ by $\underline{D}$, then the possible types of asci resulting in a translocation heterozygote are as shown in Figure 6. They are distinguished (1) by the types of spores contained and (2) by the pattern or order of the spores in the ascus. These six types have been designated as Types I through VI.

It should be noted that in Types II, III, IV and V, certain permutations of the orders given are possible, due to variations in segregation at Division I or II with relation to the ends of the ascus. If this segregation is at random, the various permutations should occur with equal frequencies. In writing ascus type formulae in the text, a



ASCUS TYPES PRODUCED BY A TRANSLOCATION HETEROZYGOTE

FIGURE 6

CROSS-OVERS IN REGION B	CROSS-OVERS IN REGION A: DOUBLES 4-str.	DOUBLES 3-str.	DOUBLES 2-str.	1	0
0	IV alt. I adj.	II	I alt. IV adj.	II	I alt. IV adj.
1	II	I 1/4 III 1/2 V 1/4	II	I 1/4 III 1/2 V 1/4	II
DOUBLES 2-str.	IV alt. I adj.	II	I alt. IV adj.	II	I alt. IV adj.
DOUBLES 3-str.	II	I 1/4 III 1/2 V 1/4	II	I 1/4 III 1/2 V 1/4	II
DOUBLES 4-str.	I alt. IV adj.	II	IV alt. I adj.	II	IV alt. I adj.

RELATION OF CROSSING-OVER & DISJUNCTION TO ASCUS TYPES

TABLE 5

CROSS-OVERS IN REGION B	CROSS-OVERS IN REGION A: DOUBLES 4-strand c	DOUBLES 3-strand $2c$	DOUBLES 2-strand c	single a	none $1-(a+4c)$
none $1-(b+4d)$	$c[1-(b+4d)]$	$2c[1-(b+4d)]$	$c[1-(b+4d)]$	$a[1-(b+4d)]$	$[1-(a+4c)]$ $[1-(b+4d)]$
single b	bc	$2bc$	bc	ab	$b[1-(a+4c)]$
DOUBLES 2-strand d	cd	$2cd$	cd	ad	$d[1-(a+4c)]$
DOUBLES 3-strand $2d$	$2cd$	$4cd$	$2cd$	$2ad$	$2d[1-(a+4c)]$
DOUBLES 4-strand d	cd	$2cd$	cd	ad	$d[1-(a+4c)]$

ALGEBRAIC TERMS FOR CROSS OVER COMBINATIONS

TABLE 6

single symbol will be used to designate a pair of sister spores, and the formula will be written with the basal spores at the right; thus +A+B represents an ascus in which there are two + spores at the tip, followed in order by two A spores, two + spores, and with two B spores at the base of the ascus. Unless otherwise noted, the formula will be used generically for all the permutations of that particular type; thus +A+B includes all Type II asci such as +A+B, +AB+, A++B, A+B+, B++A, +BA+, etc.

Crossing-over, Disjunction, and the Ascus Types

Table 5 summarizes the types of asci produced by alternate (alt.) or adjacent-I (adj.) disjunction following crossing-over in neither, one, or both interstitial segments (Regions A and B, Fig. 4) of a translocation heterozygote. Where disjunction type is not specified, either type of disjunction after that particular cross-over combination has the same result. Vertical columns include 0, 1 and 2-, 3- and 4-strand doubles in Region A; horizontal columns, those in B. Combinations of the cross-overs occurring simultaneously in the two segments result in the ascus type indicated in the square at the intersection of the two columns. Thus a single cross-over in Region A occurring simultaneously with a 2-strand double in B results in a Type II ascus, regardless of the type of disjunction. A tetrad in which no cross-over has occurred in either segment results in a Type I ascus following alternate disjunction, and in a Type IV ascus following adjacent-I disjunction.

Adjacent II disjunction results in a Type VI ascus, regardless of crossing-over preceding disjunction. Type VI asci may also conceivably

arise by other means (e.g., by trisomic segregation at Division I); these cannot at present be identified. In the present study, the frequency of Type VI asci is used as an index of the frequency of adjacent-II disjunction, with the reservation that other factors may contribute to the class.

It may be noted, in passing, that the Type numbers used in this paper are not completely correspondent to those of McClintock (1945). The equivalent types are as follows:

McClintock's	Type I	------	present	paper	Type I
"	Type II	------	"	"	Type II
"	Type III	------	"	"	Type IV
"	Type IV	------	"	"	Type VI

DERIVATION OF EQUATIONS FOR THE ASCUS TYPES

Algebraic Expressions of Cross-over Combination Frequencies

If crossing-over in one interstitial segment does not influence that which occurs in the other (i.e., if exchanges in the two segments are independent) and if 2- 3- and 4-strand double exchanges occur in random proportions, then it is possible to express the frequency of a given combination of exchanges in the two interstitial segments as the product of the frequency of the component exchanges. Thus, if 50% of the tetrads have a single exchange in Region A, and 30% have a single in B, 15% should have a single simultaneously in A and B. Of all the double exchanges occuring in one segment, 1/4 should be 2-strand, 1/2 3-strand,

and 1/4 4-strand doubles.

In all succeeding calculations, Type VI asci are omitted from consideration, since their cross-over histories cannot be deduced. The total of Types I through V is then considered as 1.0.

The symbols used to express the frequency of the various exchanges are as follows:

$a =$ that fraction of the total tetrads (I-V) in which a single exchange occurs in Region A.

$4c =$ that fraction in which a double exchange occurs in A; this is subdivided into: $c =$ 2-strand doubles

$2c =$ 3-strand doubles

$c =$ 4-strand doubles

$1.0 - (a+4c) =$ that fraction in which no exchange occurs in A.

$b =$ that fraction in which a single exchange occurs in Region B.

$4d =$ that fraction in which a double exchange occurs in B; this will include: $d =$ 2-strand doubles

$2d =$ 3-strand doubles

$d =$ 4-strand doubles

$1.0 - (b + 4c) =$ that fraction in which no exchange occurs in B.

The products of any two terms then express the frequency of simultaneous occurrence of the two types of exchanges. These are collected in Table 6. Thus the frequency of simultaneous occurrence of a single exchange in both Regions A and B is (ab), etc.

<u>Ascus Type Equations</u>

Let h = that fraction of the total tetrads (I-V) which are Type I:

j = that fraction which are type II;

m = that fraction which are Type III;

n = that fraction which are Type IV;

p = that fraction which are Type V.

Then (1) $h + j + m + n + p = 1.0$ (Note: numbers in parentheses are to facilitate reference to a particular equation.)

Let k_1 = that fraction of tetrads with a given combination of exchanges which disjoins alternately;

k_2 = that fraction of the same kind of tetrads which disjoins by adjacent-I disjunction.

(Note: since there are 25 exchange combinations, there are potentially 25 different k_1's and 25 k_2's, since there is no assurance that the frequency of a given disjunction type is the same in all exchange combinations. However, no superscript or other differentiation of the various k_1's and k_2's has been followed, since these factors soon clear from the equations.)

Since adjacent-II disjunction has been omitted from consideration, (2) $k_1 + k_2 = 1.0$ for any given exchange combination. Then the frequency of tetrads which have a 2-strand double exchange in Region A and no exchange in Region B, and in which alternate disjunction occurs, is $k_1c\ (1.0 - b - 4d)$; these tetrads will produce Type I asci. Similarly, by adjacent-I disjunction, this combination of exchanges will give rise to Type IV asci with a frequency of $k_2c(1.0 - b - 4d)$. Then by multiplying each term of Table 6 by the proper coefficient of disjunction, and collecting all terms which result in the same ascus type (from Table 5), the frequency of a given type of ascus can be expressed

in terms of the combinations of exchange and disjunction which produced it. Thus:

(3) $h = k_1(1.0 - a - 4c)(1.0 - b - 4d) + k_1d(1.0 - a - 4c) +$
$k_1c(1.0 - b - 4d) + k_1cd + k_1cd + k_2d(1.0 - a - 4c) + k_2cd +$
$k_2c(1.0 - b - 4d) + k_2cd + \frac{1}{4}[(k_1 + k_2)ab + (k_1 + k_2)2ad +$
$(k_1 + k_2)2bc + (k_1 + k_2)4cd];$

(Note: $k_1d(1.0 - a - 4c) + k_2d(1.0 - a - 4c)$ is not equal to $(k_1 + k_2)(1.0 - a - 4c)$, since the exchange combinations are different, although their frequencies are the same.)

(4) $j = (k_1 + k_2)(1.0 - a - 4c)b + (k_1 + k_2)(1.0 - a - 4c)2d +$
$(k_1 + k_2)(1.0 - b - 4d)a + (k_1 + k_2)ad + (k_1 + k_2)ad +$
$(k_1 + k_2)bc + (k_1 + k_2)bc + (k_1 + k_2)2cd + (k_1 + k_2)(1.0 - b - 4d)2c$
$+ (k_1 + k_2)cd + (k_1 + k_2)cd + (k_1 + k_2)cd + (k_1 + k_2)cd;$

(5) $m = \frac{1}{2}[(k_1 + k_2)ab + (K_1 + k_2)2ad + (k_1 + k_2)2bc + (k_1 + k_2)4cd];$

(6) $n = K_1d(1.0 - a - 4c) + k_1cd + k_1c(1.0 - b - 4d) + k_1cd + k_2cd + k_2cd$
$+ k\ (1.0 - a - 4c)(1.0 - b - 4d) + k_2d(1.0 - a - 4c)$
$+ k_2c(1.0 - b - 4d);$

(7) $p = \frac{1}{4}[(k_1 + k_2)ab + (k_1 + k_2)2ad + (k_1 + k_2)2bc + (k_1 + k_2)4cd].$

If equations (3) and (6) are added, the coefficients of disjunction of all the exchange combinations in the sum become $(k_1 + k_2)$. Since by equation (2) $k_1 + K_2 = 1.0$, the equations then become independent of disjunction frequencies. The simplified equations are then:

(8) $h + n = (1.0 - a - 4c)(1.0 - b - 4d) + d(1.0 - a - 4c)$
$+ c(1.0 - b - 4d) + 2cd + d(1.0 - a - 4c) + c(1.0 - b - 4d)$
$+ \frac{1}{4}(ab + 2ad + 2bc + 4cd);$

(9) $j = b(1.0 - a - 4c) + 2d(1.0 - a - 4c) + a(1.0 - b - 4d) + 2ad + 2bc$
$+ 8cd + 2c(1.0 - b - 4d);$

(10) $m = \frac{1}{2}(ab + 2ad + 2bc + 4cd)$;

(11) $p = \frac{1}{4}(ab + 2ad + 2bc + 4cd)$.

Collecting like terms, and reducing, these equations become:

(12) $h + n = 1.0 - a - b - 2c - 2d + \frac{5ab}{4} + \frac{5ad}{2} + \frac{5bc}{2} + 5cd$;

(13) $j = a + b + 2c + 2d - 2ab - 4ad - 4bc - 8cd$;

(14) $m = \frac{ab}{2} + ad + bc + 2cd$;

(15) $p = \frac{ab}{4} + \frac{ad}{2} + \frac{bc}{2} + cd$.

Equations (14) and (15) are included in (12); hence, for purposes of algebraic solution, only two equations in four variables are available (i.e., either (12) and (13) or (13) and (14)). This is a consequence of the impossibility of distinguishing, on the basis of ascus types produced, between single and 3-strand double exchange, or between no-exchange, 2-strand and 4-strand double exchange tetrads. However, the equations can be rewritten in terms of two variables, $(a + 2c)$ and $(b + 2d)$ (i.e., combined singles and 3-strand doubles in Regions A and B respectively):

(16) $h + n = 1.0 - (a + 2c) - (b + 2d) + \frac{5}{4}(a + 2c)(b + 2d)$;

(17) $j = (a + 2c) + (b + 2d) - 2(a + 2c)(b + 2d)$;

(18) $j = \frac{1}{2}(a + 2c)(b + 2d)$;

(19) $p = \frac{1}{4}(a + 2c)(b+2d)$.

Letting (20) $a' = a + 2c;\ b' = b + 2d$:

(21) $h + n = 1.0 - a' - b' + \frac{5}{4}a'b'$;

(22) $j = a' + b' - 2a'b'$;

(23) $m = \frac{a'b'}{2}$;

(24) $p = \frac{a'b'}{4}$.

Using equations (21) and (22) or (22) and (23), whichever combination is preferable under a given set of circumstances, it is possible to calculate from the observed frequencies of ascus types the values of the variables a' and b' (i.e., the detectable, or effective, exchanges in the two interstitial segments A and B). Since a' and b' are exchange frequencies, the genetic, or cross-over lengths of the interstitial segments are $\frac{a'}{2}$ and $\frac{b'}{2}$ respectively (i.e., a' and b' are effectively the same as data from a 2-point cross). It may be noted that values of a' and b' obtained from either set of equations should be the same; any difference is due to counting of a non-random sample of asci, so that the frequencies observed do not represent the relative frequencies of the types in the population.

In certain types of translocations (i.e., those involving the nucleolus chromosome) it may be possible to evaluate some of the variables in equations (12) to (15) from other types of data (as by counts of nucleolus types in third interphase nuclei). In such translocations the full equations may be practical.

Estimation of *a*, *b*, *c* and *d*

By making certain simplifying assumptions concerning crossing-over, it is possible to estimate the variables *a*, *b*, *c* and *d*. In spite of the

uncertainties which this estimation involves, the results are worthwhile as estimates.

Consider that the interstitial segment A consists of a left and a right segment, each one-half the length of A. A single exchange, or no exchange may occur in either the left or the right segment, independently of what occurs in the other (i.e., there is no interference between the two halves), but within a segment, no double exchanges can occur (i.e., within a half, interference is complete). Then no more than two exchanges can occur within the whole length of segment A, and these are of restricted distribution. Let $\underline{r}$ be the probability that a single exchange will occur within the left segment, or in the right segment; then $(1.0 - r)$ is the probability that no exchange will occur in the left segment, or in the right segment. The probability that a single exchange will occur in one segment and none in the other (i.e., a single exchange in Segment A, or $\underline{a}$ in the previous notation) is then (25) $a = 2r(1.0 - r)$. By the assumptions made above, $\underline{r}$ cannot exceed 1.0. The probability of an exchange occurring in both the left and the right half of A simultaneously (i.e., a double exchange in segment A, or $\underline{4c}$ in the previous notation is

(26) $4c = r^2$, hence (27) $2\,c = \frac{r^2}{2}$; $c = \frac{r^2}{4}$. Then

(28) $a + 2c = a' = 2r - \frac{3r^2}{2}$, and

(29) $r = \frac{2}{3} \pm \sqrt{\frac{4}{9} - \frac{2a'}{3}}$.

