STUDIES ON THE CYTOLOGY OF YEASTS.

1. Mitosis in *Saccharomyces cerevisiae.*

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

(*Received July 24; read November 19, 1945.*)

Introduction.

The remarkable similarity of the genetic behaviour of the yeasts to higher organisms (Winge, 1935; Winge and Laustsen, 1937, 1939, and 1940; Lindegren and Lindegren, 1943) necessitates the presence of a chromosomal apparatus. But it is precisely about this chromosomal apparatus of yeasts that very little is known. It appears as if an organised attack of the problem had not been attempted before. Much of the confusion seen in earlier literature (Wager, 1897 and 1898; Wager and Peniston, 1910) appears to be due to the presence of volutin granules in the cytoplasm. If only the earlier cytologists had carefully investigated the behaviour of yeast cells in different media, they would have found that in rapidly growing cells in wort (Subramaniam and Ranganathan, 1945*a*) volutin granules are absent in the cytoplasm and hence do not vitiate the picture of the mitotic stages seen in stained preparations. Kater (1927) states that 'occasionally cells are found with none of these granules, especially in very young cultures' (p. 438). It is surprising that all previous investigators instead of trying to get cultures of yeast with no volutin granules in the cytoplasm tried instead to perfect staining methods which at best never gave consistent results.

It has to be emphasised here that yeast could be made either to ferment or grow by controlling the composition of the medium and the investigator can have at his disposal either an active but stationary culture or a rapidly growing one. To cytologists 'the relation between nucleic acid and volutin granules of yeast is of interest, especially since the observations of Caspersson and Brandt were made not on cytological preparations but on living yeast cells by means of ultraviolet light and a quartz microscope. In resting cells volutin granules containing nucleic acid are present in the hyaloplasm, which absorbs ultraviolet light only feebly. *As the cells begin to grow the granules swell, multiply and gradually disappear.* At the same time the hyaloplasm itself absorbs more and more ultraviolet light and finally appears rather homogeneous. Observations of the cells in the dark field also show that *as the hyaloplasm becomes opaque to ultraviolet light the granules disappear*' (Mirsky, 1943, pp. 27-28).

Any cytological investigation on yeast should, therefore, be on rapidly growing cultures, when nuclear changes could be studied with ordinary staining procedures.

Material and Methods.

The material investigated was a strain of brewery yeast, *Saccharomyces cerevisiae* (Sc 9, No. 3007) in the National Collection of Type Cultures. It was realised early that to get well-stained preparations uniform smears of one cell thickness were essential. The method of handling the material assumes, therefore, a vital importance. Owing to non-availability of even a hand centrifuge, a method had to be devised by which enough material would be available at controlled intervals.

A thin layer of wort in a flask was inoculated with a loop-ful of Sc 9 from a wort-agar slant and after the lapse of 24 hours a layer of yeast would have formed at the bottom of the flask. The supernatant fermented liquid was then poured out, three times its volume of fresh wort added and the flask well shaken in order to ensure uniform distribution. The activated cells in the flask began to bud in about an hour and hence at intervals of five minutes from three quarters of an hour after addition of fresh wort, the material was smeared and fixed.

Twenty minutes after the addition of fresh wort (S.G. 1·020, *p*H 4·5–5·0) the yeast cells would begin to form a thin layer at the bottom of the flask and at the stipulated interval one need only carefully pour out the supernatant liquid, remove the yeast with a glass pippette and smear on slides lightly coated with albumin.

The fresh wet smear was then treated with ammonia vapour for 10–15 seconds and fixed in Carnoy's or Bouin's fluid. One hour's fixation in either of the above fluids was found to be ideal. Since to smear a slide took half a minute, 10 slides made at intervals of 5 minutes, from 45 to 65 minutes after changing wort, gave pictures of the changes occurring during a period of 25 minutes, at half minute intervals. The slides were stained in Heidenhain's haematoxylin. The shorter methods were found unsuitable owing to the inherent difficulties encountered in the study of such minute organisms as the yeast with the barest of equipments. Hence, the slides were mordanted overnight and kept in haematoxylin for 24 hours or more, when staining was at the control of the operator.

