STUDIES ON THE CYTOLOGY OF YEASTS.

II. Induction of Polyploidy and Heterochromatin.

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(Communicated by Sir J. C. Ghosh, Kt., D.Sc., F.N.I.)

(Original received June 14; received after revision November 14; read August 30, 1946.)

Contents.

Introduction.

The distribution of the chromosomes in a distillery yeast to the two daughter nuclei during mitosis presented some interesting variations (Subramaniam and Ranganathan, 1946b). While, in many, the distribution was equal, each getting four chromosomes, in others it was unequal, owing to chromosome lagging, and nuclei with two, three, five or six chromosomes were formed. These diploid, triploid, pentaploid and hexaploid cells appear to disintegrate after a few divisions since they could not be isolated in wort-agar plates.

From investigations on animals (White, 1945; Koller, 1943) and plants (Darlington, 1942) it appears that variations in the amount of heterochromatin not only upset the nucleic acid metabolism of the cell but even alter the timings of mitoses and often result in sterility. Mutation is suggested to be the cause of these variations. Can such an explanation be extended to include the lethal mutations in the distillery yeast? Do yeast chromosomes carry heterochromatic regions?

Though in the distillery yeast all except the tetraploid have only a short span of existence, the four original strains having distinct morphological characters isolated immediately after exposure of a brewery yeast to low temperatures for 90 days (Subramaniam and Ranganathan, 1946d) have given rise to 15 different strains having distinct characteristics.

A cytogenetical analysis of the above depends, therefore, on an elucidation of what happens in yeasts when the chromosomes are duplicated and whether yeasts have heterochromatin.

Literature.

A perusal of the literature on the reactions of yeast to biologically active substances indicates that almost similar effects could be produced by a variety of chemicals (Bauch, 1941, 1942; Fabian and McCullough, 1934; Thaysen and Morris, 1943; Levan and Sandwall, 1943; and Thomas, 1945). The conclusions drawn by

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different investigators appear, however, to be different. While Bauch (1941, 1942) claims production of tetraploids and even octoploids, Thaysen and Morris (1943) considered the change to be 'deep seated', while Thomas (1945) suggests a 'plasmagene mutation'. Levan and Sandwall (1943) consider that their results do not justify a comparison of the effects of various chemicals on yeast with the colchicine effect on higher plants.

All the above tentative conclusions are, however, uncorroborated by critical cytological investigations. Entire dependence on morphological data is often misleading, for, agencies which induce polyploidy also produce gene mutations (Kerkis, 1939; Darlington and La Cour, 1940).

Any comparison between the cell division of yeasts and cell division of higher plants should be based on a clear appreciation of the different intervals at which duplication of cells takes place in different organisms (Darlington, 1937; Darlington and La Cour, 1940). While yeasts divide almost every hour in well-aerated wort, intervals between divisions in higher plants have been known to extend in some cases to a week or more. Thus, while yeast can theoretically be converted into a tetraploid by treatment of very short duration, longer treatment is necessary to produce a tetraploid seedling. Another important observation on higher plants ignored by workers on yeast is that it has been possible to produce octoploid plants only very rarely by a single treatment (Dermen, 1940).

Our own experience with a particular strain of yeast confirms the above observation. After three months of treatment we obtained only a tetraploid (Subramaniam, 1945). Lack of realisation of the importance of the above observation has resulted in different conclusions being drawn from almost identical observations. Richards (1938) found that when colchicine is present, a maximum crop was produced in a single growth cycle. In such cultures alcohol and other products of fermentation were found to be greater. Acceptance of Richards' suggestion that colchicine not only buffers the medium but is also a food, necessarily depends on proof that the cells have not become polyploid. A careful perusal of Richards' paper shows that his colchicine cultures differed from the controls (1) in having only a single growth cycle, and (2) in their increased fermentative activity as evidenced by increased output of alcohol and increased utilisation of sugar. The difference may be as much due to presence of colchicine as due to duplication of chromosome sets.