By similar considerations:

(30) $b = 2s(1.0 - s)$;

(31) $2d = \frac{s^2}{2}$; $d = \frac{s^2}{4}$;

(32) $s = \frac{2}{3} \pm \sqrt{\frac{4}{9} - \frac{2b'}{3}}.$

It is not possible a priori to determine which of the two roots each of r and of s are the proper ones to use in further solutions. Additional information may be available in a particular translocation which will aid in this determination. From equations (29) and (32) it can be seen that when a' or b' is greater than 0.667 (i.e., $\frac{2}{3}$), the roots of r or of s are imaginary. This would indicate either (a) that the data are faulty, and that observed frequencies depart from true frequencies, or (b) that crossing-over in the translocation is not proceeding in accordance with the assumptions made above. However, the most probable departure from assumed conditions would be in the direction of a lesser number of double exchanges than assumed in the development of the equations; this would affect the result less than an increase, which is inherently less probable. The most probable explanation of imaginary roots is the first alternative above, that a non-random sample of the ascus population has been counted.

Derivation of Equations for the Frequency of Alternate and Adjacent-I Disjunction

In developing the foregoing equations, no assumptions were necessary as to the effects of crossing-over on disjunction. However, it seems probable that the type of exchange which occurs may influence the type of subsequent disjunction. Ideally, one would wish to calculate the frequency of alternate and adjacent-I disjunction for each combina-

tion of exchanges. With the data afforded by ascus type frequencies, this is impossible. However, if, in order to get an estimate of disjunction frequencies, one assumes that disjunction is not influenced by the exchanges which precede it, disjunction frequencies would be the same for all combinations of exchanges. It is then possible to derive equations for the calculation of overall or average frequencies of the two types of disjunction. This average frequency will be designated K. Then:

K_1 = the average fraction of tetrads in Types I-V which undergo alternate disjunction;

K_2 = the average fraction of these tetrads which undergo adjacent-I disjunction.

As before, (33) $K_1 + K_2 = 1.0$.

Equations (3) and (6) can then be rewritten:

(34) $h = K_1X + K_2Y + (K_1 + K_2)Z$;

(35) $n = K_1Y + K_2X$; where

X = those exchange combinations which result in Type I asci after alternate disjunction, and in Type IV asci after adjacent-I disjunction;

Y = those combinations which result in Type IV asci after alternate disjunction, and in Type I asci after adjacent-I disjunction;

Z = those exchange combinations which, with random segregation at Division II, result in Type I asci in one-quarter of the tetrads in which they occur, following either alternate or adjacent-I disjunction (i.e., simultaneous singles, simultaneous 3-strand doubles, or a single and a 3-strand double occurring simultaneously in both A and B).

The reduced forms of X, Y and Z, in terms of a, b, c and d, are:

(36) $X = 1.0 - a - b - 3c - 3d + ab + 3ad + 3bc + 10cd;$

(37) $Y = c + d - ad - bc - 6cd;$

(38) $Z = \frac{ab}{4} + \frac{ad}{2} + \frac{bc}{2} + cd.$

Since by equation (33), $K_1 + K_2 = 1.0$, $K_2 = 1.0 - K_1$. Then:

(39) $n = (1.0 - K_1)X + K_1Y = X - K_1X + K_1Y = K_1(Y - X) + X;$

(40) $K_1 = \frac{n - X}{Y - X} = \frac{X - n}{X - Y}.$

If the values of X and Y given in equations (36) and (37) are substituted in (40), it expands to:

(41) $K_1 = \frac{1.0 - a - b - 3c - 3d + ab + 3ad + 3bc + 10cd - n}{1.0 - a - b - 4c - 4d + ab + 4ad + 4bc + 16cd},$

where a, b, c and d are calculated from equations (12) to (15) and (25) to (32), and n is the observed frequency of type IV asci.

Since double sets of values for the variables a and c, b and d are obtained by solutions based on the present data, four separate values of K_1 must be solved for. A more convenient equation for this purpose can be derived in terms of a', c, b', d, and the observed frequencies of ascus types, h, m, and n. To eliminate the factor Y from equation (40):

(42) $h = K_1X + (1.0 - K_1)Y + Z = K_1X - K_1Y + Y + Z$ (from equation 34);

(35) $n = -K_1X + K_1Y + X;$

(43) $h + n = X + Y + Z;$

(44) $Y = h + n - X - Z.$

Substituting this value in (40):

(45) $K = \frac{X - n}{X - (h + n - X - Z)} = \frac{X - n}{2X + Z - (h + n)};$

(46) $Z = \frac{m}{2}$ (where m is the observed frequency of Type III asci, cf. equations (14) and (30)); hence

(47) $K_1 = \dfrac{X - n}{2X - (h + n - \frac{m}{2})}$. Expressing X in terms of a' and b', c and d:

(48) $X = (a' + c - 1.0)(b + d - 1.0) + cd$; then

(49) $K_1 = \dfrac{(a' + c - 1.0)(b' + d - 1.0) + cd - n}{2\,[(a' + c - 1.0)(b' + d - 1.0) + cd] - (h + n - \frac{m}{2})}$.

Summary of Available Equations

(12) $h + n = 1.0 - a - b - 2c - 2d + \frac{5ab}{4} + \frac{5ad}{2} + \frac{5bc}{2} + 5cd.$

(13) $j = a + b + 2c + 2d - 2ab - 4ad - 4bc - 8cd.$

(14) $m = \frac{ab}{2} + ad + bc + 2cd.$

(15) $p = \frac{ab}{4} + \frac{ad}{2} + \frac{bc}{2} + cd.$

(20) $a' = a + 2c$; $b' = b + 2d.$

(21) $h + n = 1.0 - a' - b' + \frac{5a'b'}{4}.$

(22) $j = a' + b' - 2a'b'.$

(23) $m = \frac{a'b'}{2}$

(24) $p = \frac{a'b'}{4}$

(25) $a = 2r(1.0-r).$

(27) $c = \frac{r^2}{4}.$

(29) $r = \frac{2}{3} \pm \sqrt{\frac{4}{9} - \frac{2a'}{3}}$.

(30) $b = 2s(1.0 - s).$

(31) $d = \frac{s^2}{4}$.

(32) $s = \frac{2}{3} \pm \sqrt{\frac{4}{9} - \frac{2b'}{3}}$.

(33) $K_1 + K_2 = 1.0; \quad K_2 = 1.0 - K_1$.

(41) $K_1 = \frac{1.0 - a - b - 3c - 3d + ab + 3ad + 3bc + 10cd - n}{1.0 - a - b - 4c - 4d + ab + 4ad + 4bc + 16cd}$.

(49) $K_1 = \frac{(a' + c - 1.0)(b' + d - 1.0) + cd - n}{2\,[(a' + c - 1.0)(b' + d - 1.0) + cd] - (h + n - \frac{m}{2})}$.

Validity of the Equations

The validity of the values for the various factors which are determined by use of the preceding equations depends upon two factors, the reliability of the data which are used, and the correctness of the assumptions involved in the derivations.

The reliability of the data obtained from counts of ascus types depends in part upon the nature of the translocation. Inability to distinguish A-type spores from normal spores results in failure to separate Type II and Type III asci. This reduces the number of available equations to two. Inability to distinguish the A-type and B-type spores results in failure to separate Type IV and Type V asci; this does not reduce the number of usable equations. A more serious error is introduced if the observed frequency of a particular type or types of asci is lower or higher than their actual frequency in the population. This may be due either to the more accurate classification of some types, or, more often, to differential breakage of the various types. When asci are pressed out of the perithecium onto a slide for counting, consider-

able breakage occurs, especially if the asci are approaching maturity. Type I asci are probably particularly prone to breakage of this kind. Types IV, V and VI are least likely to break. Since Types II and III are alike in content, though not in order, of spores, the frequencies of the two relative to each other are not so likely to be changed by breakage, but may be higher than they should be in relation to Type I asci.

The assumptions involved in deriving the equations in the preceding section may be discussed with relation to the variables concerned. In deriving equations for the calculation of $\underline{a'}$ and $\underline{b'}$, that is, for the frequency of effective cross-overs or exchanges in the two interstitial segments, only two assumptions were necessary: (1) that exchanges in one interstitial segment were independent of those in the other segment, and (2) that 2-,3- and 4-strand double exchanges occurred in the random proportions of 1:2:1.

So far as the writer is aware, there are no critical data bearing on assumption (1) above. Assumption (2), which apparently holds in Drosophila, has been challenged by Lindegren and Lindegren (1937, 1939) in Neurospora crassa. Of a total of 90 double exchanges which they identified genetically, 52 were 2-strand, 34 were 3-strand, and 13 were 4-strand doubles. The expected distribution would have been 22.5:45:22.5. Thus a considerable excess of 2-strand doubles occurred, while both the 3-strand and 4-strand classes were below expectation. Whitehouse (1942), upon reanalyzing the data of the Lindegrens, concluded that (a) there was negative chiasma interference and positive chromatid interference across the centromere, and (b) there was a slight tendency toward positive

chiasma interference within a chromosome arm. Thus while assumption (2) is a necessary one, in the absence of sufficient critical data to construct weighting factors for the various types of double exchanges, it may be incorrect. No attempt has been made to estimate the magnitude of the error which would thus be introduced.

In derivation of equations for calculating *a*, *c*, *b* and *d* from the calculated frequencies of *a'* and *b'*, a very arbitrary situation as regards exchanges within an interstitial segment was assumed to prevail. The segment was considered to be composed of two cross-over regions, within which interference was complete, and between which there was no interference. Since no information is available as to the actual distribution of exchanges within such segments, nor as to the amount of interference, and since such factors will vary from segment to segment in various translocations, the values of *a*, *b*, *c* and *d* obtained thereby must be regarded as very rough approximations. Nor can any criteria be adopted to distinguish between the two sets of values which are obtained for the variables *a* and *c*, and *b* and *d*, other than arbitrary ones growing out of the assumptions involved in calculating the values themselves, or by considerations involving other possible data on particular translocations. Calculated values of *c* and *d* will undoubtedly be higher than their true values.

The equations by which exchange frequencies are calculated involve no assumptions concerning the effect of exchanges upon subsequent disjunction. However, such assumptions are necessary for the calculation of disjunction frequencies. In the absence of data upon which to construct weighting factors for the various combinations of exchanges, it

was necessary to assume that the relative frequencies of adjacent-I and alternate disjunction were the same for all combinations of exchanges. The work of Brown (1940) and Pipkin (1940) would indicate a correlation between the type of exchange and the type of subsequent disjunction. Thus the values actually obtained from calculations involving the foregoing equations represent at best average values of the frequency of the two disjunction types. It should be pointed out that when none, or a very low frequency of no-exchange tetrads are formed, it becomes impossible to deduce the frequency of disjunction types, if the frequencies are approximately the same for both 2- and 4-strand double exchanges, which are assumed to occur with equal frequency, and which compensate each other in regard to the types of asci produced (cf. Table 5). In fact, the frequencies of alternate and adjacent-I disjunction, as calculated from the equations, or as determined directly by inspection of the raw data, are primarily related to the frequencies of the two in no-exchange tetrads. Extension of these frequencies to the other classes of tetrads is a consequence of the assumption that exchanges do not influence disjunction.

CROSSING-OVER AND DISJUNCTION IN T45502

McClintock (1945) presented data on the frequencies of ascus types in T45502. These are summarized in Table 7. The counts were made in the early ascospore stage; A-type spores were not distinguishable from normal spores at that time. Thus Type II and Type III asci would not have been distinguishable. But Type V asci, which would have contained pairs of apparently normal spores alternating with pairs of defective spores, were not found. It may be assumed that Type III asci were

TABLE 7

FREQUENCIES OF ASCUS TYPES IN T45502 AND T4637

	T 45502*			T 4637	
ASCUS TYPE	NUMBER COUNTED	% OF TOTAL	% OF TYPES I - V	NUMBER COUNTED	% OF TOTAL
I	197	22.7	25.4	65	18.7
II	381	43.8	49.1	242	69.8
III	0	0	0	39	11.2
IV	198	22.8	25.5	1**	0.3
V	0	0	0	0**	0
VI	93	10.7	---	0**	0
Total	869	100.0	100.0	347	100.0

* Data from McClintock (1945)

** These frequencies are undoubtedly low, because of criteria adopted for counting. Type VI asci probably constitute from 15% to 20% of all asci produced by the heterozygote of T4637; frequencies of the other two types are undoubtedly higher than recorded.

likewise absent, and that the Type II asci recorded did not include Type III's.

Then Types I + IV = (1) h + n = 0.509

Type II = (2) j = 0.491

Type V = (3) p = o

From (1) and (2), a' = 0.491, b' = 0. Then 49.1% of the tetrads in Types I - V resulted from a single or 3-strand double exchange in the longer interstitial segment; 50.9% resulted from 2- or 4-strand doubles or no exchanges in the longer segment; no tetrads resulted from exchanges in the shorter interstitial segment. (Insofar as exchanges in the two segments are separable only on the basis of their simultaneous occurrence (i.e., from Types III and V), this conclusion may be slightly in error. A frequency of exchange in the shorter segment of the order of 0.1% is not excluded.)

Since $\underline{b'}$ = 0, $\underline{b}$ and $\underline{d}$ are likewise zero. Solving for $\underline{a}$ and $\underline{c}$:

r = 1.009 or 0.325

a = -0.018 or 0.439

c = 0.255 or 0.026

Since $\underline{r}$ is in one case greater than 1.0, either (a) the probability of single exchanges in one-half of the interstitial segment is greater than 1.0, hence the assumptions made in deriving the equations are contravened, or (b) the data represent a sample of a population in which the true value of $\underline{a}$ is 0 (i.e., every tetrad hasmore than one exchange in the longer interstitial segment), in which case $\underline{r}$ is 1.0 and $\underline{c}$ is 0.25. The alternative values then are: 100% of the tetrads with a double exchange in the longer interstitial segment, or 43.9% with a single ex-

change and 10.4% with a double exchange in the segment. The longer segment in this case comprises almost all except a small terminal portion of the long arm of chromosome 1. It seems not improbable, on the basis of observations of diakinesis configurations in standard strains, that two chiasmata would normally be formed in this region. On the other hand, asynapsis might reduce chiasma frequency in this region below its normal value.

When $\underline{c} = 0.026$, $K_1 = 0.499$ and $K_2 = 0.501$; when $\underline{c} = 0.25$, $K_1 = 0.444$ and $K_2 = 0.556$. The true values of K_1 and K_2 probable lie somewhere between these two extremes.

To sum up, of the total tetrads produced by the translocation heterozygote: 10.7%resulted from adjacent-II disjunction; between 39.6% and 43.6% resulted from alternate disjunction, and between 49.7% and 45.7% resulted from adjacent-I disjunction. Of the total tetrads, 10.7% were of undetermined cross-over history; in 43.8% a single or 3-strand double exchange had occurred in the longer interstitial segment, and in 45.5% a 2- or 4-strand double or no exchange had occurred in this segment. Apparently no exchanges occurred in the shorter interstitial segment.