All the observations recorded are from such series of preparations.

Observations.

In active but stationary cultures the cells vary in shape from oval to round and have the following range of measurements:

Table 1.

Length in μ ..	5·00	5·00	5·72	5·72	6·44	7·15	7·15	7·15
Width in μ ..	4·65	5·00	4·65	5·00	5·72	5·00	5·72	6·44

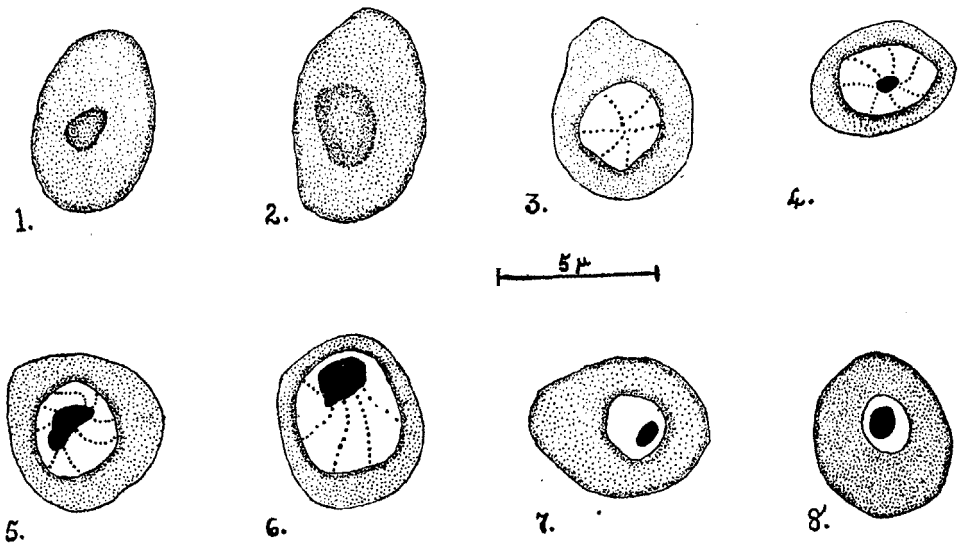
45–50 *Minutes.*

In slides fixed between 45–50 minutes after changing wort many cells still in the quiescent condition may be made out. These have a clear cytoplasm and a nucleus which stands out by its slightly deeper shade (Figs. 1 and 2). No stained granules are visible either in the cytoplasm or inside the nucleus. The comparative sizes of the cell and the nucleus in a representative series are given below (Table 2).

Table 2.

Length of Cell in μ	4·65	5·00	5·72	6·00	6·44
Width of Cell in μ	4·30	4·30	4·30	5·00	5·72
Length of Nucleus in μ ..	2·14	2·14	2·14	2·86	2·14
Width of Nucleus in μ	1·43	1·76	1·76	2·14	1·76

The next change observed is the appearance of lightly stained granules arranged linearly and appearing to radiate to the periphery (Fig. 3). The nucleus becomes globular and the



relation between cell size and nuclear size appears to undergo a change as will be evident from the following measurements (Table 3):

TABLE 3.

Length of Cell in μ ..	3·57	4·30	4·30	5·72	5·72	7·15
Diameter of Nucleus in μ ..	1·43	2·80	3·57	2·53	2·86	5·00

The increase in size of the nucleus in relation to that of the cytoplasm continues (Table 4) and at the same time a deeply staining granule puts in its appearance in the centre and begins to increase in size (Fig. 4).

TABLE 4.

