12 h prior to the mitosis in the starved controls, and in combinations in which the number of interphase nuclei within the mixed plasmodia was up to 3.6 times that of the nuclei from the premitotic plasmodia; e.g. in the experiment shown in Figure 3, the proportion in the mixed plasmodium of interphase nuclei to premitotic nuclei was between 2.4/1 and 3.6/1, as determined in smear preparations of explants taken from different parts of the plasmodium, and the interphase nuclei in the mixed plasmodium entered prophase, along with the nuclei from the premitotic plasmodium, approximately 30 min after the beginning of prophase in the premitotic control and 12.4 h before the beginning of mitosis in the starved interphase control.

Once initiated, mitosis in the mixed plasmodia went to completion in most of the nuclei. In the nuclei which did not divide, the nucleoli failed to disintegrate during prophase, and chromosomes and nucleoli clumped in the middle of the nuclei which became pycnotic.

The onset of prophase within the mixed plasmodia, without considerable delay as compared to the premitotic controls and in a number of interphase nuclei more than twice that of the premitotic nuclei present, suggests that prophase was initiated by a diffusible factor(s) which was either present in the premitotic plasmodia at the time of coalescence or which was produced, after coalescence, under the influence of constituents, including nuclei, that were contributed by the premitotic plasmodia.

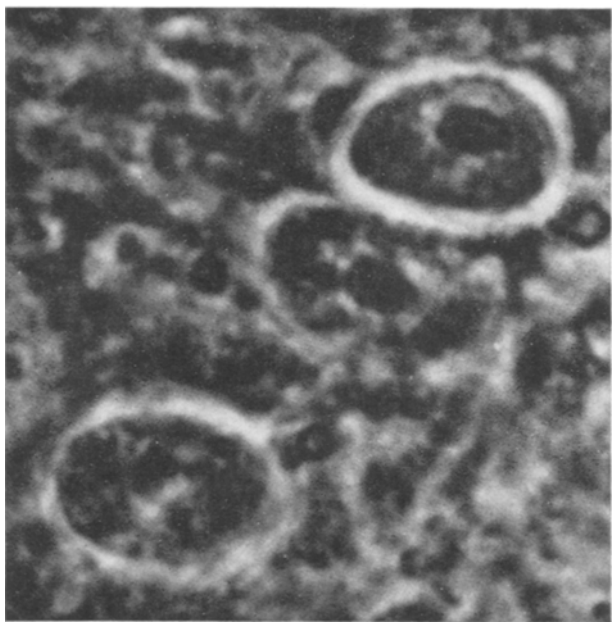

Fig. 4. Nuclei of starved plasmodium, early prophase. Nucleoli surrounded by condensed mass of chromosomes. × 5750.

In a number of other, seemingly similar combinations, no such initiation of mitosis was observed, and the interphase nuclei which were contributed by the starved plasmodia showed no morphological change in the presence of the premitotic nuclei. The morphological appearance of the latter gradually reverted to that of interphase nuclei (nucleolus in the middle of the nucleus, chromosomes evenly distributed in proximity to the nuclear membrane), and they decreased in size until, approximately 8–10 h later, they were no longer distinguishable from the interphase nuclei that were contributed by the starved plasmodium. In this case, observation was discontinued approximately 12 h after coalescence. This negative result could have been due to an extremely short duration of the initiating state in the premitotic plasmodia and the resulting difficulty in achieving coalescence at the right time.

Attempts to initiate mitosis, also by sandwiching, by fusion of premitotic plasmodia with growing, rather than starved, plasmodia, were unsuccessful. It would appear, therefore, that in starved plasmodia, but not in growing plasmodia, mitosis is preceded by a sufficiently long period during which an initiating factor(s) is the last remaining requirement for the beginning of prophase. Starved plasmodia, therefore, during the latter part of their intermitotic period, or plasmodia which are maintained on deficient, defined medium, would provide suitable indicator systems to determine the presence of mitosis initiating factors either in vivo or in subcellular fractions.

Zusammenfassung. Es wird gezeigt, dass eine Kernteilung innerhalb sehr kurzer Zeit eintritt, wenn Teile eines Plasmodiums von *Physarum polycephalum*, das zur Teilung ansetzt, mit Teilen von Plasmodien verschmolzen werden, die sich in Ruhe befinden. Daraus wird auf eine stoffliche Induktion zur Mitose geschlossen.

E. GUTTES and SOPHIE GUTTES

Department of Biology Loyola University, Chicago (Illinois 60626, USA), 30 May 1969

Monocentric Nature of the Chromosomes of *Ranatra* (Heteroptera) Verified by the Induced Fragmentation Experiments

Recently from our observations on the morphology and the anaphase configurations of the chromosomes of a heteropteran insect *Ranatra elongata*, we (DESAI and DESHPANDE[1]) have proposed that the chromosomes of this insect are monocentric and not polycentric as is generally believed. The one sure test to demonstrate the polycentric nature of the chromosomes is to induce fragmentations in them by irradiation techniques and see if the fragments survive as independent chromosomes during cell divisions. In the present work, results of such experiments on adult males of *Ranatra filiformis* Fabr. having the chromosome number $n = 23$ ($19A + 3X + 1Y$) are described. Groups of 10 were irradiated at various dosages as 500r, 800r, 1000r, 2000r, 3000r, 5000r, 8000r, 10,000r, 12,000r and 15,000r, using the source 'Gamma cell 200' at the rate of 4.45 Kr/min. Owing to their extremely small size, these chromosomes could be fragmented only at the dosages of 5000r and above. Dosages up to 10,000r induced fragmentations considerably, and hence numerous cells showed anaphase bridges. Yet the

[1] R. N. DESAI and S. B. DESHPANDE, Experientia *25*, 384 (1969).

cells were not damaged. Higher dosages than this, however, caused severe damages to the cells, and such materials were unsuitable for our studies. The insects were sacrificed for their testes squash preparations at 3 intervals, viz. 24, 48 and 72 h, after irradiation. The second stock gave better results than the other two.