Though many substances have been known to induce 'colchicine mitosis', few are useful for the production of polyploids. Apart from colchicine the largest number of polyploids have been produced by treatment with acenaphthene. Even in the selection of a suitable chemical for induction of polyploidy in yeasts several factors have to be taken into consideration. Recent experiments have shown an antagonism of ethyl alcohol to colchicine. Levan and Ostergren (1943) found that while the threshold value of colchicine for *Allium* was 0·0055%, in the presence of 0·5% alcohol even 0·008% was found to be ineffective. The increasing percentages of alcohol produced during fermentation may therefore desensitize the yeast to the action of colchicine.

Organisms differ in their sensitivity to different polyploidizing agents. The Gramineae differ from the Leguminosae in their sensitivity to acenaphthene (Kostoff, 1938b). While C-mitosis could be induced in wheat by different substances, flax reacts only to colchicine (Simonet and Guinochet, 1939). Ulva, a green alga, reacts only to acenaphthene but not to colchicine (Levan and Levring, 1942).

Thus, if we take the four factors into consideration, viz. (1) that yeast cells should be compared to whole plants and not to individual cells of higher organisms; (2) that action of a short duration may bring about a duplication of chromosomes in yeasts; (3) that biologically active substances do not generally produce higher polyploids among treated seedlings by a single treatment; and (4) that the tetraploids and octoploids may have a shorter generation time; then, the observations of various workers show a remarkable coincidence.

Results on higher plants indicate that chromosome duplication may produce entirely different types of tetraploids. '(1) There may occur an appreciable increase in size of each vegetative cell in the tetraploid individual while the total number of cells making up the plant remains relatively the same as in the diploid form; consequently, the tetraploid plant appears larger than the diploid individual. Most of the changes following polyploidy appear to fall into this category. (2) An increase in cell volume may follow a doubling of chromosomes, but there may be a decrease in the total number of cells making up the tetraploid plant; therefore, the tetraploid individual will not appear different from the diploid. (3) The doubling of the chromosomes may not have any effect on the size of the cells. The polyploid individuals remain indistinguishable except probably in sexual and in some obscure physiological behaviour' (Dermen, 1940).

It would be well to remember that volume changes in induced polyploids depend not merely on an increase in the number of chromosomes but also on the occurrence of particular genetic factors in the organism. The possibility of different types of gene mutations being induced by different biologically active substances has also to be kept in view.

The results of previous workers on yeast, when analysed in the light of the above considerations fall into two separate groups: (1) polyploidy with augmentation of size appears to be likely when cultures are treated with camphor and borneol, and (2) polyploidy without any remarkable increase in size in cultures treated with colchicine (Levan and Sandwall, 1943; Thaysen and Morris, 1943; Richards, 1938; Bauch, 1941, 1942).

Though a large number of observations have been recorded on variation in yeasts, *the absence of cytological data has resulted in an absolute lack of criteria for any rational analysis of the changes occurring in yeasts when treated with biologically active substances.* Many workers have recorded the tendency of the variants to revert to their original morphological condition. A careful perusal of the literature shows that variants themselves are of two types: (1) 'saltants' and (2) 'Dauermodifications' (Henrici, 1941). Both these seem to occur in cultures either spontaneously or when exposed to cold or the action of polyploidizing or carcinogenic substances. We do not know yet how many of these variations are the result of changes in chromosome number and how many to causes such as deficiency (duplication) and gene mutations (Subramaniam and Ranganathan, 1946a). Even in higher plants the origin of aneuploid individuals and chimeras after colchicine treatment still awaits a rational explanation. While some consider that such aberrations are the result of partial instead of total arrest of chromosome division, others have shown origin of such individuals by multipolar divisions of some polyploid nuclei (Dermen, 1940; Kostoff, 1938b).

The unstable nature of some of the mutants and the 'sporting' behaviour of the distillery yeast observed in this laboratory (Subramaniam and Ranganathan, 1946b) led me to a closer study of the problem of chromosomal behaviour in yeast and compare it with that in higher plants and animals. One curious observation on the structure of the chromosomes in the distillery yeast appeared to be significant. In Carnoy iron haematoxylin as well as in Feulgen slides the chromosomes were 'glistening'. The cause of this 'glistening' appears to be due to the chromosomes having a lightly stained core and a chromophile cortex. While in the control Sc 9 (Subramaniam, 1946a) such a phenomenon was not observed, in material stained while the cultures were undergoing treatment with acenaphthene a similar staining reaction of the chromosomes was observed.