Length of Cell in μ ..	3·57	4·30	4·30	4·30	5·72	5·72
Diameter of Nucleus in μ ..	2·14	2·86	3·20	3·57	4·30	5·00

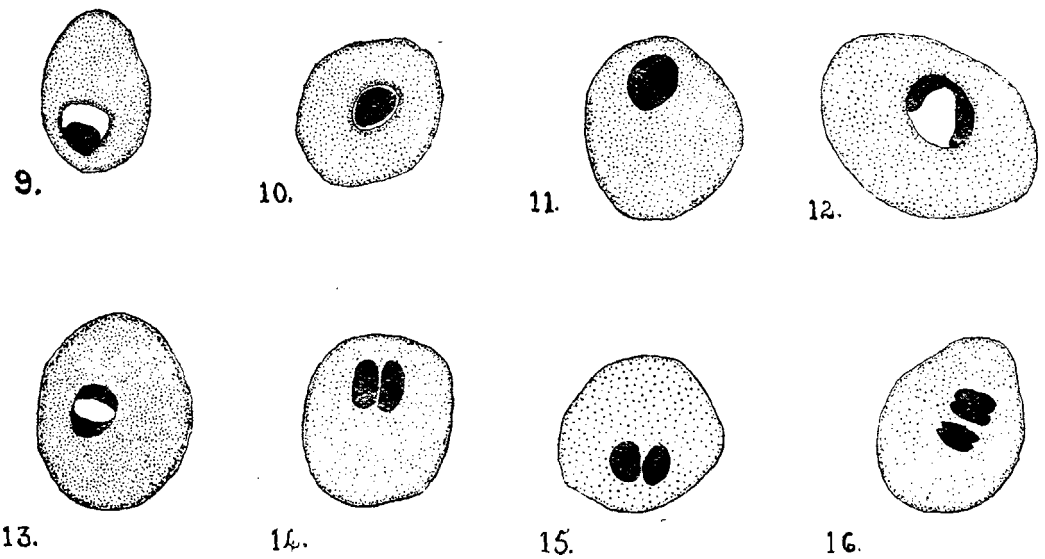
The radiating linear rows of granules slowly disappear with the increase in size of the central chromatin grain which finally (Figs. 5, 6, 7 and 8) occupies the entire area of the nucleus. During this period the nucleus appears to undergo a reduction in size (Table 5; Figs. 9, 10 and 11).

TABLE 5.

Length of Cell in μ ..	4·30	5·72	5·72	5·72	6·44	6·44
Diameter of Nucleus in μ ..	1·43	1·43	1·76	2·14	1·76	2·14

50–55 *Minutes.*

In preparations made between 50 and 55 minutes the chromatin mass while retaining its relative size to that of the cell (Table 6) appears to become bilobed and later to divide into



two, the products of division, however, remaining very close together (Figs. 14 and 15). Usually the nuclear membrane would have disappeared by this time, but two such chromosomes enclosed by a nuclear membrane may be seen occasionally in the preparations (Fig. 18). It is during these stages that one observes the chromatin mass appearing as plastered to the nuclear membrane, as a horse-shoe shaped mass (Figs. 12; cf Badian, 1937) or as two crescents (Fig. 13).

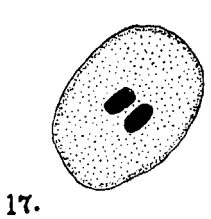
TABLE 6.

Length of Cell in μ	5·00	5·72	5·72
Diameter of Chromatin Mass in μ	1·76	1·76	2·14

The two chromosomes separate from each other and occasionally there are pictures (Fig. 16) which give one the impression that each of these prophase chromosomes is double.