CROSSING-OVER AND DISJUNCTION IN T4637

Counts made in a cross of Chilton a x T4637A (grown at 25° C.) are summarized in Table 7. These data are presented because they illustrate some properties of the equations, and because a further breakdown of the counts, as presented in Table 8, reveals an interesting indication

of non-random segregation in certain classes. Insofar as they purport to represent exchange frequencies, no confidence can be placed in the data. In the first place, a considerable amount of differential breakage is known to have occurred in the samples. Type I asci are probably present in considerably higher frequency in the perithecium than the data would indicate. Secondly, in order to make an accurate classification of the various types of asci, only asci which had started to mature were counted. This automatically excluded Type VI asci, which never reach any appearance of maturity, and which are known to be present in the perithecia in considerable quantity. Type IV and Type V, in this translocation, frequently resemble immature Type I. Unless the wall of the A-type spore had begun to deposit the rib system, such asci would not have been classified. The result of these two factors, differential breakage and differential classification, has probably been to increase the relative frequencies of Type II and III at the expense of the other types of asci. This is further shown by analysis of the data through the equations.

From Table 7: Types I + IV = (1) $h + n = 0.190$

Type II = (2) $j = 0.698$

Type III = (3) $m = 0.112$

Type V = (4) $p = o$

Since, if segregation at Division II is at random, Type V should occur with a frequency half that of Type III, it is probable that p is not 0, but that Type V asci were misclassified. The low frequency of Type IV is probably also in error; Type IV resembles Type V, except in order of spores, and was probably also misclassified.

Solving for a' and b' with (1) and (2): a' = 0.813, b' = 0.184. Using (2) and (3): a' = 0.896, b' = 0.250. The discrepancy between these two sets of figures is another clear indication that the relative frequencies in the raw data are incorrect.

Since a' is in either case greater than 0.667, the roots of r are imaginary, and it is impossible to solve for a and c. The roots of s are 1.320 and 0.014, hence the alternative values of b and d are b = 0.344 or 0.028 and d = 0.446 or 0.000. This behavior indicates either (a) that crossing-over in the interstitial segments is in excess of that considered in deriving the equations concerned, or (b) that the detectable cross-over tetrads in the sample are in excess of their proportions in the population. The latter is very probable, in view of the considerations already mentioned.

Since the class of tetrads which is an indicator of adjacent-I disjunction (Type IV) was probably grossly under-counted in the sample, estimates of the relative frequency of the disjunction types are useless.

NON-RANDOM SEGREGATION IN T4637

Table 8 summarizes the complete counts of ascus types found in T4637, with all subtypes shown. Within Type III asci, all four subtypes should occur in equal frequencies, if segregation at Division I and II is at random with respect to the two ends of the ascus. While there is a slight bias in favor of the types with deficient spores basal in the ascus, it is not significant (P from X^2 between .30 and .05).

TABLE 8

FREQUENCIES OF ASCUS SUBTYPES IN T4637

ASCUS TYPE	SUBTYPE	SPORE ORDER	NUMBER COUNTED	ASCUS TYPE	SUBTYPE	SPORE ORDER	NUMBER COUNTED
I	none	++++	65	II	1	+A+B	25
IV	1	AABB	1		2	A+B+	20
	2	BBAA	0		3	+B+A	53
III	1	AB++	8		4	B+A+	27
	2	++BA	13		5	+AB+	28
	3	BA++	8		6	A++B	14
	4	++AB	10		7	+BA+	37
					8	B++A	38

Likewise, within Type II asci, all subtypes should occur in equal frequencies if segregation is at random. Asymmetrical (subtypes 1-4) and symmetrical (subtypes 5-8) distributions do occur with approximately equal frequencies (125:117, P from X^2 between .70 and .50). However, subtypes in which the two A-type spores are on the apical side of the Division I plane occur in about one-half the frequency of those in which the A-type spores are on the basal side of the plane of Division I (87:155, P from X^2 much less than .01). There is also a considerable excess of asci in subtype 3, as compared with similar subtypes (e.g., subtypes 4, 7 and 8).

Two possible explanations of the excess of subtypes 3, 4, 7 and 8 over subtypes 1, 2, 5 and 6 might be considered. First, there could have been selection of a non-random sample. This does not seem probable, for the following reasons: (1) none of the types is more easily classifiable than any other; (2) all types contain the same kinds of spores, differing only in arrangement, and would be expected to break with equal ease, so that non-random breakage is probably not effective, and (3) the non-randomness of the two classes of asci appears in all three samples (three separate slides) and in about the same amount in each.

The other possibility is that of some kind of directed orientation of the translocation complex on the Division I spindle, or of directed segregation at first anaphase. Mechanisms which might accomplish the latter are obscure. Orientation of the complex on the spindle might conceivably be affected by the spatial relationships of the chromosomes at the time of synapsis; thus a particular portion of the

complex might tend to lie toward the apical end or basal end of the ascus. This, in turn, could conceivably be related to the spatial orientation of the two parental nuclei; if one type of nucleus (e.g., the one carrying the translocated chromosomes) usually lay apical to the other at the time of fusion, then the chromosomes carried by it might maintain this orientation through the time of segregation at first anaphase. The relative positions of the parental nuclei might in turn be related to the manner in which the cross was made.

While the data are such as to strongly suggest a non-random segregation, further counts are needed to firmly establish the reality of the phenomenon. A comparison of data from the cross Chilton a x T4637A (Chilton a used as the protoperithecial parent) and the reciprocal, T4637A x Chilton a (Chilton a used as the conidial parent), should give some indication of the pertinence of the conjectures made in the preceding paragraph (i.e., one would expect, on the basis related above, that the distributions in the two crosses would be the reverse of each other).

A comparable case of non-random (polarized) segregation in ascomycetes has been analyzed by Catcheside (1944). The mechanisms involved in this case are not understood. The ascomycetes offer favorable material for the detection of such behavior because of the persistent order of the meiotic products and because of the distinctive ascus ends.

SUMMARY

Translocation heterozygotes in _N. crassa_ may produce as many as

six distinct types of asci, characterized by the nature and position of the spores contained. These types are related to crossing-over and disjunction in the heterozygous fusion nucleus. Equations were derived by means of which it is possible to calculate the frequency of effective exchanges (i.e., single and 3-strand double exchanges) in the two interstitial segments of a translocation. With accurate data on the frequencies of the various types of asci, it is also possible to estimate the distribution of exchanges between singles and doubles, and to estimate the frequency of alternate and adjacent-I disjunction. The frequency of adjacent-II disjunction is obtained directly from the frequency of one ascus type (Type VI), but possibly also includes other phenomena, such as trisomic segregation.

In T45502, from analysis of the data of McClintock (1945), crossing-over occurs in only one interstitial segment. Within this segment, either 100% of the tetrads have a double exchange or 44% have a single exchange and 10% a double exchange. It is not possible to differentiate between the two alternatives. In tetrads which have had no exchange or a 2- or 4-strand double exchange in the segment, alternate and adjacent-I disjunction are about equally frequent; adjacent-II disjunction occurs in about 11% of the total asci produced.

In T4637, the available data probably represent a highly non-random sample of the ascus population. On the basis of the data, effective exchanges in one segment occur either in 90% or 81% of the tetrads, and in the other segment in either 25% or 18% of the tetrads. The equations for calculation of the distribution of exchanges between singles and doubles, and for calculation of disjunction frequency are useless, pro-

bably because the data are heavily overweighted with cross-over ascus types. Adjacent-II disjunction occurs in an estimated 15-20% of the tetrads.

In Type II asci of T4637, a non-random distribution of subtypes was found. Types with a particular deficient spore (A-type spore) on the apical side of the first division plane occurred in approximately half the frequency of the alternative types, in which the A-type spore was on the basal side of the first division plane. The difference was highly significant. It is thought that the difference is not due to possible non-randomness of the sample, but rather to some type of directed orientation of the translocation complex on the first division spindle or to directed segregation at first anaphase.

PART EIGHT. SUMMARY

Since the observations presented in each section of the paper have been summarized at the end of that section, no detailed summary of the entire paper will be presented.

The literature pertaining to the nuclear cycle in the life history of Neurospora is reviewed.

Stocks and culture methods used in the study are described. Several modifications of the squash technique were tested; those which were most useful are summarized as schedules of treatment.

The morphology of the mitotic and pachytene chromosomes is described and diagrammed. A correspondence between the centromeres of the mitotic chromosomes and the heavy chromomeres of the pachytene chromosomes is suggested.

A detailed description of the chromosome cycle during the development of the ascus and the formation of ascospores is presented. Points of special interest in this cycle are discussed, including the problem of synapsis, chiasma frequency, and the behavior of the nucleolus and of the centriole.

A number of chromosome abnormalities are described. A gene present in the Abbott strains and in certain of their derivatives causes a high degree of spore abortion when present in the homozygous condition in the

ascus. The cytological basis of the abortion appears to lie in a generalized "stickiness" of the chromosomes which results in anaphase bridges and in hypo- and hyperploid spore complements, as a consequence of chromosome non-disjunction. The three translocations found by McClintock (1945) and two others found by the writer are described and identified, in some cases tentatively, as to the chromosomes involved. The known correlations between chromosomes and linkage groups are summarized.

The spore types and ascus types resulting from crossing-over and disjunction in a translocation heterozygote are described. Equations were derived by means of which, given the frequencies of the various types of asci, it is possible to calculate the frequency of effective exchanges in the two interstitial segments of a translocation, to estimate the distribution of single and double exchanges, and to estimate the relative frequencies of the various types of disjunction. The validity of the results obtained through the use of the equations, and the limitations of the equations are discussed. The equations are applied to data on ascus type frequencies in T45502 and T4637. An indication of non-random segregation in the translocation T4637 following a single or 3-strand double exchange in a single interstitial segment was found. A possible mechanism is suggested.

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EXPLANATION OF FIGURES 9 - 24.

Note: All photographs are of aceto-orcein preparations, unless otherwise designated.

Fig. 9 -- Diagrammatic representation of the life cycle of _N. crassa_, after Beadle (1946a).

Fig. 10 -- (a) Ascogenous hyphae (croziers) in prophase of the conjugate mitosis. x2400

(b) At right, two croziers in prometaphase and metaphase of the conjugate mitosis; center, paraphysis cell with several small, compact nuclei; at left, ascus in early stage of synapsis. Cell shapes distorted in flattening. x2700

(c) Left, crozier at metaphse of the conjugate mitosis; right, ascus in pre-fusion stage. Both cells stem from a common base. x1800

(d) Group of young asci. Upper right, pre-fusion ascus, with nucleus of tip cell apparently in process of moving back into basal cell; center, stages of synapsis; left, early pachytene. x1800

(e) Left and right of center, two croziers in telophase of the conjugate mitosis, spindles differentiated after HCl hydrolysis, followed by cytase digestion. Center, paraphysis cell. x2400

Fig. 11 -- (a) Ascus in pre-synaptic stage, with 14 condensed, unpaired chromosomes, shape undistorted. x1800

(b) Ascus in (a), retouched to clarify chromosome re-

lationships.

(c) Pre-synaptic ascus with 14 condensed, unpaired chromosomes, flattened after cytase digestion. x2400

(d) (c), retouched (d is rotated 180° with respect to c).

(e), (f) Upper and lower focal levels of pre-synaptic ascus. x2400

(g) Ascus in (e) and (f), retouched to show all chromosomes in composite figure.

(h) Camera-lucida drawing of ascus in pre-synaptic stage. The two nuclei are fused in the central region of overlap, but still retain their individual outlines. Note grouping of 14 chromosomes into two sets of seven. x1790

(i) Camera-lucida drawing of ascus in slightly later stage. x1790

(j) Camera-lucida drawing of early synapsis. Seven completely paired, but still much condensed bivalents. Terminal portion of ascus broken off. x1790

(k) Camera-lucida drawing of ascus with seven bivalents, condensed. Note slight asynapsis at one end of chromosome 1 (longest) and at satellite end of chromosome 2 (on nucleolus). x1790

(l) Ascus much flattened after cytase digestion; chromosomes in early pachytene, about twice as long as in (c), but still considerably condensed. x2400

(m) Ascus in (l), retouched.

(n) Ascus in early pachytene, shape undistorted. Note asynapsis of end of chromosome 1 (at 9 o'clock). x1800

NOTE: All subsequent pachytene figures are from crosses between standard strains, unless otherwise designated.

Fig. 12 -- (a) Pachytene, showing chromosome 7 at lower left. Note chromomere pattern, and characteristic "spear-end" at 6 o'clock. Lacto-orcein. x3600

(b) Pachytene, Chilton a x 5936A; translocation configuration at right of nucleolus. x1800

(c) (b), retouched.

(d-f) Three focal levels of pachytene, showing chromosome morphology. x2700

(g) Composite diagram of (d-f). Note heavy b chromomeres (nucleolus organizer?) at nucleolus end of chromosome 2; heavy a chromomeres and heterochromatic region at loop of chromosome 1.

Fig. 13 -- (a-c) Three focal levels of pachytene. x2700

(d) Composite diagram of (a-c). Note a chromomeres near nucleolus end of chromosome 2; a chromomeres and heterochromatin of chromosome 1.

(e-g) Three focal levels of pachytene. x2700

(h) Composite diagram of (e-g). Note a and b chromomeres on chromosome 1; chromosome 5 is stretched, as is dotted region of chromosome 7.

Fig. 14 -- (a-c) Three focal levels of pachytene. x2700

(d) Composite diagram of (a-c). Note tiny satellite on chromosome 2 (Fig. 14a), just above b chromomere.

(e) Pachytene. x2700

(f) Diagram of (e). Note chromosome 1, with a chromomeres.

Fig. 15 -- (a-b) Two focal levels of pachytene. x2700

(c) Composite diagram of (a-b). Note a chromomeres on chromosome 1; a and b chromomeres on chromosome 2.

(d-e) Two focal levels of pachytene. x2700

(f) Composite diagram of (d-e). Note a and b chromomeres on chromosome 1, with heterochromatic regions.

Fig. 16 -- (a-b) Two focal levels of pachytene. x2700

(c) Composite diagram of (a-b). Note chromosome 1.

(d) Diplotene. x2400

(e-f) Early diakinesis, from cold-treated material; two focal levels. Nucleolus is at 6 o'clock. x2700

(g-h) Two focal levels, mid-diakinesis, from cold-treated material. Nucleolus at 6 o'clock. x2700

(i) Composite diagram of (g-h).

(j) Diakinesis, Chilton a x 5936A; five bivalents and a quadrivalent. x2400

(k) Diakinesis, Chilton a x 5936A; five bivalents and a ring-of-four. x2400

(l) Diagram of (k).