Levan (1945) has reported recently deviations in the staining qualities of chromosomes when treated with inorganic salt solutions. Not only could he observe the relational spirals of the two half chromatids clearly, but the heterochromatic regions retained the stain when the other regions of the chromosomes had lost their colour.

To understand the different staining reactions of the chromosome when it becomes duplicated, we have to turn to the results on higher plants and animals for an explanation. *Even there the whole matter remains still as a speculation.* Pontecorvo (1944) says: 'It is implicit in the view expressed here that a heterochromatic segment should arise every time that a minute euchromatic region undergoes repeated duplications in the genotype and the replicas remain adjacent to each other on the chromosome.' Mather (1944), on the other hand, states: 'The functional distinction between the two kinds of gene must not, however, be held to imply that one type can never change into the other. So far as the effect on the phenotype is concerned it would not seem impossible that the polygene of relatively small effect could become the major gene of relatively large effect if the developmental history of the organism became elaborated in an appropriate way.'

Are we seeing in yeast chromosomes a transformation of a part of the euchromatin into heterochromatin? Is the chromophobic interior of the chromosomes the heterochromatin?

Increase in the rate of cell division is said to be associated with increased synthesis of nucleic acids by the cell, this again being supposed to be regulated by the action of heterochromatin (Thomas, 1945). The shorter generation time of the major strain of *Torulopsis* produced by Thaysen and Morris (1943) necessarily presupposes such a series of events. Since the amount of alcohol produced by any strain depends on its genic make-up and since duplication of the genes should in all probability lead to increased alcohol output, it necessarily follows that the cells should produce increased quantities of nucleic acids. It is only the ribose nucleic acids which are concerned in cellular syntheses (Caspersson and Schultz, 1940, p. 512).

If we extend the concept of the important rôle of nucleoproteins in cellular synthesis to yeasts also (Henneberg, 1916), then the increased concentration of both types of nucleic acids in the major strain of Thaysen and Morris (1943) appears intelligible. Production and regulation of nucleic acids is said to be intimately associated with the heterochromatic regions, for, it is stated: 'Heterochromatic regions have the capacity (1) to form large amounts of thymonucleic acid (or better perhaps, thymonucleoproteins) in the chromosomes themselves; (2) to form or affect the composition of the nucleoli; (3) to affect the characteristics of neighbouring regions translocated to them in such a way as to change the developmental effects of these regions in somatic cells; and (4) to affect the content of the ribonucleic acids in the egg cytoplasm of *Drosophila*' (Schultz *et al.*, 1940, p. 521).

Looked at from different angles, the occurrence of heterochromatin in polyploid yeasts appears to be a possibility.

Material and Methods.

The accidental choice of acenaphthene for experiments on yeast necessitated by non-availability of even colchicine was a happy one, for, its valuable feature appears to be its lack of toxic properties (Levan and Ostergren, 1943). As qualitative investigations should precede quantitative ones, experiments were devised more with an idea of producing polyploids than to gauge the effect of differing concentrations of acenaphthene on the mitotic cycle. The workers who discovered that acenaphthene could successfully be used for the induction of polyploidy in plants have stressed the importance of having an undissolved excess of the substance, as a 'saturated solution alone was not sufficient to induce chromosome doubling' (Kostoff, 1938a, p. 753).

Yeast could be made either to grow or ferment. Therefore an attempt was made to bring as far as possible growing cells under the action of acenaphthene. Tubes containing 24-hour cultures were well shaken and the contents poured out. Fresh wort was added to the tube and the few cells left in the tube were distributed uniformly throughout the medium by vigorous shaking. A loop from the above,

which would usually contain not more than 50 cells, was inoculated into the experimental tube containing a few crystals of acenaphthene and about 10 c.c. of wort. Every day most of the material was discarded and the same quantity of wort was added and the crystals of acenaphthene were renewed. By this method actively growing cells were exposed to the action of acenaphthene and the above procedure was continued for 90 days. Bauch (1942) has stressed the importance of temperature at the time of treatment, but the control of temperature was thought unnecessary in the present instance because of the prolonged treatment. Examination of the contents of the tube was carried out every day. After the third day, every 24-hour culture would show a layer of small cells at the top.