55–60 *Minutes.*

Contraction of the chromosomes proceeds (Table 7; Figs. 17, 18 and 19) till finally the two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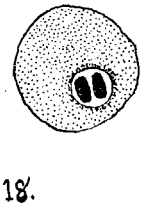
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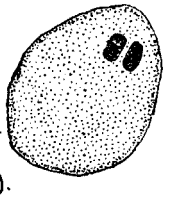
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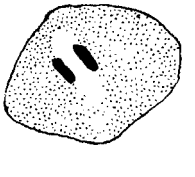
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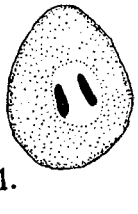
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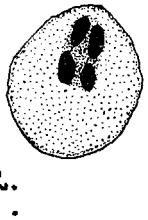
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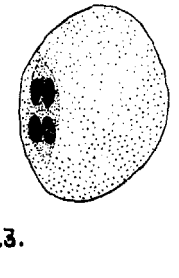
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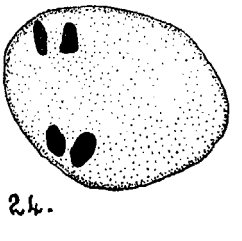
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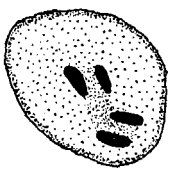
rod-like chromosomes appear in what may be considered as the metaphase stage (Figs. 20 and 21).

TABLE 7.

Length of Cell in μ	5·00	5·72	5·72	5·72	7·15
Length of Chromosomes in μ ..	1·43	1·43	1·76	1·76	1·76
Width of Chromosomes in μ ..	0·71	0·71	1·08	0·71	0·36

The next stage observed is one in which the chromosomes have become four in number and are separating in pairs (Figs. 22, 23, 25, 26 and 27). A spindle like structure may be seen in deeply stained cells (Figs. 23, 26) but in others what appears to represent the spindle appears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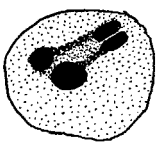
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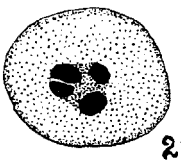
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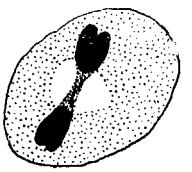
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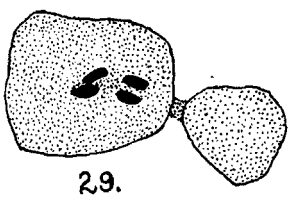
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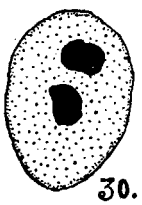
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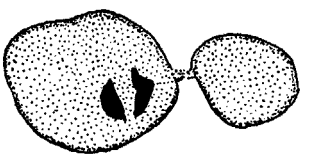
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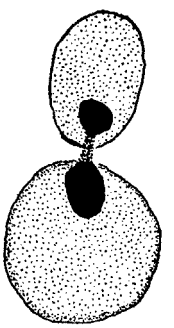

32.

either as an unstained area of the cytoplasm (Figs. 20 and 21) or a lightly tinted one (Figs. 22, 25 and 27). The late anaphase stages when deeply stained give the misleading impression

(Fig. 28) that the nucleus is dividing amitotically. The chromosomes have at these phases a characteristic but almost uniform size (Table 8).

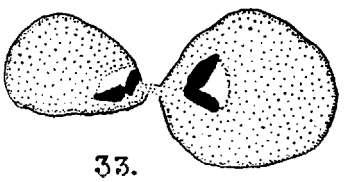
TABLE 8.

Length of Chromosomes in μ	..	1·33	1·33	1·33	1·00	1·00
Width of Chromosomes in μ	..	0·33	0·33	0·33	0·33	0·33

One curious picture shown in fig. 24 was seen among cells at early metaphase stage. Whether this is a naturally occurring tetraploid or an abnormal one, is very difficult to judge.