Fig. 17 -- (a-b) Two focal levels, diakinesis in Chilton a x 5936A; five bivalents and a ring-of-four. Note chromosome 2 at the nucleolus (6 o'clock). x2400

(c) Late diakinesis, Chilton a x 45502A; five bivalents and a quadrivalent. x1800

(d) Metaphase of first meiotic division; seven bivalents. Nucleolus at 3 o'clock. x2400

(e) Early anaphase of Division I; 14 dyads. x2400

(f) Mid-anaphase of Division I; note differentiation of spindle after HCl hydrolysis. x2400

(g) Mid-anaphase of Division I. x1800

(h) Telophase of Division I. x2400

Fig. 18 -- (a) Metaphase, side view, of Division II; note aberrant orientation of upper spindle. x1800

(b) Polar view of metaphase, Division II; seven typical dyads in each nucleus. x2400

(c) Metaphase of Division II; arrow points to nucleolus from Division I, still present between the two second division nuclei. Carmine, followed by orcein. x1800

(d) Second division spindles at anaphase. Typical orientation. x1800

(e) Telophase nuclei of second division; arrow points to persistent nucleolus left at old equatorial plate. Carmine plus ferric acetate. x1800

(f) Ascus with interphase nuclei, following Division II. Note heterochromatic region around congressed centromeres. Typical orientation of sister nuclei. Tip of ascus is differentiating ring through which ascospores are later expelled. x1800

Fig. 19 -- (a) Sister nuclei at interphase after Division II; note heterochromatin at centromere regions. Chromosome arms show some coiling. x2400

(b) Early prophase of third division; centrioles (at c') about half-way arougd the nucleus toward their metaphase position. x1800

(c) Prometaphase of Division III. x2400

(d) Diagram of (c). Note small nucleolus on chromosome 2; chromosome 2 is less condensed than the rest of the complement.

(e) Third prometaphase. x1800

(f) Diagram of (e)

(g) Four third division spindles, side view, in the ascus. Note centrioles (dark rods) at poles of spindles. x900

(h) Three Division III spindles, side view; anaphase separation just begun. x900

Fig. 20 -- (a) Same ascus as Fig. 19h. x1800

(b) Ascus in telophase of Division III, much flattened after cytase digestion. Note eight nuclei, in two rows; second pair from top were oriented in plane at right angles to the rest. x1800

(c) Ascus in interphase following third division; only four nuclei shown. Note that all nuclei are now arranged in single row, with centromere regions oriented toward ascus wall. Darker area between nucleus and wall is centriole, in face view. Small nuclei to left of ascus are from paraphyses; note heterochromatin at centromere side of each nucleus. x2400

Fig. 21 -- (a-c) Three focal levels of ascus following Division III; spores are just starting to cut out. Note triangular centriole at top of ascus; third nucleus from top has centriole on edge; others are in intermediate positions. x900

(d) Ascus in which spores have just been cut out; note heterochromatic region at centromere side of each chromosome group. In lower-most nucleus, this is clearly bilobate. x1800

(e) Early prophase of Division IV in spores; much flattened after cytase digestion. Note relic orientation of centromere regions. x2400

Fig. 22 -- (a) Prometaphase of Division IV in spores; marked relic orientation of chromsomes. x2400

(b) Prometaphase of Division IV in spore. x2400

(c) Diagram of (b). Note tiny mucleolus in chromosome 2.

(d) Metaphase, polar view, of Division IV, x2700

(e) Diagram of (d).

Fig. 23 -- (a) At left, telophase of Division IV; note spindles differentiated by HCl hydrolysis. At right, binucleate spores, showing centromere heterochromatin, and sister-nuclei orientation. x1800

(b) Telophase of Division IV in ascus with eight spores. All sixteen nuclei are shown in this ascus, much flattened after cytase digestion. Spore at 3 o'clock is in late anaphase. x1800

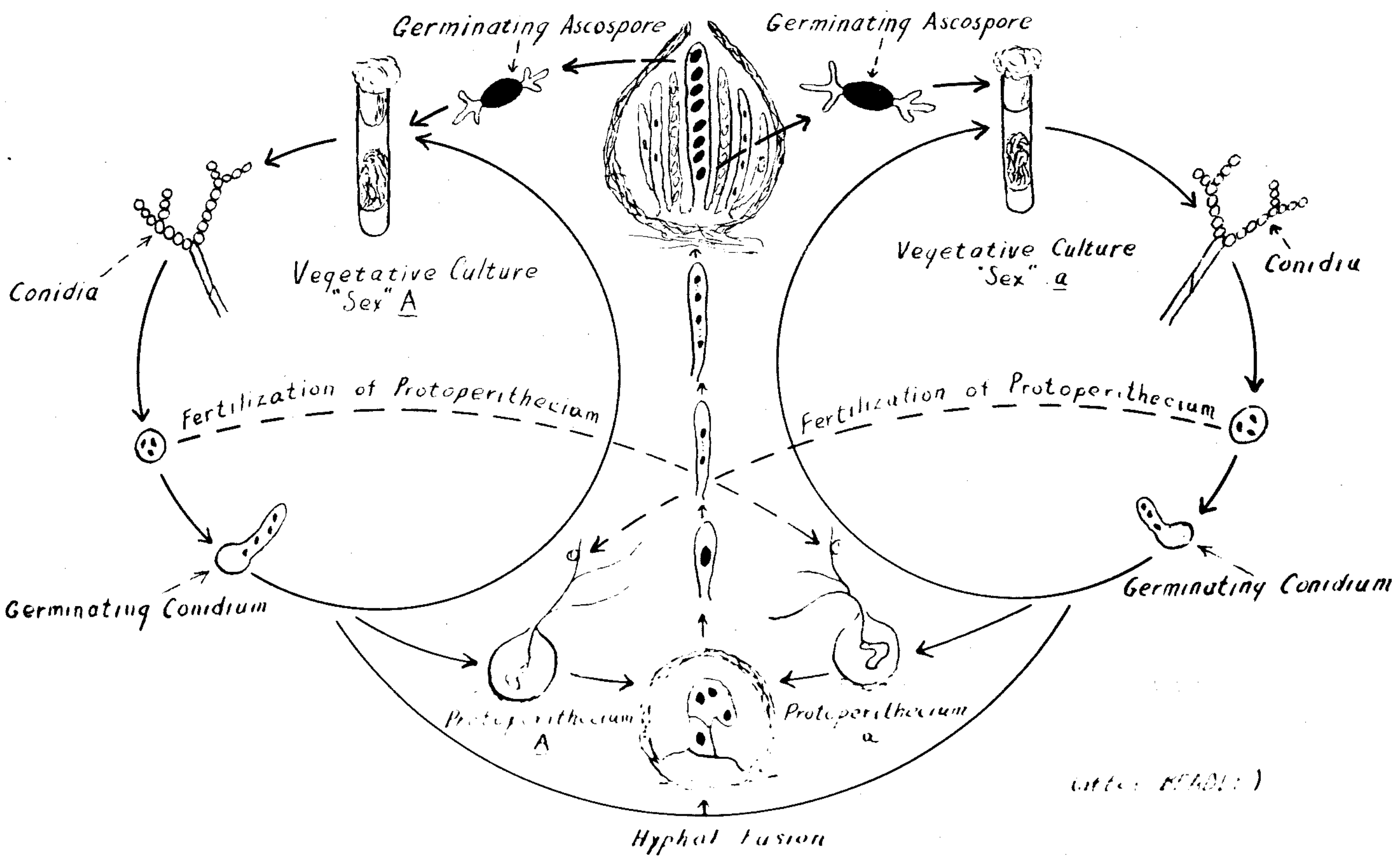
(c) Binucleate spores, showing heterochromatin and sister-nuclei orientation. Lower ascus illustrates differential maturity of spores; spores at left are maturing ahead of those at right. x1800

Fig. 24 -- Camera-lucida diagrams of pachytene configurations in translocation heterozygotes.

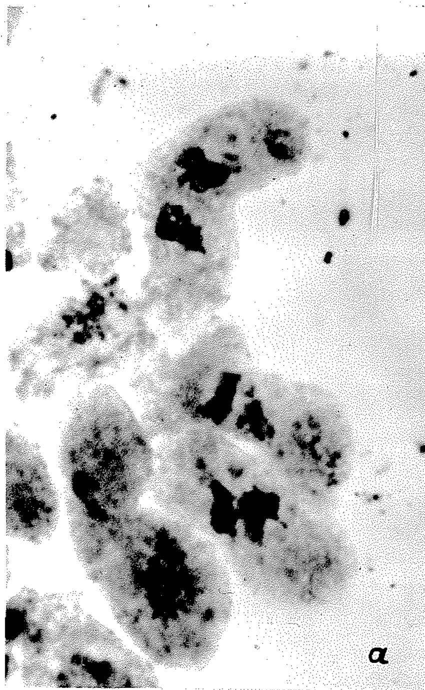
(a) Chilton a x 45502A. x4000

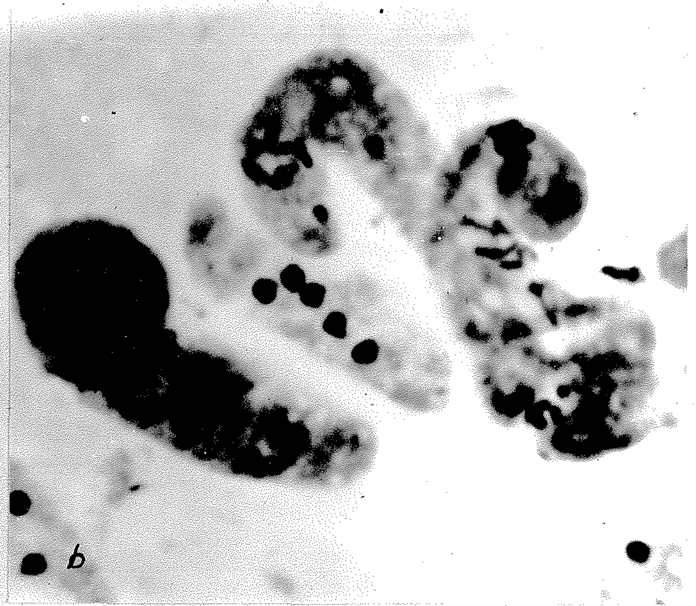
(b) Em 5297a x 4637A. x3600

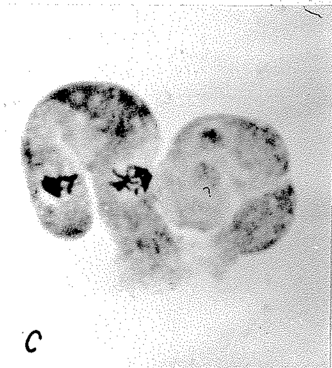
(c-f) Em 5297a x 5936A. (c) Note asynapsis. x4000 (d) x2950 (e) x4000 (f) x4000 (cf. Fig. 12b)