Since vigorous growth has been noticed in higher plants immediately on return to normal environment after treatment with polyploidizing agents (Kostoff, 1938c; Nebel and Ruttle, 1938b; Muntzing and Runquist, 1939), the acenaphthene treated culture was kept in an active condition in wort before isolating the various types of cells by their distinct colony characteristics in wort-agar plates.

A description of the immediate cytological effects of acenaphthene on active cells of the brewery strain Sc 9 (N.C.T.C. 3,007) having only two chromosomes (Subramaniam, 1946a) was held up pending a preliminary analysis of the cytology of the new types produced (Subramaniam, 1945; Subramaniam and Ranganathan, 1946a) by 90 days' treatment. This was just to confirm the suspicion based on a careful perusal of the literature that higher polyploids may not occur even on continuous treatment. It was thought that if such a confirmation was available it may enable a correlation of the contradictory results obtained by different workers on the effect of colchicine on yeasts.

The observations recorded in this paper, however, are the results of a cytological investigation of the immediate effects of acenaphthene on growing cells of the above two chromosome strain. Since few cells are introduced at the beginning, the time of fixation was arbitrarily fixed at six hours in order to get sufficient material for preparing a few slides. The contents of the tubes were centrifuged at regular intervals to get a series showing the chromosome stages (Subramaniam, 1946a). Unsolved technical difficulties have prevented a study of the first mitosis under acenaphthene treatment and hence the results recorded are those of later divisions from acenaphthene cultures.

Confusion of volutin with chromatin was avoided by choosing cultures of cells with clear cytoplasm showing no granular inclusions (Caspersson and Brandt, 1941) and fixed in Bouin or Carnoy to prevent the mitochondria vitiating the picture. The slides were stained in Heidenhain's haematoxylin.

Observations.

The nucleus of the yeast preparing for division may be observed as a vacuole enclosing a chromophile mass having an irregular shape (Fig. 3). The staining of this chromatin mass is uniform and it divides into two and soon after into four as the indentation in one of the chromophile masses in Fig. 4 would suggest. Or, the nuclear vesicle disappears and the chromatin mass first assumes an irregular vesicular shape with a chromophile rim and a chromophobic interior (Fig. 5) before dividing into two very similar bodies having an identical shape and structure (Fig. 1). The differentiation into chromophilic and chromophobic regions appears at this stage even in those chromophile bodies which appeared uniformly stained at the time of division of the initial chromatin mass (Figs. 3 and 4). Division of the two bodies is not simultaneous as Figs. 2 and 7 would indicate. One of the bodies divides first (Fig. 2) and then the other (Fig. 7). This appears to be the typical tetraploid metaphase condition which appears to be succeeded by the typical anaphase illustrated in Fig. 12. Among the large number of cells seen in any field, though four chromosome stages predominate (Figs. 7 and 13), cells showing other

chromosome numbers are also present. The two initial chromophile bodies may give rise by division to four chromosomes, two of which may differ in size (Fig. 6). One pair of these chromosomes may differ not only in size but also in structure. A pair of vesicular chromosomes and two chromatin grains occur in the cell illustrated in Fig. 8. In Fig. 10 may be seen three vesicular chromosomes and three granular ones. On careful examination of large numbers of cells it appears as if these granular chromosomes are the result of unequal division or breakage of the chromosomes and that they eventually disintegrate. The vesicular shape of the chromosomes in cells undergoing treatment with acenaphthene renders it impossible to judge the exact cause for such inequality in size between the two daughter chromosomes. Since the mutant isolated from cultures grown under normal conditions after undergoing treatment for 90 days with acenaphthene showed two unequal chromosomes (Subramaniam and Ranganathan, 1946a), the question of fragmentation and translocation of bits of chromosomes have to be seriously considered.