60–70 *Minutes.*

A bud may begin to develop when the chromosomes are in the anaphase state (Fig. 29) or it may appear only after the two nuclei have been reconstituted (Fig. 30). The telophasic reconstitution of the nuclei and the migration of one to the daughter cell present some interesting variations. One pair of chromosomes may migrate to the bud when the components of the other pair retain their individuality (Fig. 33) or the mother cell may have a reconstituted nucleus while the pair of chromosomes in the bud remain separate (Fig. 34). Or finally one of the two reconstituted nuclei may be seen passing to the bud while the other remains in the mother cell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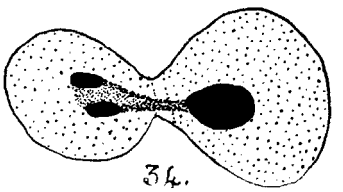
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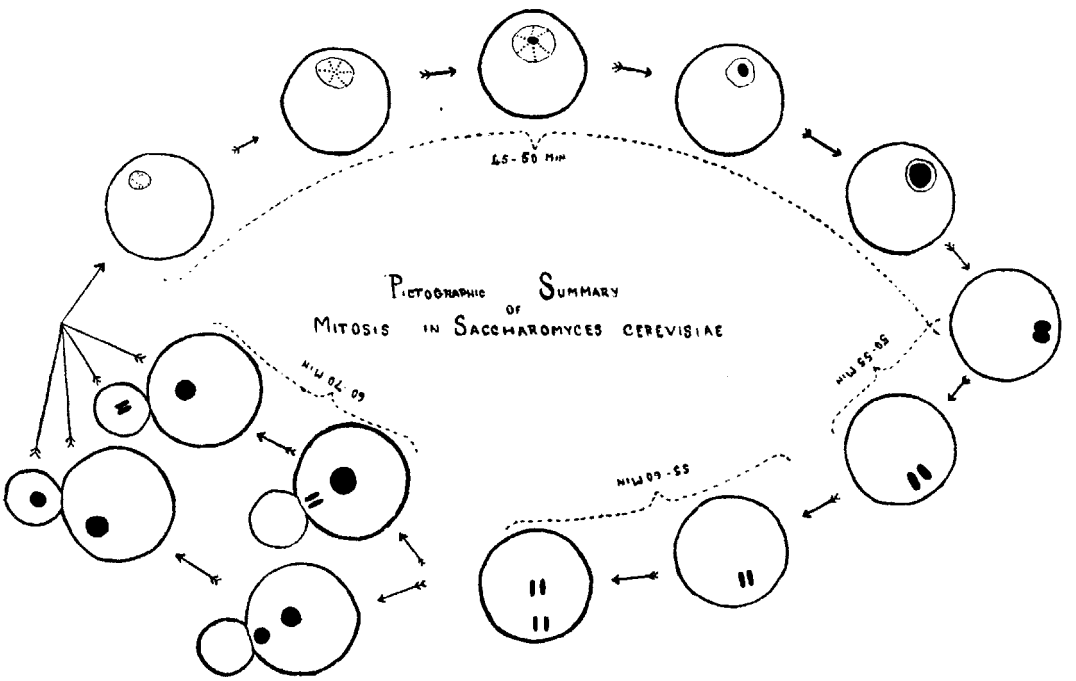
(Figs. 31 and 32). In all the above stages unless the slide is carefully differentiated one may get the impresssion that he is observing the phenomenon of amitosis.

A pictographic summary of the mitotic cycle is given in fig. 35.



35.

Discussion.

The presence of a nucleus itself was doubted in the early years of the present century and the identification of a particular structure in the yeast cell as the nucleus was suspected because Feulgen failed to obtain a 'nuklealreaktion'. However, modification of Feulgen technique especially by Rochlin (1933) is said to have given beautiful pictures and Badian (1937) seems to have been able to confirm the observations.

The demonstration of anaphase stages is the crucial test for any claim of mitosis in yeasts and the very fact that these could be demonstrated in material fixed and stained in the ordinary way shows that failure of the earlier workers to obtain the mitotic stages was more due to inherent difficulties in the handling of material for cytological investigations than in the lack of availability of a suitable technique for demonstration.