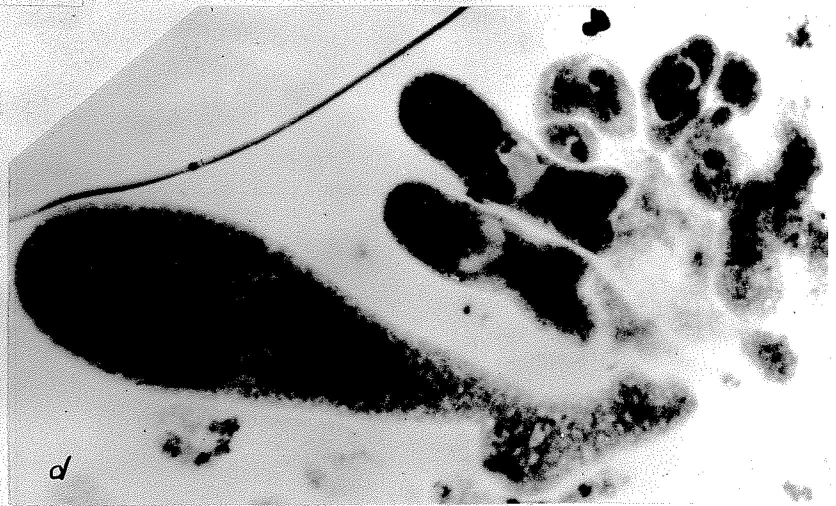


LIFE CYCLE OF NEUROSPORA CRASSA
FIGURE 9









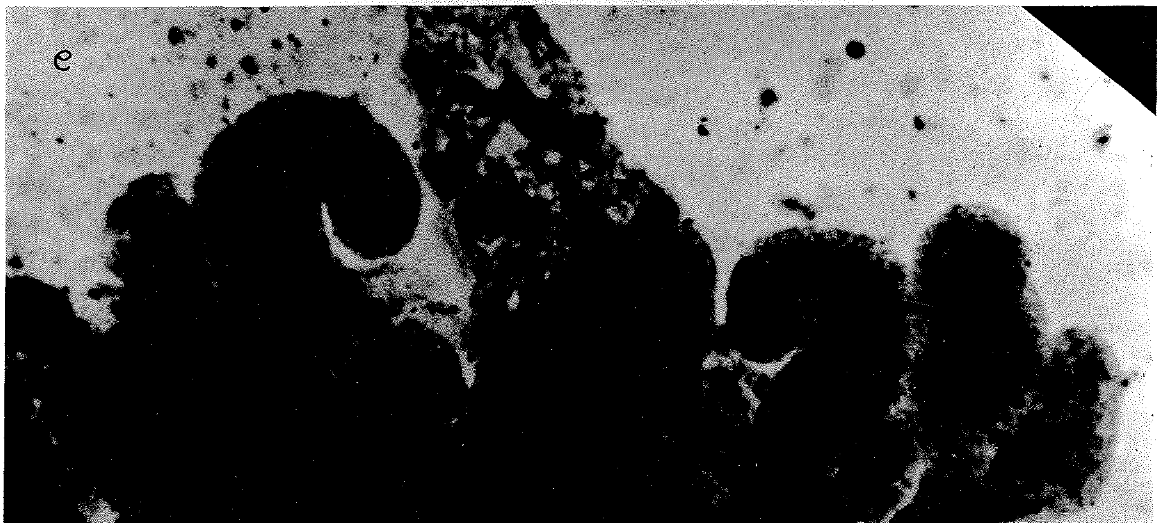


FIGURE 10

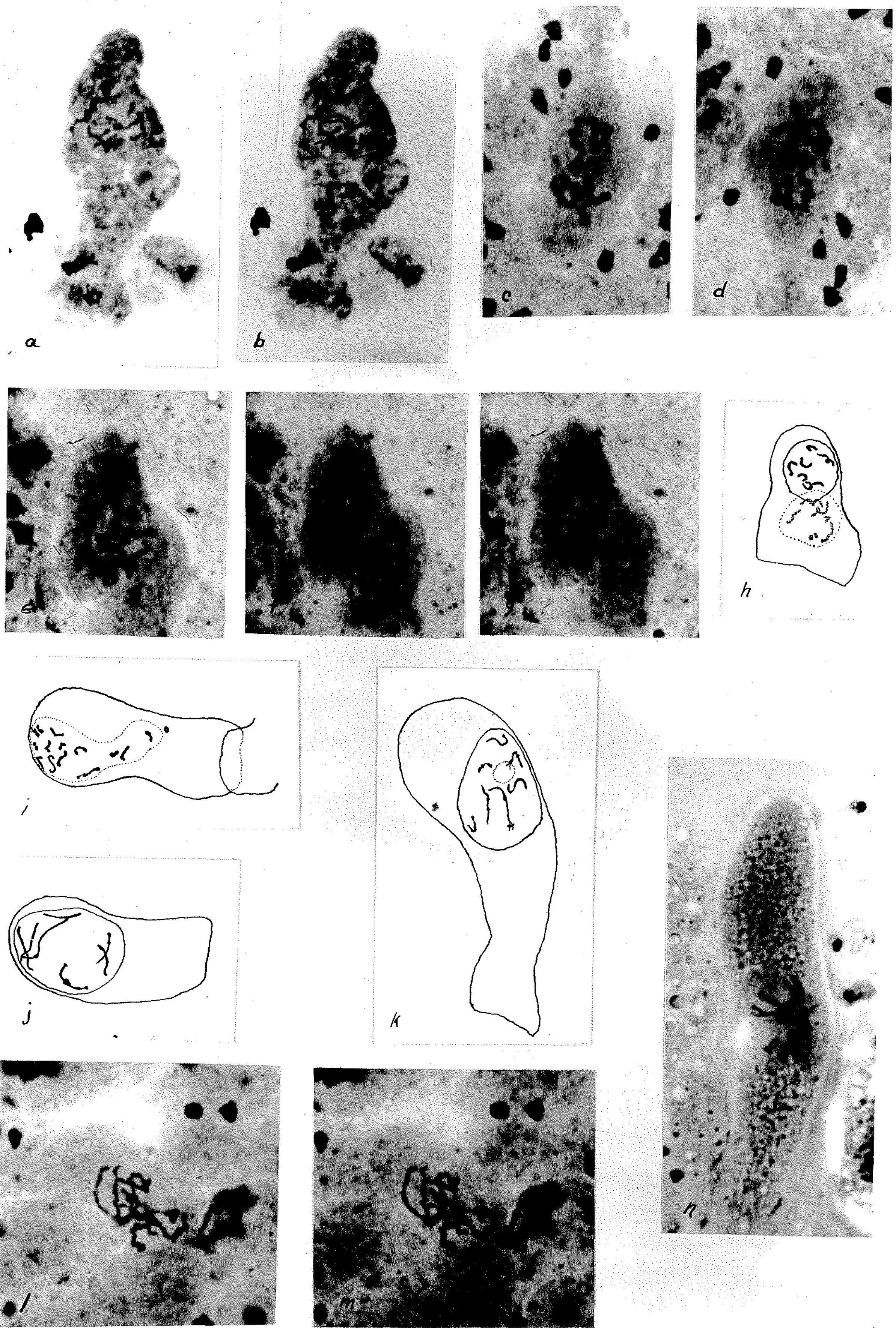
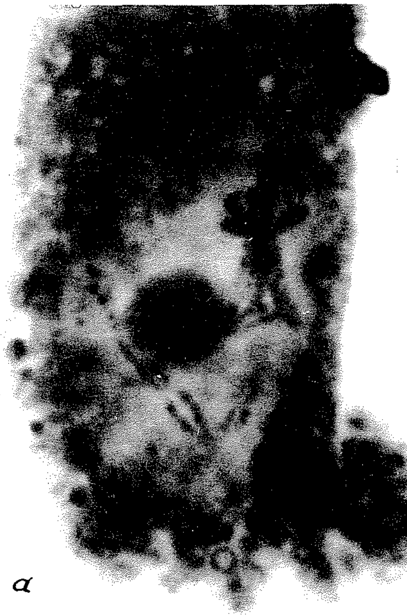
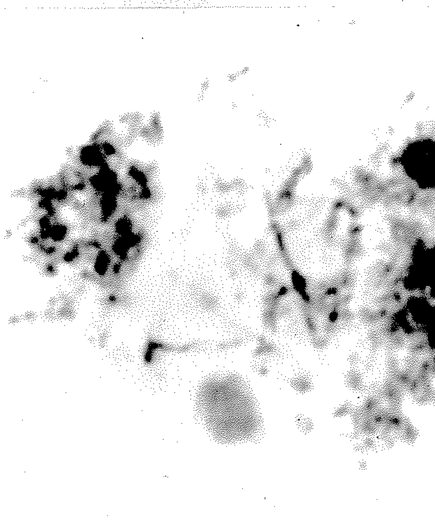
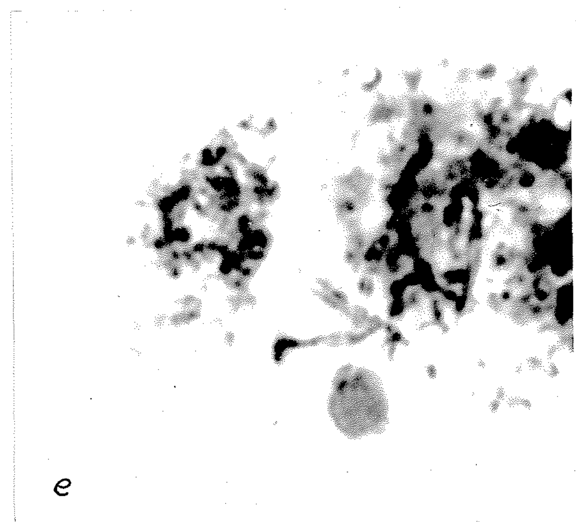
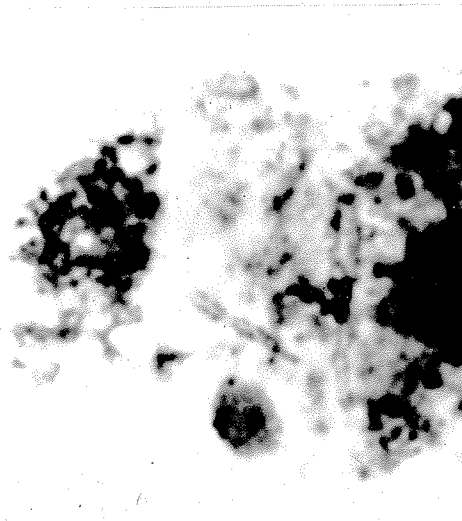


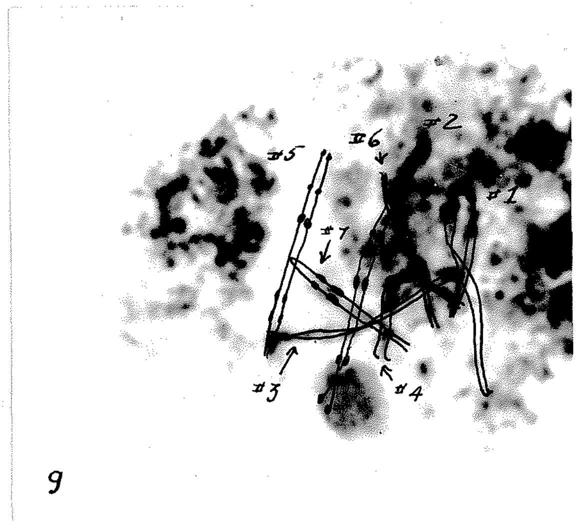
FIGURE 11











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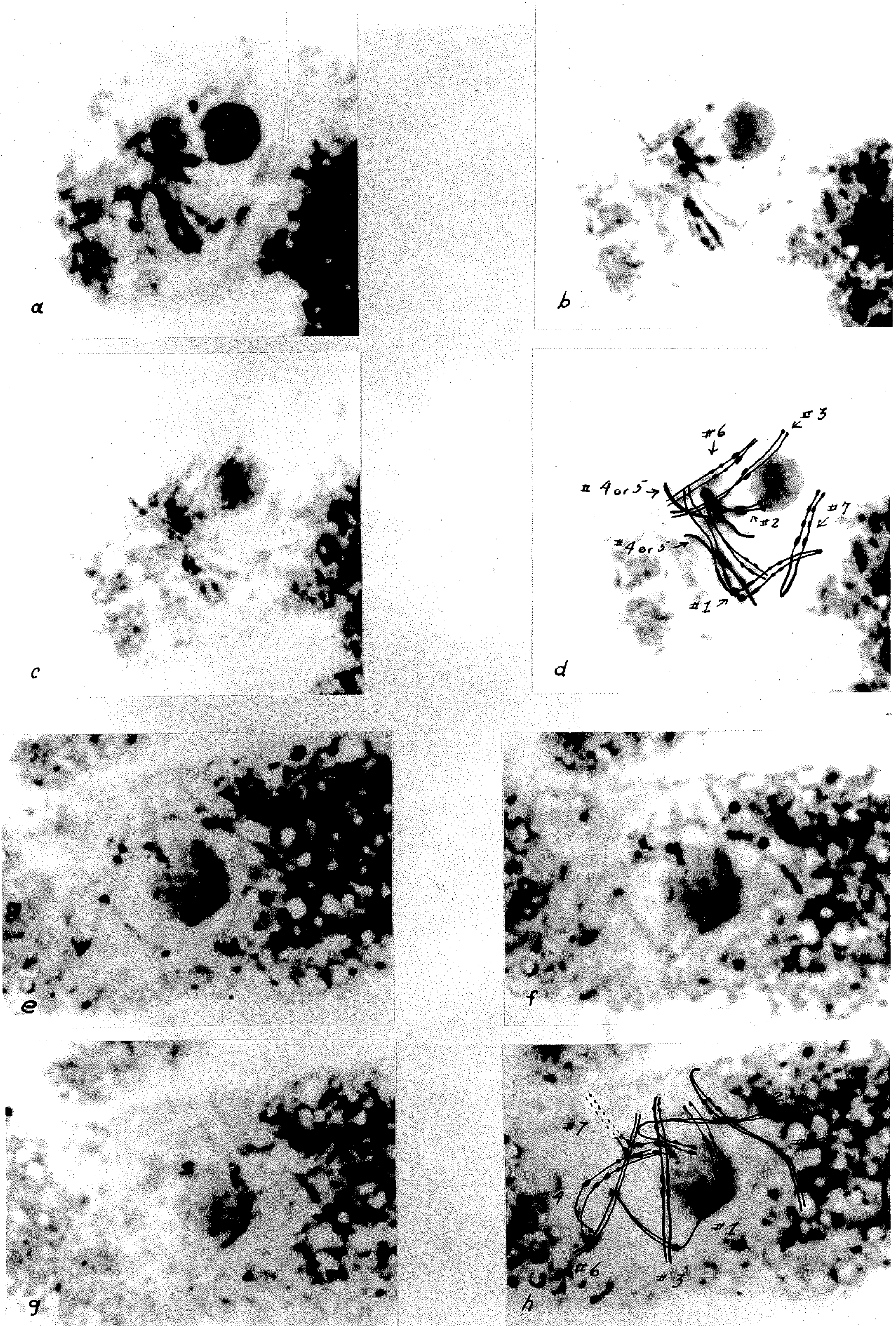
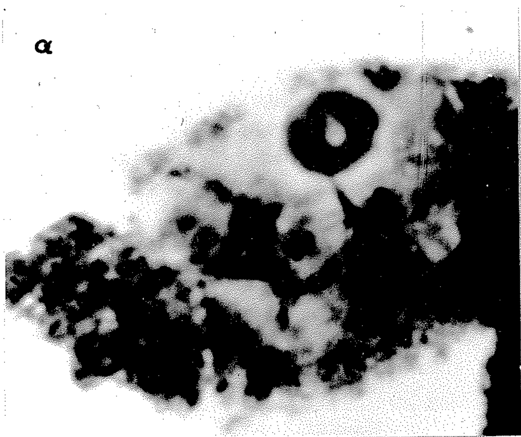
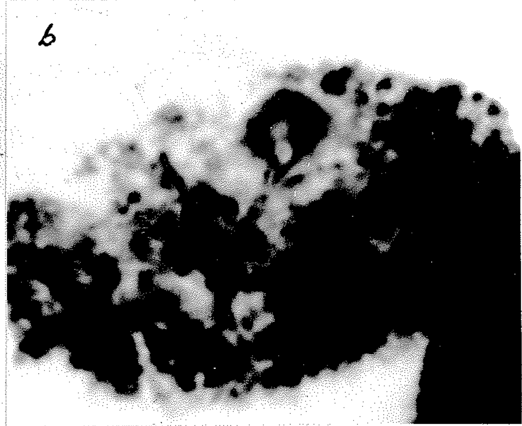
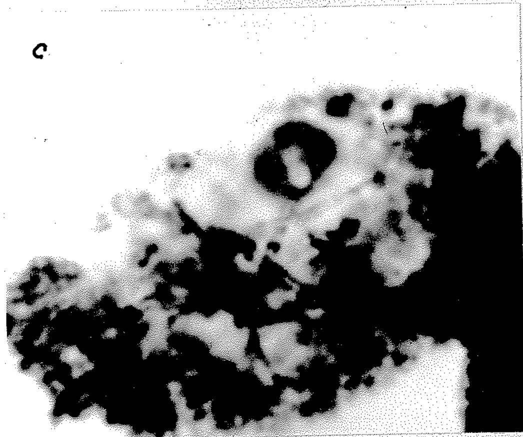
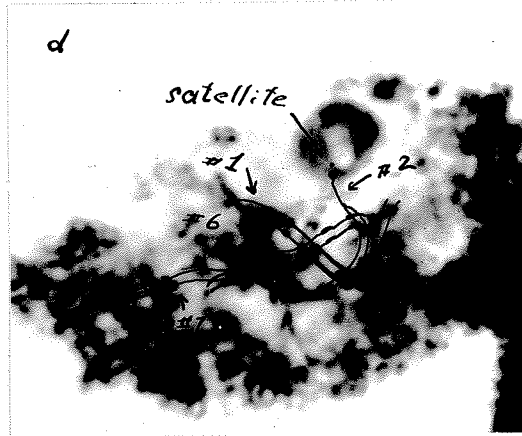