The occurrence of triploid anaphases (Fig. 16) suggests either disintegration and loss of one chromosome or the non-division of some chromosomes, the later segregation of the six chromosomes into two groups and the reconstitution of two nuclei each with three chromosomes. The occurrence of pentaploids and triploids (Figs. 9, 10, 11, 14, 15 and 16) suggests the latter possibility. Colchicine and acenaphthene though they inhibit spindle formation do not, however, have any effect on the streaming movements of the cytoplasm (Nebel and Ruttle, 1938a). Observations on the control suggest that apart from the spindle, the streaming movements also play an important rôle in the distribution of the daughter chromosomes or the reconstituted nucleus to the bud (see Pictographic summary, Subramaniam, 1946a). Unco-ordinated division of the chromosomes into chromatids and streaming movements may explain the curious disposition of the chromosomes in Figs. 9, 14 and 15. Thus in Figs. 14 and 15 one of the chromophile bodies appears to have divided giving an odd number of chromosomes. The possibility that in the majority, the ultimate division of all the bodies may finally be followed by the anaphase illustrated in Fig. 12 is worth consideration since pentaploid anaphases have not been noticed in the material. While in Figs. 9, 14 and 15 five chromosomes are present, in Fig. 17 there are six, two of which appear to be considerably bigger than the rest. In Fig. 18 there are seven bodies, the result probably of non-division of one of the chromosomes, which when completed would probably proceed later to the anaphase shown in Fig. 12.

The behaviour of the chromosomes in both plants and animals under continued treatment appear to be similar. 'The sequence of events is similar to that described by Levan (1938) in *Allium*. The chromatid attraction lapses and the division of the centromere takes place, but the two chromatids remain parallel. Either a single resting nucleus, which will be tetraploid is produced or several unbalanced nuclei which will degenerate' (Barber and Callan, 1943, p. 264; Kostoff, 1938b).

The vagaries of different types of cells from different plants to identical concentration of these chemicals are slowly coming to light. It is well known that after treatment with colchicine aberrant forms with unchanged chromosome numbers also occur.

The results recorded above of the action of acenaphthene on a brewery strain of yeasts do not appear to be unusual. The mutant with two unequal chromosomes (Subramaniam and Ranganathan, 1946a) may be the result of a simple division of the cell shown in Fig. 6, or the multipolar division of a cell containing eight unequal chromosomes. The occurrence of only a tetraploid even after 90 days' treatment suggests that the efficiency of the chemical as a polyploidizing agent ceases once a duplication of the chromosomes had occurred. It is quite likely that several types with unbalanced chromosome numbers may occur in the cultures. But all these various forms may have only a short span of existence since they did not appear in wort-agar plates.

Discussion.

Heteropycnosis of entire chromosomes or parts of chromosomes have been known for a long time. In fact, the 'chromomeres' of Wenrich (1916) and the 'prochromosomes' of Rosenberg (1909) seem to belong to this category. Chromosomes show different types of heteropycnosis during the various stages and members of the same set may differ from one another even at the same stage. The X-chromosome of Acrididae show positive heteropycnosis during the prophase of meiosis and negative heteropycnosis during the early spermatogonial divisions. The autosomes of the same set, however, are positively heteropycnotic during the meiotic prophase but show no reversal in the early spermatogonial divisions. Even among these autosomes, the 'precocious chromosome' resembles in its staining capacity the X-chromosome itself (White, 1945).

In many plants (Darlington and La Cour, 1940) such a reversibility has been demonstrated. Particular regions which show negative heteropycnosis during metaphase at low temperatures appear deeply stained during the resting stages. The Y-chromosome of *Drosophila* is completely heteropycnotic and genetical evidence suggests that it does not carry the major genes. On this basis chromosomes or chromosomal regions are classified into eu- and heterochromatic regions. As in the case of the Y-chromosome, in the autosomes also the heterochromatic regions have been shown to exhibit a different type of genic behaviour. Apart from all this Caspersson (1941) has shown that the protein synthesized by the genes are less complex in the heterochromatic regions.