The account of mitotic stages given above is perhaps the first complete account and naturally differs in details from the description of Kater (1927) and Badian (1937). A detailed comparison of the results presented above with that of others was not attempted since there was a doubt whether different investigators had not investigated different races passing under name of *S. cerevisiae* (Subramaniam and Ranganathan, 1945*b*). Since it was possible to induce polyploidy in yeasts (Subramaniam, 1945) it was considered that a knowledge of the mitotic phenomena in polyploids is an essential prerequisite for any detailed comparison or generalization.

The idea that during budding the nucleus of the yeast cell divides by mitosis is nothing new. An intermediate form of mitosis was described by Janssens (1893 and 1902) and Janssens and Leblanc (1898), while a true mitotic process was described by Swellengrebel (1905) and Fuhrmann (1906). Kater (1927) states: 'Although these last two articles are in the main correct they have not been generally approved and the ideas of Guilliermond (1904, 1912, 1917 and 1919) which gain weight by the *mere bulk of his work on yeast* seem to meet with more favour' (p. 438). This appears to be largely true even to-day (Kater, 1940; Guilliermond, 1940) in spite of Kater's and Badian's discoveries. It is time that a new approach is made in this direction in order that planned cytogenetical investigations are possible.

Summary.

1. Details of the technique of handling yeast for cytological investigations are given and it is shown that in rapidly growing cultures in wort, volutin granules do not vitiate the picture of the mitotic stages seen in finished preparations.

2. The resting nuclei, which show no stained structure inside, on becoming active have chromatin granules radiating to the periphery. A central chromatin granule develops, occupies the entire nucleus and divides into two chromosomes.

3. The rod-shaped chromosomes split longitudinally and measure at the anaphase stage 1·00μ by 0·33μ.

4. A pictographic summary of the mitotic cycle during budding is given.

Acknowledgments.

I am very grateful to Sir J. C. Ghosh and Mr. M. Sreenivasaya for their active interest and encouragement and to Messrs. the K. C. P. Ltd., Vuyyur, for the award of a studentship.

References.

Badian, J. (1937). Sur la cytologie des Levures. *Bull. Internat. Acad. Polonaise. Sci. et Lettr. Cl. Sci. Math. et Nat.*, Ser. B. Sci. Nat. (1) (Bot). 1/5, 61–87.

Fuhrmann, F. (1906). Die Kernteilung von Saccharomyces ellipsoideus I. Hansen bei des Sprossbildung. *Centralbl. f Bakt.*, II Abt., **15**, 769–777.

Guilliermond, A. (1920). The Yeasts. (Trans. F. W. Tanner.) John Wiley & Sons, 1920.

——— (1940). Sexuality, developmental cycle and phylogeny of Yeasts. *Bot. Rev.*, **6** (1), 1–24.

Janssens, F. A. (1893). Beitrage zu der Frage uber den Kern der Hefezelle. *Centralbl. f Bakt.*, **13**, 639.

——— (1902). A propos du noyeau de la levure. *La Cellule*, **20**.

Janssens, F. A., et A. Leblanc (1898). Recherches cytologiques sur la cellule de levure, **14**.

Kater, J. McA. (1927). Cytology of Saccharomyces cerevisiae with especial reference to nuclear division. *Biol. Bull.*, **52** (6), 436–448.

——— (1940). Amitosis. *Bot. Rev.*, **6** (4), 164–180.

Lindegren, C. C. and G. Lindegren (1943). Selecting, Inbreeding, Recombining and Hybridizing Commercial Yeasts. *Journ. Bact.*, **46** (5), 405–419.

Mirsky, A. E. (1943). Chromosomes and Nucleoproteins. *Advances in Enzymol.*, **3**, 1–34.

Rochlin, E. (1933). Uber die Nuklealreaktion bei Hefen. *Centralbl. Bakt.*, II Abt., **88**, 304–306.