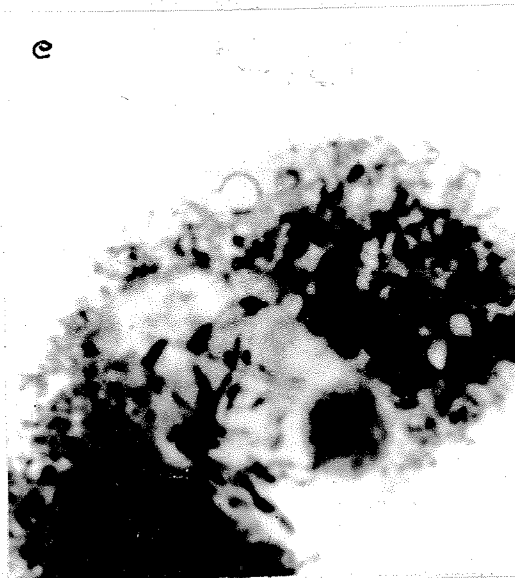
FIGURE 13











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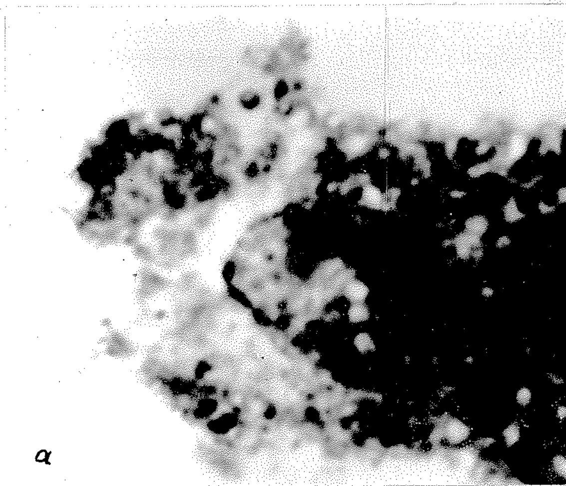

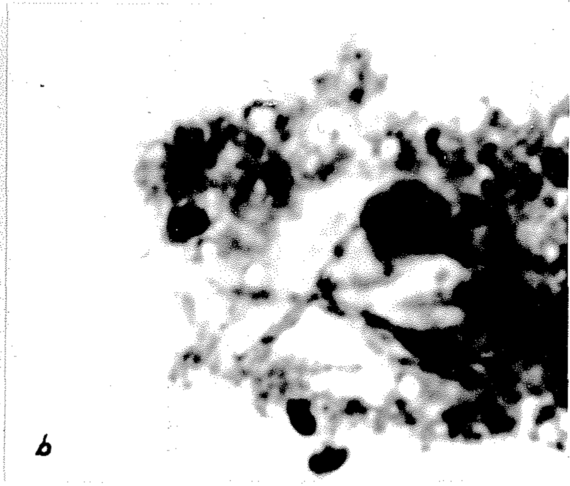

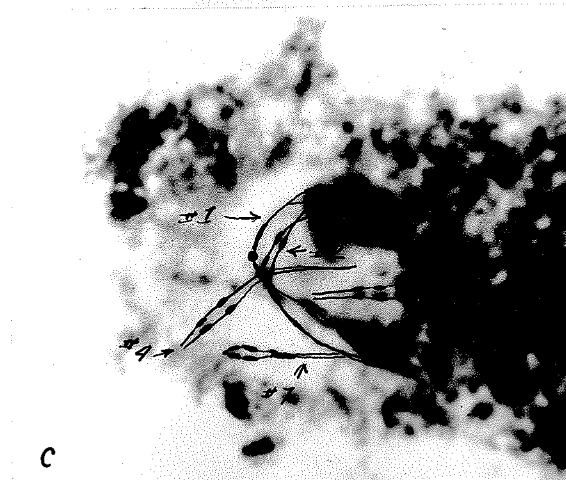

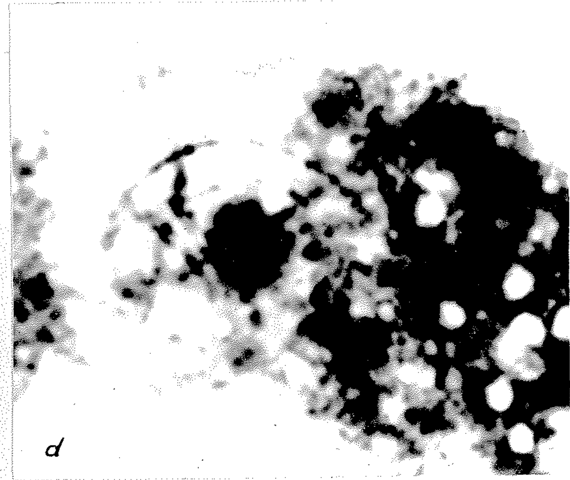

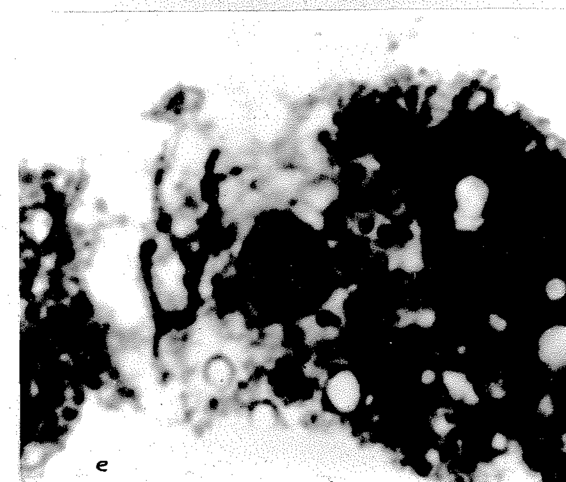

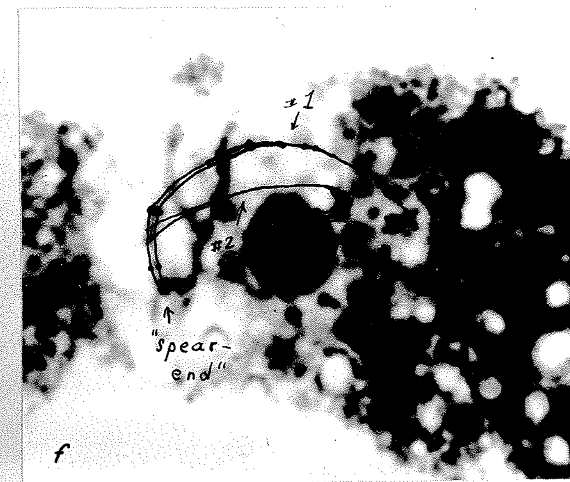

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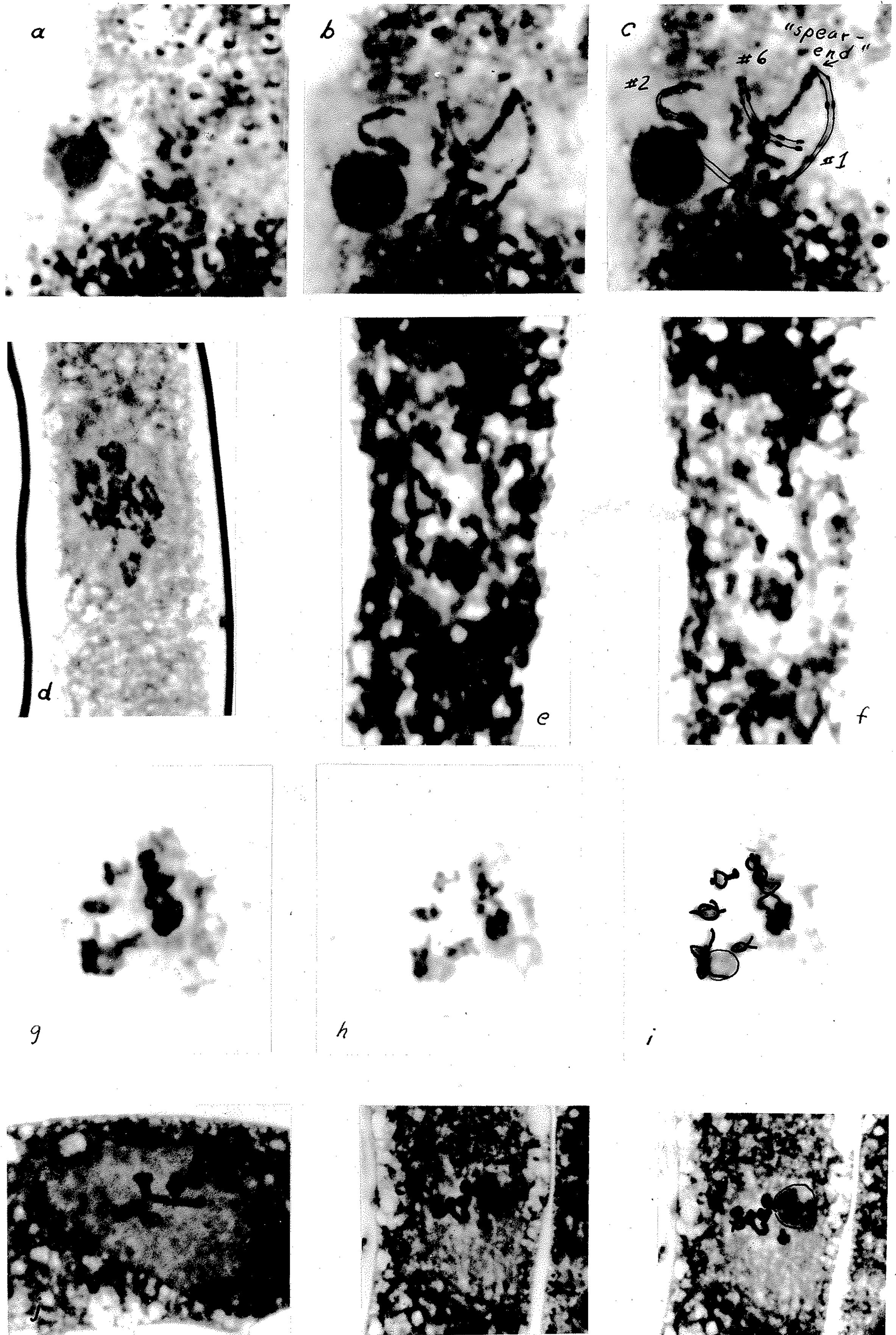


FIGURE 16

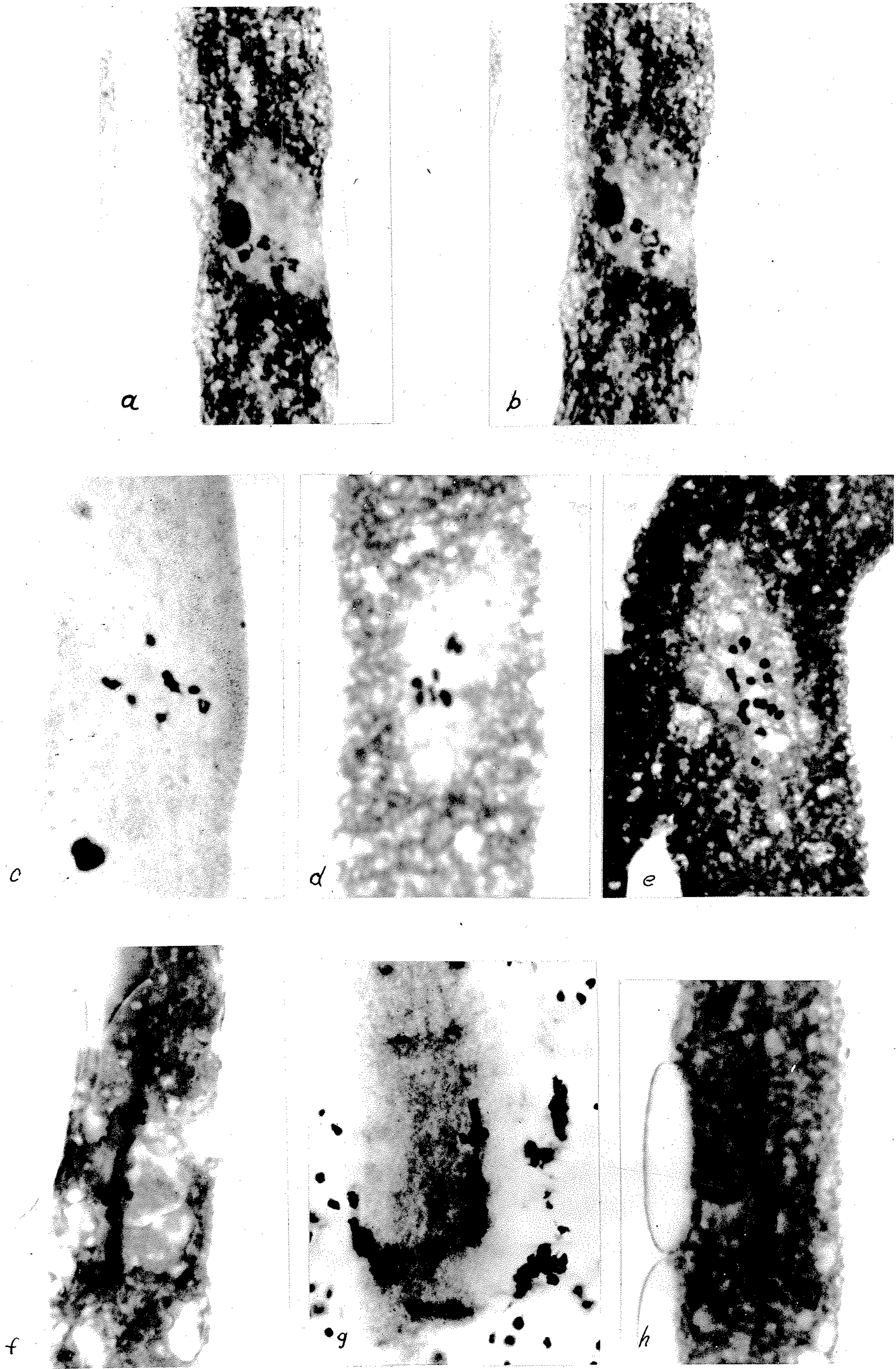
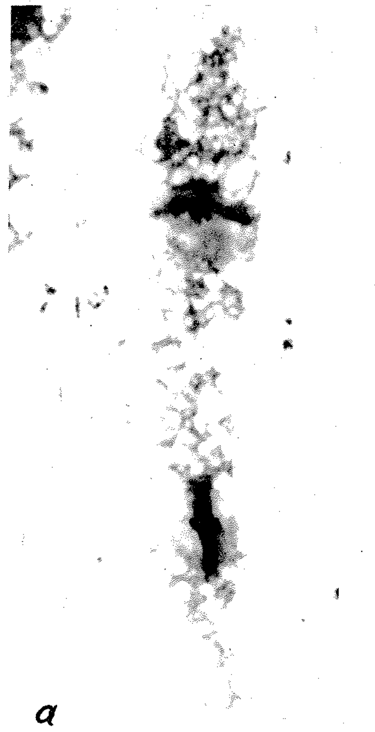
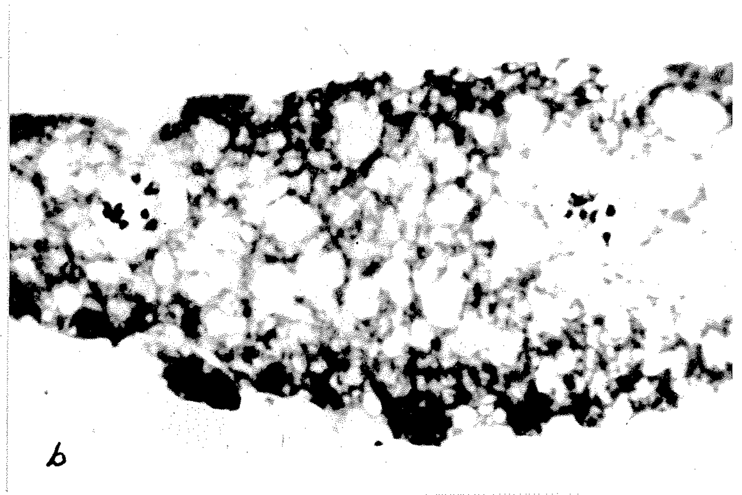
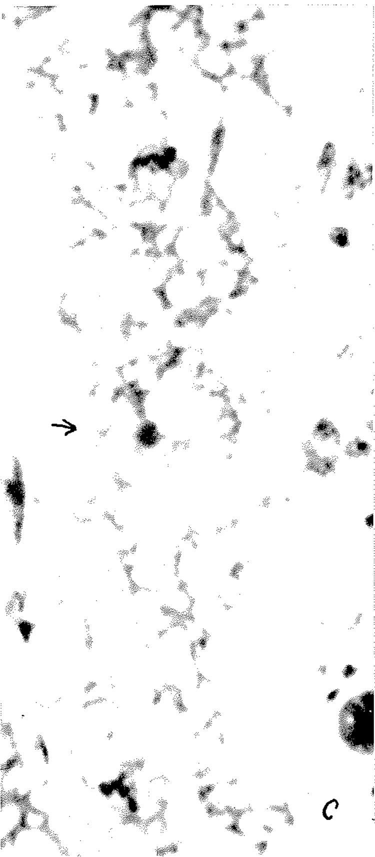
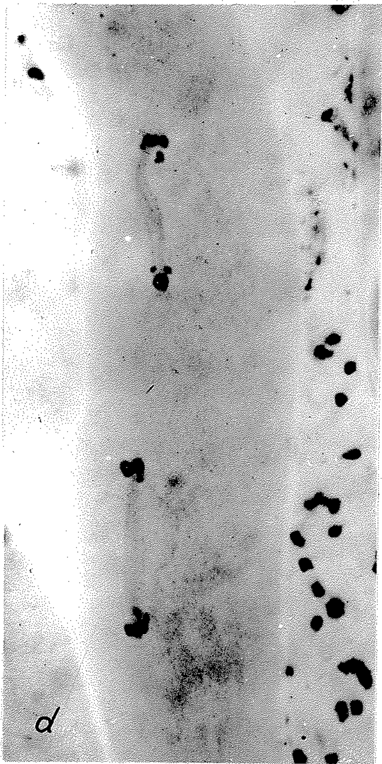