The identification of heterochromatic regions in chromosomes does not appear to be an easy affair (Callan, 1942). Though in certain plants the chromosomes show the differential segments when mitosis or meiosis takes place at low temperatures, this method is not of universal application. The number of chromocentres in the resting nuclei (Manton, 1935) may not also be a safe guide. Darlington and La Cour (1940) found the number of chromocentres to correspond to the heterochromatic segments in *Paris polyphylla* but not in others. In *T. erectum*, the number of chromocentres were fewer than the heterochromatic segments. They conclude that it is not always possible to distinguish the intercalary segments (Kaufmann, 1939) and that the possibility of two neighbouring differential segments appearing as a single chromocentre should be kept in view.

Mirsky (1943) suggested that heterochromatin may be characterised as 'that portion of a chromosome which retains its high content of nucleic acid in the interphase when the rest of the chromosome (the so-called euchromatin) loses much of its nucleic acid' (p. 28). The realisation of the fact that the detection of heterochromatin being not an easy affair, the failure to locate such regions by cytological methods need not necessarily indicate its absence, has led Darlington to re-define it as 'parts of chromosomes which are liable to remain charged with thymonucleotides in the resting stage' (Mather, 1944).

This naturally leads to a consideration of the position of heterochromatin in the chromosomes. The supernumerary chromosomes in many plants and animals (White, 1945; Darlington and Thomas, 1941) are almost wholly composed of heterochromatin. Even different tissues in the same plant or animal may have different numbers of heterochromatic supernumerary chromosomes. The supernumeraries are limited to the germ track in *Sorghum*. In *Sciara* the male and female have seven and eight chromosomes in their soma while the cells of the germ line contain in addition a pair of supernumeraries, the 'limited chromosomes'.

Even in the same autosome the heterochromatic regions may be limited to the areas around the centromere or they may have in addition such regions at their ends as also minute ones distributed at intervals. Translocation of a gene to the heterochromatic segment leads initially to irregularities in reproduction before a mutation to the heterochromatic type (Caspersson and Schultz, 1938). Since in

Drosophila the inert regions contain most of the repeats (Kaufmann, 1939) it has been tentatively suggested that inertness may be the cause of reduplication. Since additions or deletions of the heterochromatic segments have only slight phenotypic effects and since heterochromatin carries 'polygenes' it has been surmised that 'by virtue of its complement of polygenes it must play an important part in the fine adjustment of the phenotype to the immediate environment and in the storing of the variability on which will depend the future adaptation and evolution of the organism' (Mather, 1944). In animals *repeats* represent 'an important kind of "raw material" for evolution', for, 'mutations which would be lethal or at any rate lower the viability of the organism if they occurred in a non-repeated region, may in many cases have no such disastrous consequences if they occur in a tetraploid segment' (White, 1945, p. 48). Polyploidy appears to have played a major rôle in the evolution of plants and the yeast is perhaps no exception.

The specific question therefore is—whether the chromophobic portions of the chromosomes in yeast cells undergoing treatment with acenaphthene represent the heterochromatin? Any duplication of the chromosome sets should make some set of genes more or less superfluous.

Before discussing the possibility of the chromophobic portion of the chromosomes being heterochromatin, the question whether such a staining reaction may not be a mere indication of the structure of the chromosome itself has to be considered. Chambers (1925) has shown that in favourable material the chromosomes during certain stages possess a cortex which can be optically differentiated from a central core. 'This structure is significant in view of the way in which the artificially induced chromatin filaments come to view in the prophase spermatocyte of the grasshopper. Granules appear out of the hyaline nuclear material and align themselves in rows. As the granules increase and accumulate, their arrangements about a hyaline non-granular core becomes more and more appreciable. The definitive chromosome finally results by a shortening of the core and the fusion of the granules into a hyaline cortex' (p. 274). Levan (1945) found that treatment with many salts produced clear pictures of the internal structure of the chromosomes.

That the lightly stained region in the chromosome in acenaphthene material is not merely a clear picture of the internal structure would be evident owing to the following reasons: (1) The control strain shows no such differential staining. (2) In actively growing cells of the distillery yeast under normal conditions the chromosomes show such a differential staining. (3) In Carnoy or Bouin iron haematoxylin and in Feulgen's nucleal reaction an identical picture is obtained. Since the cells under discussion differ from the control in that they are polyploid, the differentially understained region is in all probability the heterochromatin.

