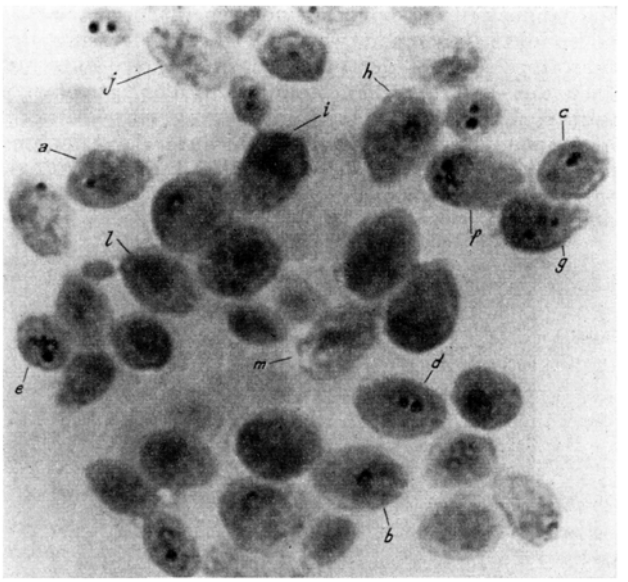
Further Evidence for Mitosis in Yeasts

The mitotic behaviour of our two-chromosome control strain has been under constant observation for the past six years. The cytological pictures observed depend upon the physiological condition of the cells. Division is mitotic in aerobic liquid cultures[1] while during fermentation the majority become endopolyploid[2]. For the past one year attention has been directed towards devising a technique whereby the presence of fermenting endopolyploid cells could be entirely avoided. It is known that many yeast strains require a long period of adaptation to galactose before fermentation can occur. This characteristic, which is shown by our control strain also, was employed to obtain pictures of mitotic stages uncomplicated by other phenomena. The material was smeared as usual, fixed for 20 min in osmic vapour, hydrolysed for 6 min at 60°C and stained according to the Feulgen method[3].

The crucial stages presented as camera lucida drawings by SUBRAMANIAM[1] can be seen in the photomicrograph. At *a* and *b* are cells showing a single chromatin mass, delimited from the surrounding cytoplasm by a hyaline area. This is the early prophase stage when the chromatin mass (chromocentre) becomes visible. At *c*, this chromatin mass is seen dividing into two chromosomes. Separation of these discrete chromosomes and their characteristic arrangement at early metaphase appears in the cell at *d*. The splitting of the two chromosomes to form four chromatids becomes obvious at early anaphase (*e*, *f*). The pairs of chromosomes separating is seen in the cell at *g* in which one of the chromosomes is in a different optical plane. That cytokinesis does not parallel karyokinesis would be evident from the presence of discrete chromosomes in the bud (*h*) while the mother cell has a reconstituted nucleus. This lack of correlation is further exemplified by the cells at *i* and *j*, where the mother cells have reverted to the complete resting condition whereas the buds still show two discrete chromosomes. At *l* and *m* are cells with buds which, because they do not show any chromatin, must be presumed to be in the resting condition.

A comparison of the above, unretouched photomicrograph with those published by LEVAN[4], DE LAMATER[5], and WINGE[6] would prove conclusively that the techniques perfected in this laboratory give much superior pictures. In a recent criticism of investigations reported from this laboratory, WINGE[6] comments: "On the basis of a number of limited observations of supposed chromosomes, these investigators have been led to some very far-reaching conclusions ..." (p. 86). It is rather surprising that an extended and connected series of studies should be characterized as "limited observations". The photomicrograph presented should be sufficient evidence to justify our claim that the control two-chromosome strain still retains the chromosome constitution first reported by SUBRAMANIAM and RANGANATHAN[7] in 1945.

WINGE[8] observes: "The drawings in their various papers dealing with cytology lack sufficient detail but nevertheless show a few to several stained bodies which are regarded as the chromosomes, in spite of the fact that they bear little resemblance to such structures" (p. 94). This scepticism is apparently due to the fact that only camera lucida drawings were presented in our earlier publications. Photographic materials and equipment were rare commodities as a result of the dislocations caused by the war. Under the circumstances, we had to content ourselves with presenting only Indian ink illustrations. WINGE's criticism of our identification of chromosomes in yeasts appears to be based on preconceived ideas regarding size and shape. We would in this connection invite attention to the following comments of SHARP[1]: "As they appear in the metaphase or anaphase of somatic mitosis, the chromosomes of different organisms show a great range in size. In some cases they are extraordinarily minute, being less than 1 μ, long while in others they may reach a length of 20 μ or more; the breadth may vary likewise. Some natural groups, such as fungi and certain insect orders, have small chromosomes as a rule, while in others, notably amphibia, grasshoppers, and liliaceous plants, they are characteristically large" (p. 114). That there is no justification for WINGE's assumption would be obvious to investigators who have examined different genera of plants and animals (cf. DARLINGTON[2], Table 12, p. 85).



Key to the lettering in the photomicrograph
a and *b*, early prophase. *c*, late prophase. *d*, early metaphase. *e* and *f*, early anaphase. *g*, anaphase. *h*, *i* and *j*, telophase. *l* and *m*, resting stage. (Magnification ca. 2,500:1.)

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Zusammenfassung

In einer früheren Arbeit wurde die Mitose einer Bierhefe mit nur zwei Chromosomen beschrieben. Dieser Befund kann jetzt auch mikrophotographisch belegt werden. Alle Phasen der Mitose lassen sich ohne irgendwelche Störung durch andere Phänomene beobachten, wenn die Hefe in einem Galaktosemedium kultiviert wird.

[1] M. K. SUBRAMANIAM, Proc. Nat. Inst. Sci. India *12*, 143 (1946).
[2] M. K. SUBRAMANIAM, Proc. Nat. Inst. Sci. India *14*, 325 (1948).
[3] M. K. SUBRAMANIAM, Proc. Nat. Inst. Sci. India *14*, 315 (1948).
[4] A. LEVAN, Nature *158*, 626 (1946).
[5] E. D. DE LAMATER, J. Bact. *60*, 321 (1950).
[6] O. WINGE, C. r. Lab. Carlsberg, Ser. Physiol. *25*, 85 (1951).
[7] M. K. SUBRAMANIAM and B. RANGANATHAN, Curr. Sci. *14*, 131 (1945).
[8] O. WINGE, C. r. Lab. Carlsberg, Ser. Physiol. *25*, 85 (1951).

[1] L. W. SHARP, *Introduction to Cytology*, 3rd Edit. (McGraw Hill Book Co., New York, 1934).
[2] C. D. DARLINGTON, *Recent Advances in Cytology* (J. A. Churchill, London, 1932).