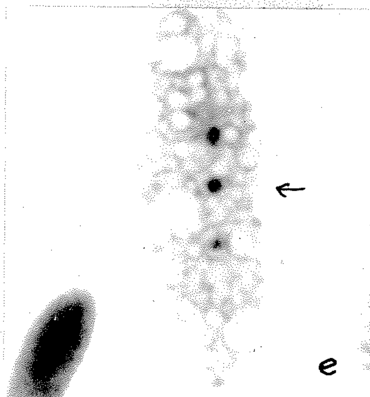
FIGURE 17











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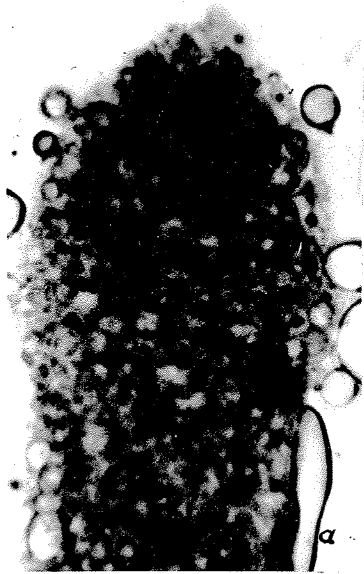
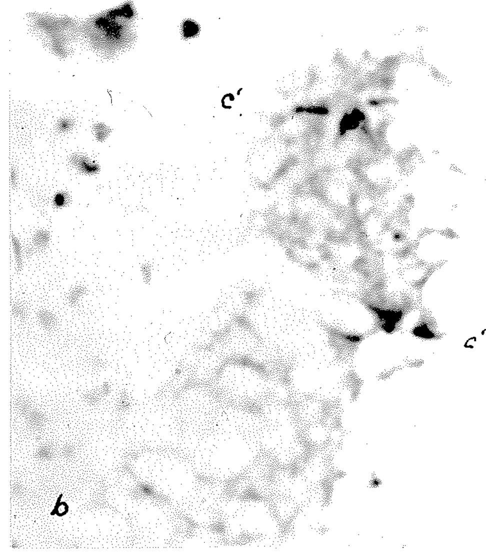
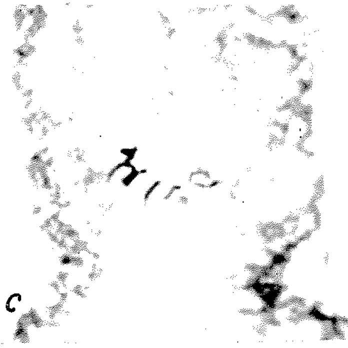
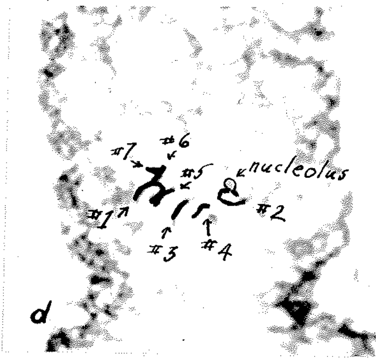
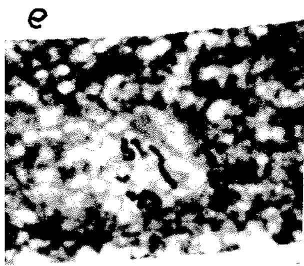
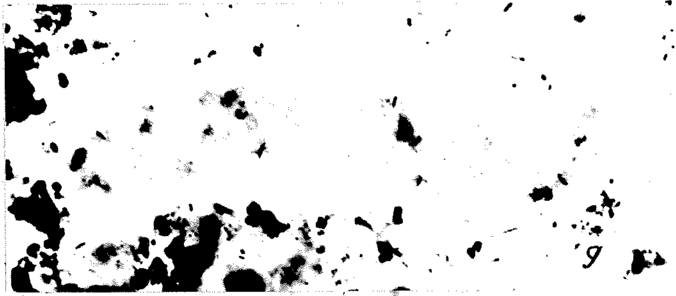


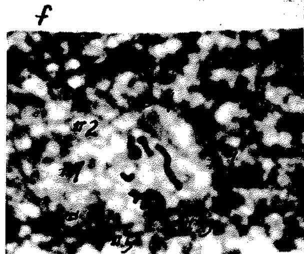












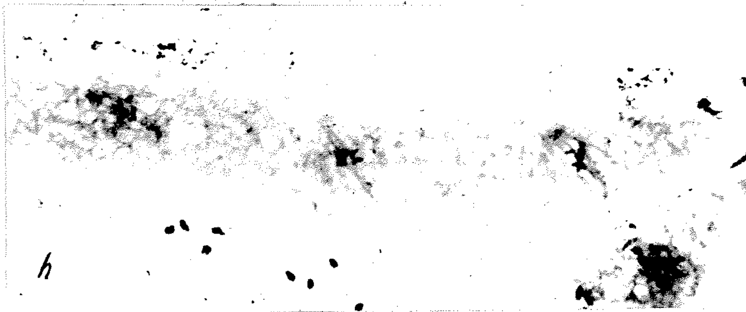
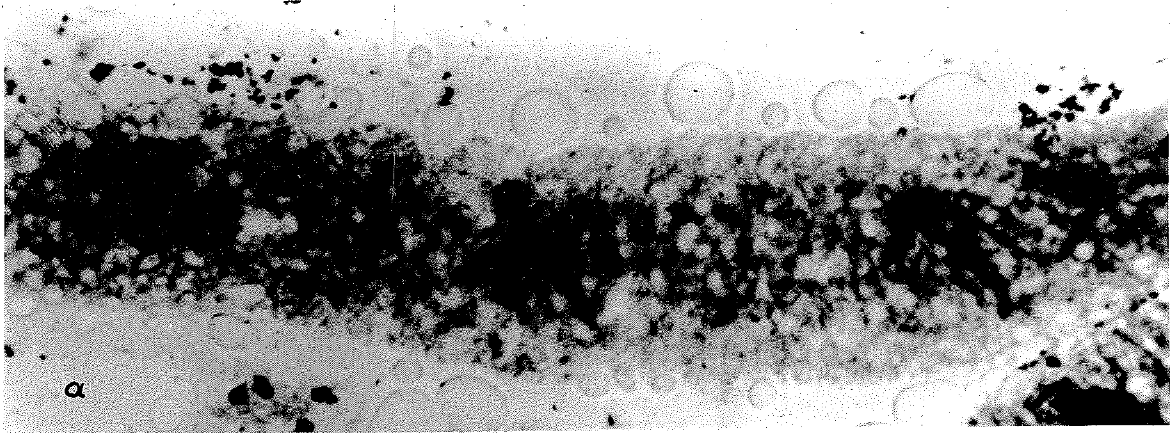


FIGURE 19



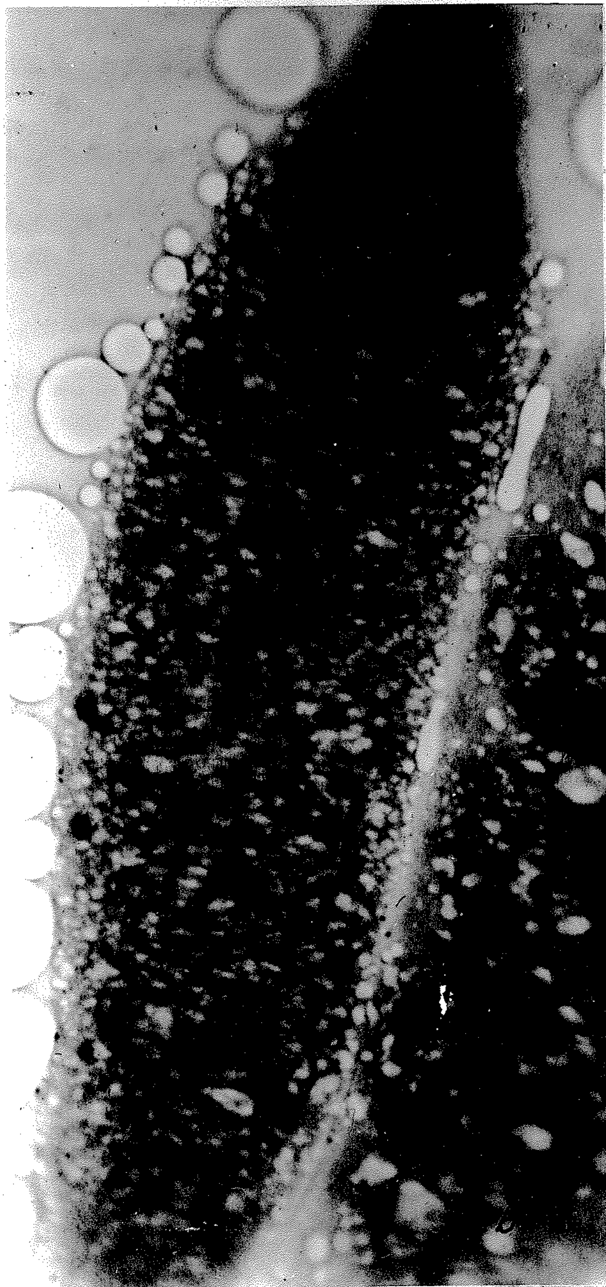
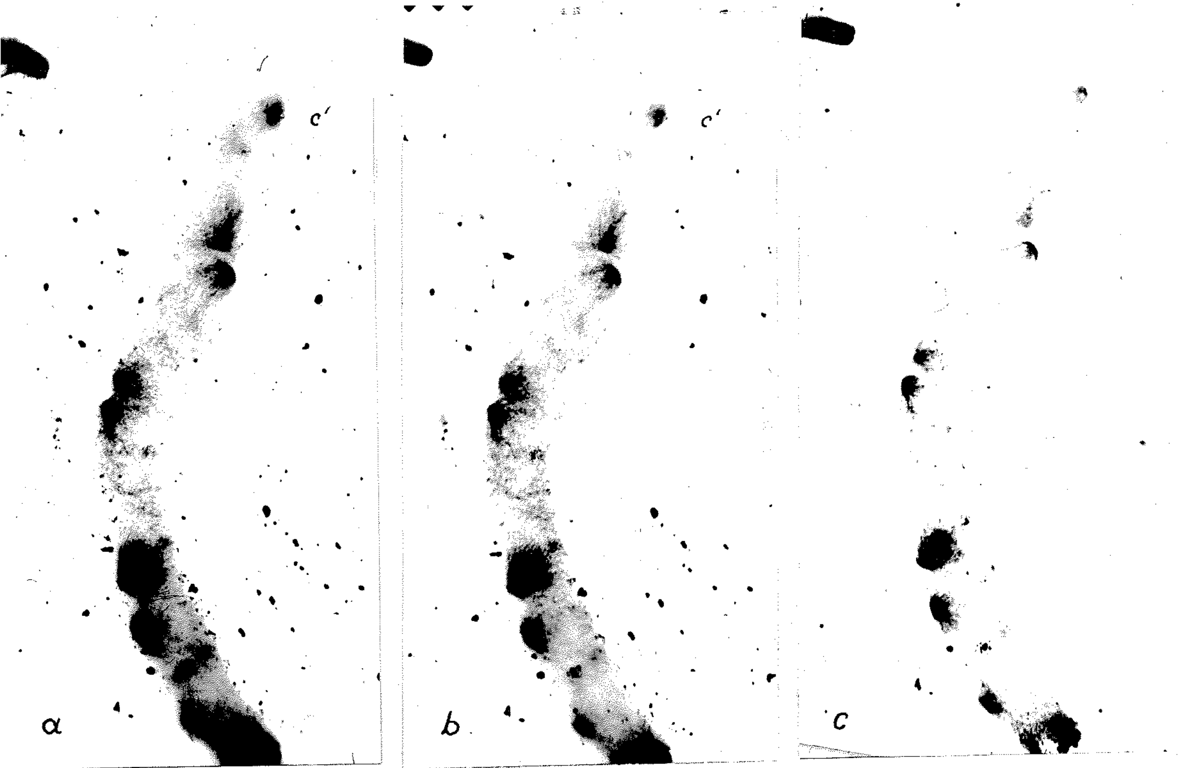
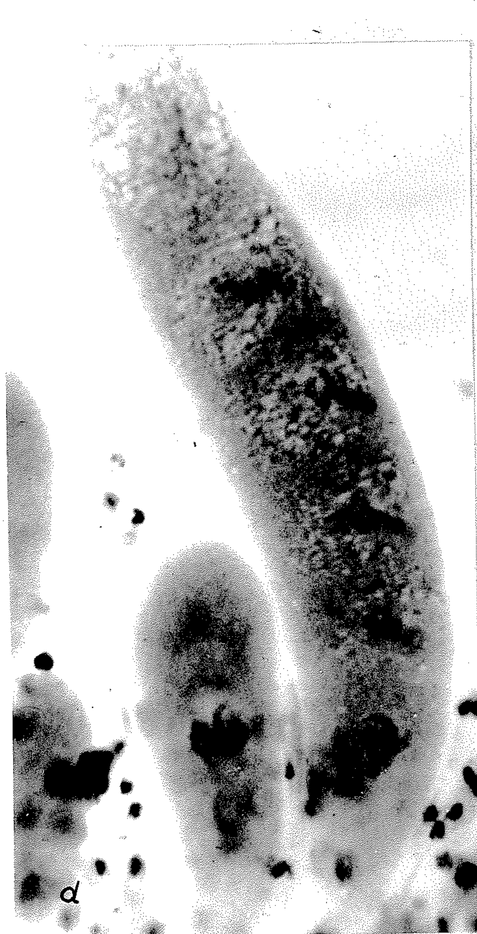