In rod-shaped chromosomes the heterochromatin occurs either intercalated or as continuation of the euchromatin. Not only does the amount of heterochromatin differ in some groups from individual to individual owing to duplications and deletions, but also they show variations in amount in different tissues. In *Drosophila melanogaster* the heteropycnotic region which is about one-third the length of the X-chromosome at mitosis is represented in the salivary chromosomes by less than one-tenth its length. While, in *Drosophila* salivary glands the heterochromatic regions around the centromeres fuse to form the chromocentre, in the Chironomidae no such fusion occurs and intercalary and terminal heterochromatic regions have been observed.

The heterochromatin of the autosomes have been known to differ from that of the sex chromosomes and it seems as if 'there is difference between "compact heterochromatin" in which chromomeres still form bands and "loose heterochromatin" in which the regular arrangement of the chromomeres is entirely lost' (White, 1945, p. 44). Thus the range of variations observed in the position and distribution of heterochromatin in animals and plants renders it possible to consider the lightly

stained interior of the yeast chromosomes as heterochromatin. Very little attention seems to have been paid to the location of heterochromatin in granular chromosomes. We have an example of such a type in *Drosophila melanogaster* itself. The 'dot' chromosome appears in salivary glands as a short strand attached to the chromocentre. Often both ends of the 'dot' chromosome may be attached to the chromocentre showing the existence at the ends of heterochromatic segments.

The differentially stained region in the yeast chromosomes thus appears in all probability to be the heterochromatin. In certain species of Chironomidae, Bauer has observed that 'single bands of large "vesicular" chromomeres occur in the middle of a chromosome. These he interprets as heterochromatic segments consisting of only one band; they may also occur at the end of a chromosome' (White, 1945). The resemblance in structure of these heterochromomeres to the chromosomes in the yeast exposed to the action of acenaphthene is rather striking.

The peculiar position of the heterochromatin in the yeast chromosomes militates in no way against its identification since Painter and Taylor (1942) describe in the toad discrete granules of heterochromatin entirely removed from the chromosomes and still appearing to function. Caspersson and Brandt (1941) suggested that the volutin granules and thymonucleic acid of yeast cells may correspond respectively to the hetero- and euchromatin of animals and plants.

The demonstration of a change from eu- to heterochromatin on duplication of chromosomes appears to be of considerable significance. Since gradations between polygenes and major genes as well as transformation of one into the other have all been envisaged, definite statements based on observations on yeast would be precarious. Only planned experiments on higher plants on the effect of induced polyploidy on eu- and heterochromatin may furnish us with any rational explanation.

Summary.

1. Lethal mutations observed in a distillery yeast necessitated an elucidation of what happens in yeasts when the chromosomes are duplicated and whether yeasts have heterochromatin.

2. A review of the effects of polyploidizing agents on yeasts and higher plants is presented. It is suggested that if we take the four factors into consideration, viz. (1) that yeast cells should be compared to whole plants and not to individual cells of higher organisms, (2) that action of a short duration may bring about a duplication of chromosomes in yeasts, (3) that biologically active substances generally do not by a single treatment give higher polyploids among treated seedlings, and (4) that the tetraploids and octoploids may have a shorter generation time, then the observations of various workers show a remarkable coincidence.

3. The observations and speculations on the problem of heterochromatin are reviewed and the possible occurrence of heterochromatin in polyploid yeasts is indicated.

4. Details of the method of treatment of actively growing yeast cells with acenaphthene are given.

5. The various chromosome pictures seen during mitosis in cells undergoing treatment with acenaphthene are described. The chromosomes have a chromophilic cortex and a chromophobic interior.

6. The possibility of a change from eu- to heterochromatin on induction of polyploidy is discussed and it is suggested that the chromophobic core of the chromosomes may correspond to the heterochromatin of higher plants and animals.

7. It is shown that the peculiar position of the heterochromatin militates in no way against its identification since Caspersson and Brandt suggested a correspondence of volutin granules of yeast to heterochromatin, while Painter and Taylor describe in the toad discrete granules of heterochromatin entirely removed from the chromosomes and still appearing to function.

Acknowledgments.