The chromosome fragments, so produced at metaphase or earlier, show to a large extent refusion at random either with centric fragments or acentric ones, and give rise to bridges during anaphase movements of the chromosomes (Figure 1). Further, at late anaphase the centric fragments or the chromosomes which have escaped irradiation reach the poles as usual, while there are also some fragments which have failed to fuse either with normal chromosomes or with centric fragments. These are extruded out from the spindle and are left in the middle (Figure 2); and such fragments together form irregular chromatin mass (Figure 3) without being included in the telophase nuclei. All these irregular chromatin masses or the left-out fragments are finally eliminated from the cell. It is therefore sufficient to believe that such fragments are the ones without their own centromeres for which reason they are thrown out of the spindle. On the contrary if the chromosomes of this insect happen to be really polycentric, their fragments too should be maintained in the spindle apparatus during cell divisions. But such is not the case here. These results lend a strong support to our earlier contention that at least in *Ranatra* spp. the chromosomes are monocentric and not polycentric[2].

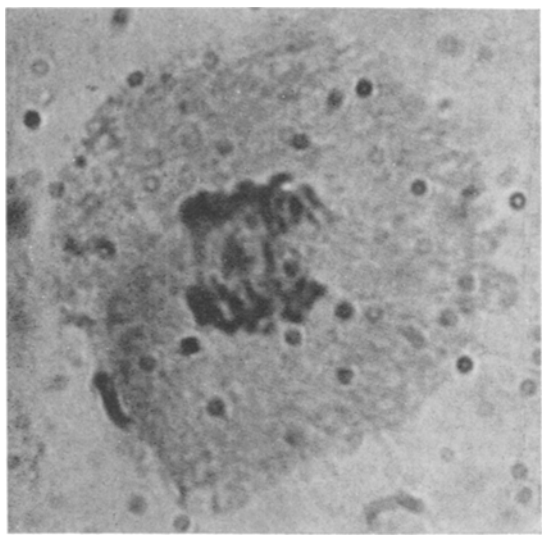

Fig. 1. An anaphase plate showing chromosome bridges. × 4500.

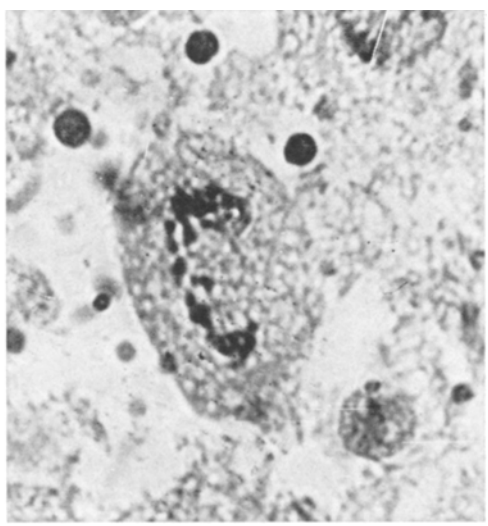

Fig. 2. A late anaphase plate showing acentric fragments reaching neither of the poles. × 4500.

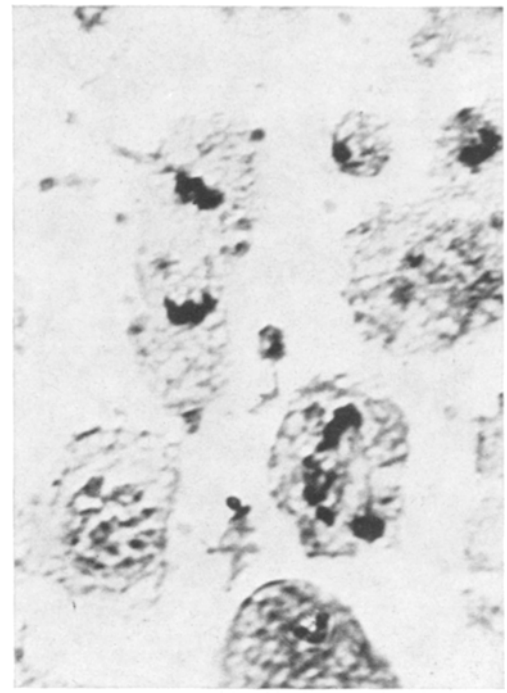

Fig. 3. A telophase plate with irregular chromatin outside the spindle. × 4500.

Résumé. Les chromosomes de *Ranatra filiformis* Fabr. (Heteroptera) a été exposé à la radiation gamma de 5000 à 10000r à raison de 4,45 Kr/min. Dans beaucoup de cellules, les fragments du chromosome, ainsi produits, atteignent le pôle ou bien forment des ponts durant l'anaphase ou des masses irrégulières de chromatine. Un pareil comportement des fragments indiquent nettement que les chromosomes de ce genre sont monocentriques et non polycentriques.

R. N. Desai

Department of Zoology, Karnatak Science College, Dharwar (Mysore State, India), 8 July 1969.

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