FIGURE 20





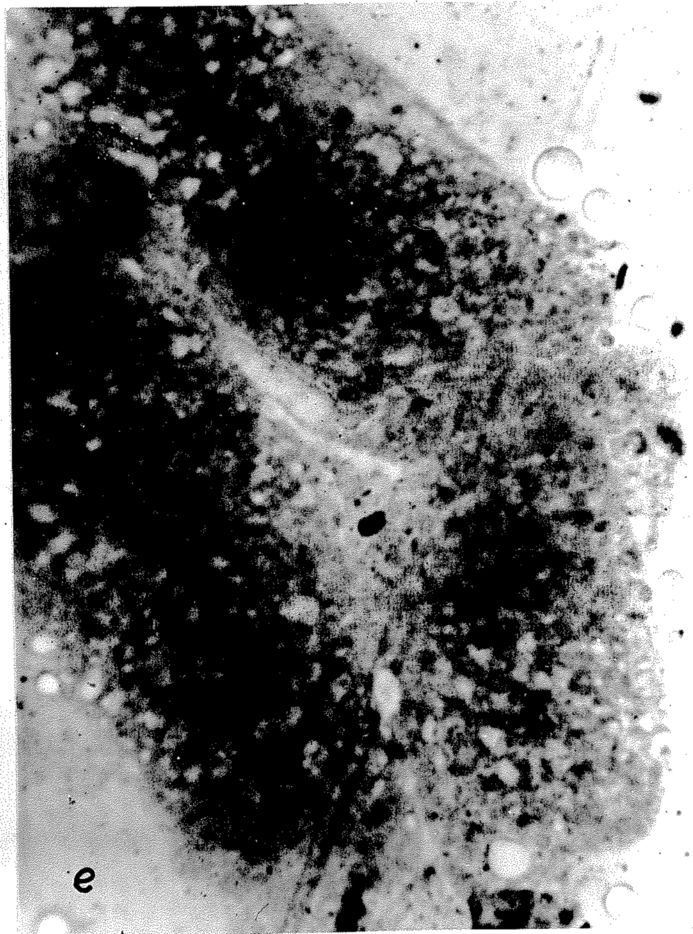
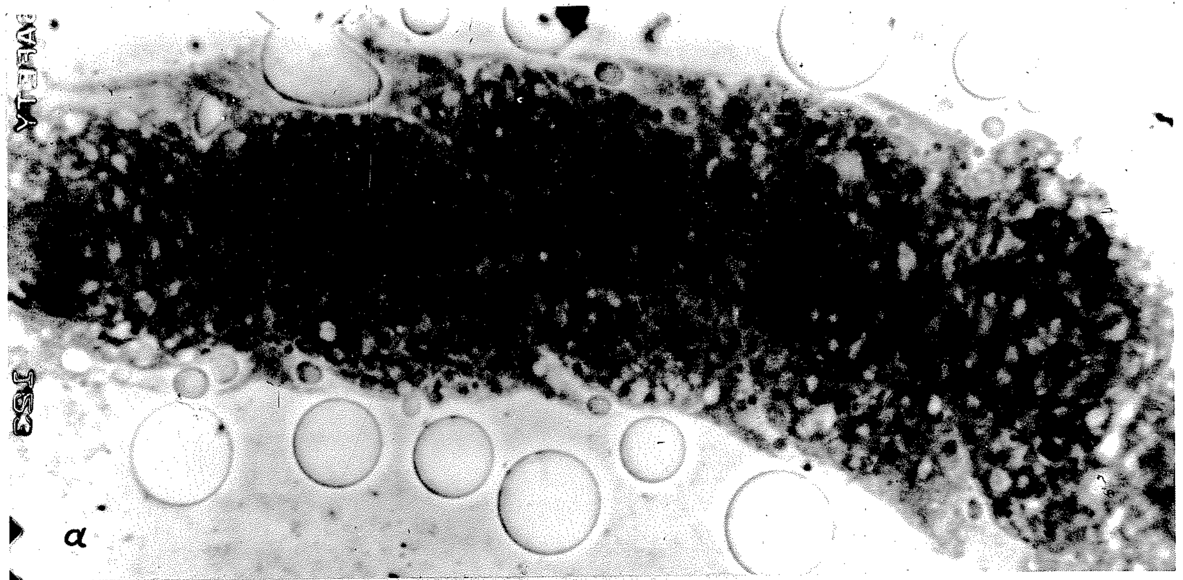
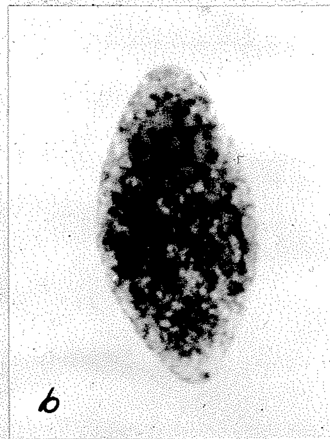
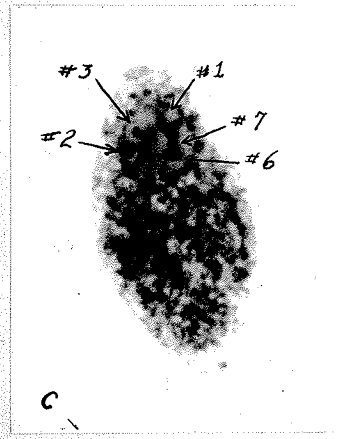
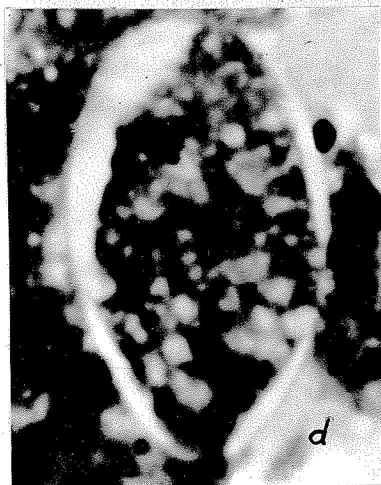


FIGURE 21









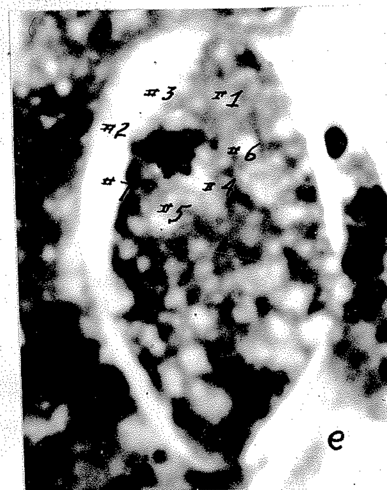
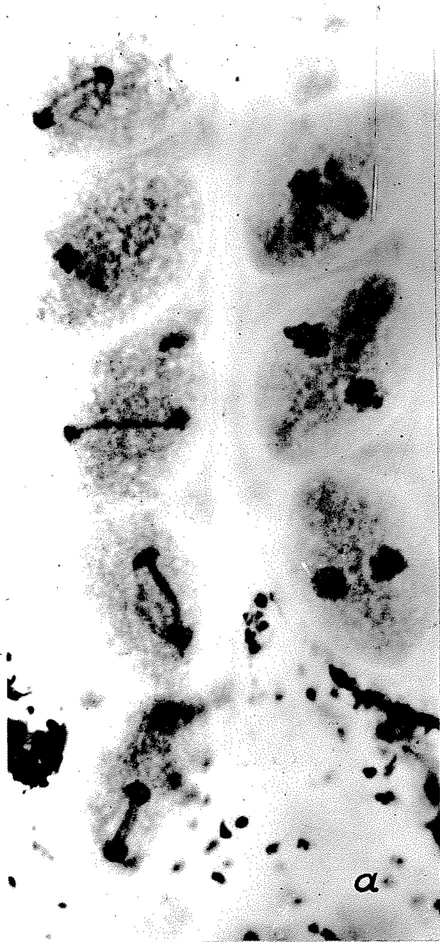
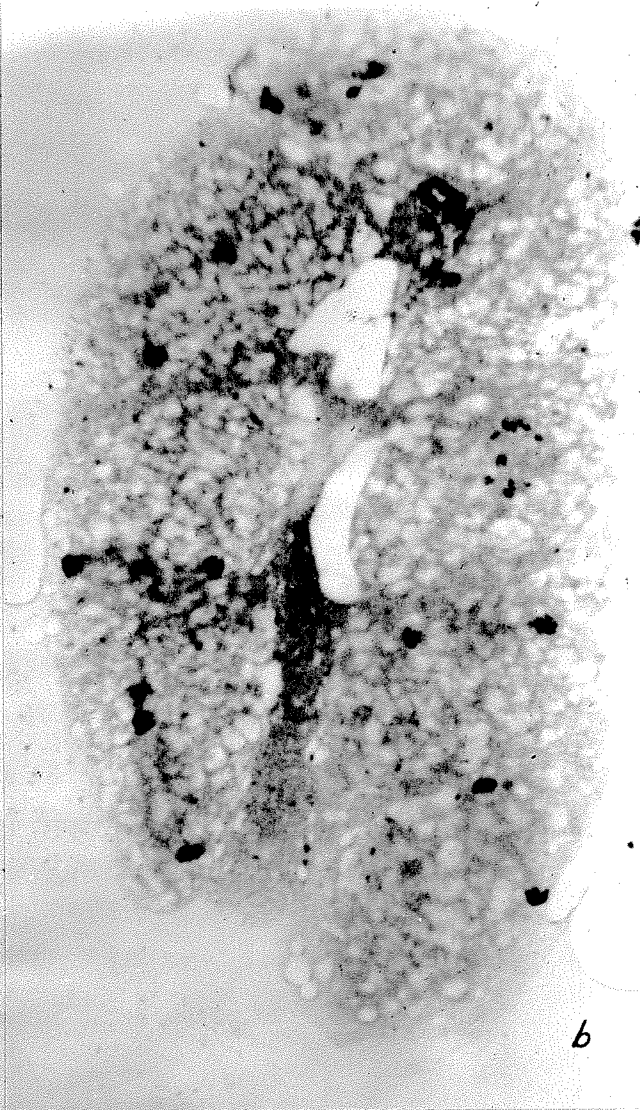


FIGURE 22





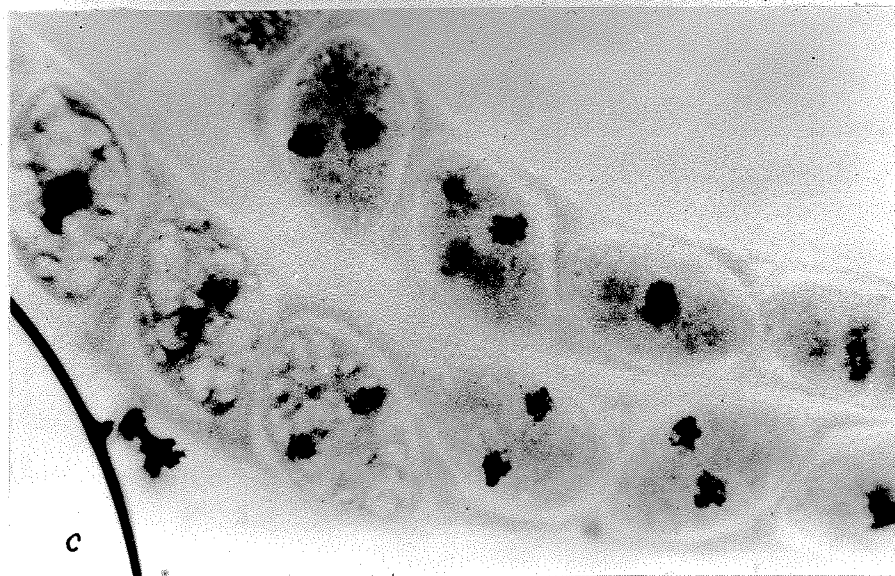
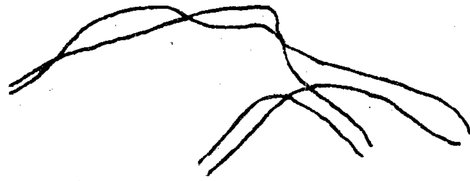


FIGURE 23



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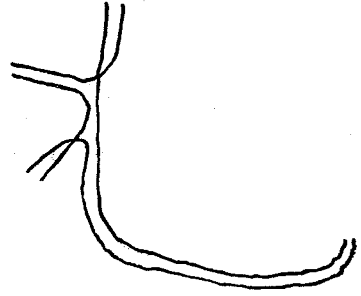

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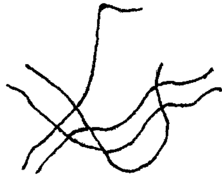

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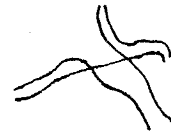

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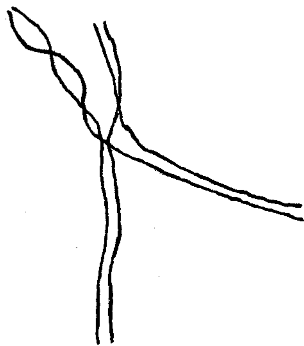
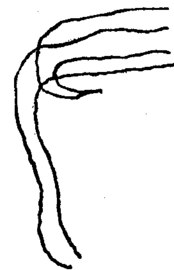


e



f

FIGURE 24