I am very grateful to Sir J. C. Ghosh, Kt., D.Sc., F.N.I., and Mr. M. Sreenivasaya for their active interest and encouragement and to the Council of Scientific and Industrial Research for generous financial assistance.

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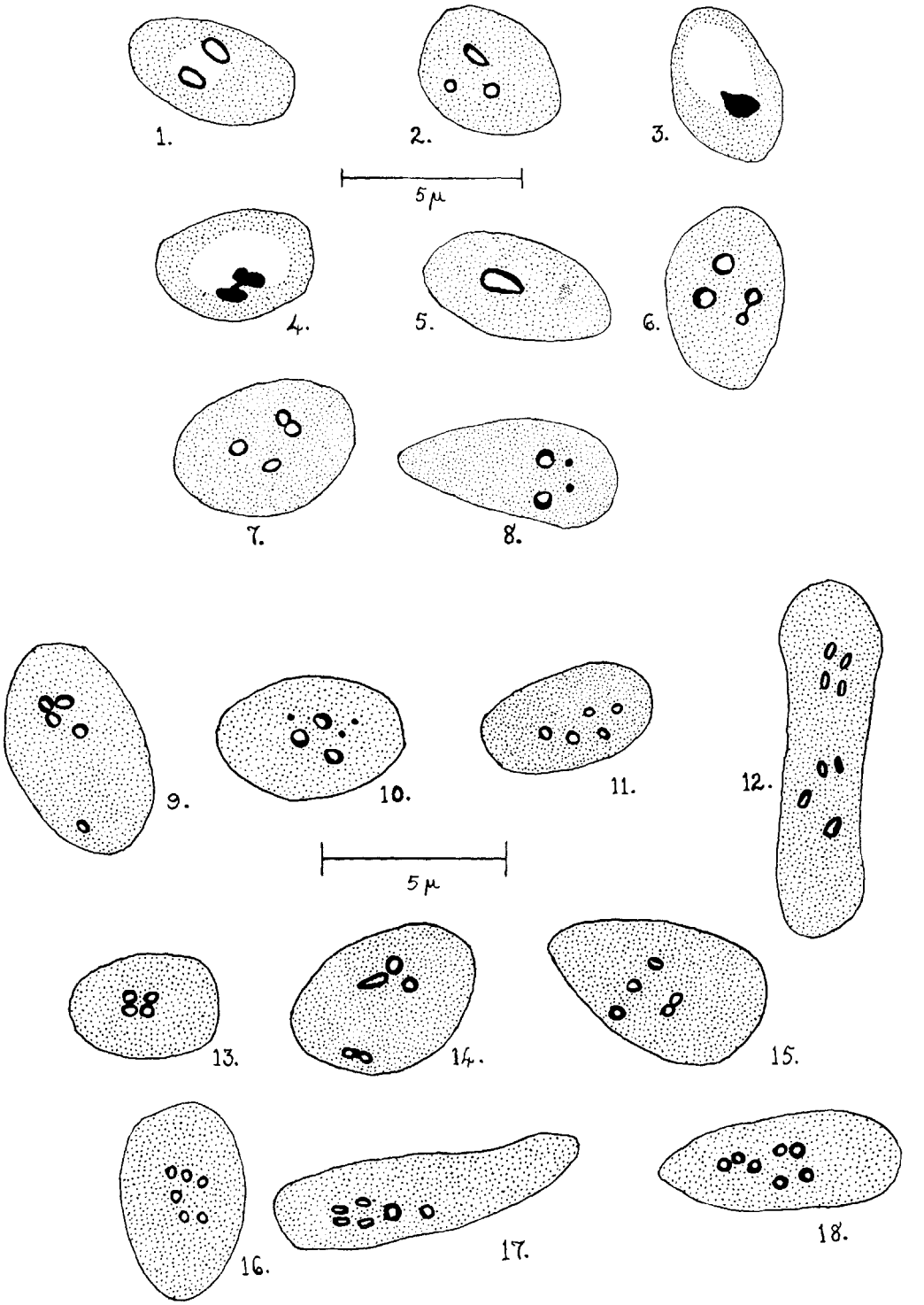
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