Subramaniam, M. K. (1945). Induction of Polyploidy in Saccharomyces cerevisiae. *Curr. Sci.*, **14**, 234.

Subramaniam, M. K. and B. Ranganathan (1945*a*). Mitosis in Saccharomyces cerevisiae. *Curr. Sci.*, **14**, 78.
——— (1945*b*). Chromosome Number of Saccharomyces cerevisiae. *Curr. Sci.*, **14**, 131.
Swellengrebel, M. (1905). Sur la division nucleaire de la levure presse. *Ann. Inst. Past.*, **19**, 503.
Wager, H. (1897). The Nucleus of the Yeast Plant. *Rept. Brit. Assn. Toronto*, 860-61.
——— (1898). The Nucleus of the Yeast Plant. *Ann. Bot.*, **12**.
Wager, H. and A. Peniston (1910). Cytological Observations on the Yeast Plant. *Ann. Bot.*, **24**.
Winge, O. (1935). On Haplophase and Diplophase in some Saccharomycetes. *Compt. Rend. Trav. Lab. Carlsberg* (Copenhagen), **21**, 77–112.
Winge, O. and O. Laustsen (1937). On two types of Spore Germination and on Genetic Segregations in Saccharomyces demonstrated through single spore cultures. *Ibid.*, **22**, 99–120.
——— (1939*a*). Artificial species hybridization in Yeast. *Ibid.*, **22**, 235–248.
——— (1939*b*). On 14 New Yeast Types produced by hybridization. *Ibid.*, **22**, 337–356.
——— (1940). On a Cytoplasmic Effect of Inbreeding in Homozygous Yeast. *Ibid.*, **23**, 17–39.

All the drawings have been drawn at a uniform magnification with an Abbe Camera lucida using an × 14 eyepiece and an × 100 oil immersion.

Note added in proof.

Very recently claims have been advanced (Srinath, K. V., *Curr. Sci.*, **15**, pp. 25 and 50-51, 1946) that yeasts form an exception to the general rule and that they have Feulgen positive centrioles and Feulgen negative chromosomes. A perusal of Mirsky's paper (1943) would have *shown that the identification of a nucleus in yeast was dependent on a positive Feulgen reaction* (pp. 5-6). That yeast nucleus contains thymonucleic acid and that yeast nucleic acid occurs in the cytoplasm of the yeast have already been demonstrated by Delaporte, 1939 (*see* Mirsky, p. 24, 1943). Badian's claim that yeasts have the conventional Feulgen positive chromosomes (*see* Mirsky and Pollister, *Biol. Symp.*, **10**, p. 258, 1943, for the composition of chromatin and Darlington, 1937, p. 574, for definition of chromosomes and chromatin) has recently been confirmed by Robinow (*J. Hyg.*, **43**, p. 420, 1944) and Thomas (*Nature*, **156**, p. 739, 1945).

The Feulgen positive bodies ranging in number from one to five described in the first Note and said to remind one of the heteropycnotic areas in nuclei are now considered doubtful and two perfectly spherical 'centrioles' have been suggested in the second contribution. No critical evidence is presented in support of the identification of certain structures in the yeast cell as chromosomes and centrioles. If they had been the bodies they are identified to be (*see* Fig. 2, p. 51) then, their orientation should be entirely different. There should be a 'spindle' between the two centrioles and the chromosomes should be orientated on the spindle (*see* text-books: Wilson, 1904; Sharp, 1926; and Darlington, 1937).

Mitochondria contain ribo-nucleoproteins (Claude, *A. Biol. Symp.*, **10**, p. 111, 1943) and until criteria are offered to distinguish the 'Feulgen negative chromosomes' from the above 'living' inclusions (compare his Figs. 1A and 2, pp. 50 and 51 with Guilliermond's Fig. 98A, p. 232, 1920) we have to consider that mitochondria have been confused with chromosomes and nuclei with centrioles.