Further work is now under progress in order to correlate the accumulation of excretory products in the kidney of snails under different diets and conditions of estivation and hibernation.

Résumé. La composition de l'urine du pulmoné terrestre *Strophocheilus oblongus musculus*, spécialement le taux de l'urée, est un reflet des différences du contenu protéique des aliments. Ce fait confirme que le fonctionnement du rein des mollusques et des vertébrés est semblable.

F. B. DE JORGE, J. A. PETERSEN and A. S. F. DITADI

Department of Medicine, School of Medicine, Deptartment of Animal Physiology and Department of Zoology, University of São Paulo (Brazil), 28 January 1969.

Synchronization of Mitosis in *Physarum polycephalum* by Coalescence of Postmitotic and Premitotic Plasmodial Fragments[1]

In the coenocytic slime mold, *Physarum polycephalum*, the nuclei undergo mitosis in synchrony[2]. When microplasmodia growing in agitated culture and representing all stages of the mitotic cycle[3] at random are allowed to coalesce on filter paper, the nuclei of the resulting surface plasmodia are mitotically synchronized[4–6]. Postmitotic nuclei which become part, by plasmodial coalescence, of a predominantly premitotic plasmodium, enter the next mitosis along with the nuclei of the latter[7]. We report in the following on mitotic synchronization resulting from fusion between pre- and postmitotic plasmodia in such a ratio that nuclei of both stages were present in approximately equal numbers in the resulting composite plasmodia. Of special interest to us were those combinations in which the premitotic plasmodia, at the time when coalescence was underway, were at a stage of the intermitotic period as close as possible to prophase. We employed for this purpose suspensions of plasmodial fragments which were freshly prepared from mitotically synchronized surface plasmodia.

The organism was grown in form of microplasmodia in agitated culture[8]. Mitotically synchronized surface plasmodia were prepared as described previously[6]. Cultures of microplasmodia from several flasks were pooled, redistributed, and returned to the shaker. One set of surface plasmodia was prepared immediately, and a second set was prepared 1, 2, and 4 h later, respectively, in different experiments. Both sets of plasmodia were made from 1-ml aliquots of identically prepared, concentrated suspensions of microplasmodia[6]. Since the cultures were approaching the plateau of growth at the time of fusion, the increase in the number of microplasmodia during the intervals between preparing the 2 sets of plasmodia was negligible. Immediately upon the first postfusion mitosis, the plasmodia were placed on fresh growth medium. The growth medium added to the first set of plasmodia contained thymidine-^{3}H (5 μc/ml; from Schwarz Bioresearch, Inc.). When the plasmodia of the first set had just completed the second postfusion mitosis (anaphase to late telophase), plasmodia from both sets were floated, along with the supporting filter paper, on balanced salt solution[8] and removed from the filter paper with the help of a spatula. 4 premitotic (each containing approximately N nuclei) and 2 postmitotic (each containing approximately $2N$ nuclei) plasmodia were pooled in 30 ml of balanced salt solution (in 500-ml Erlenmeyer flasks) and fragmented by vigorous shaking for 15 min on an Eberbach alternating shaker (250 reciprocations/min, stroke length 4.5 cm). In order to have an estimate of the effect of fragmentation followed by fusion upon the beginning of mitosis, other premitotic and postmitotic plasmodia were fragmented separately and the fragments were reunited by fusion. After fragmentation the pieces were concentrated by centrifugation at low speed and aliquots of the concentrated suspension were placed on filter paper (this procedure is referred to in the following as 'fusion'). Growth medium was added approximately 2 h later. The actual proportions of postmitotic nuclei/premitotic nuclei in the mixed plasmodia were determined, after addition of growth medium, in autoradiographs (Kodak AR-10 stripping film) prepared from ethanol-fixed smear preparations (incubation in the dark for 3 days). Under these conditions, the heavily labelled postmitotic nuclei of the first set of plasmodia were clearly distinguishable from the premitotic nuclei.

Fragmentation of surface plasmodia caused inhibition of mitosis as seen in Figure 1. Only if the nuclei were

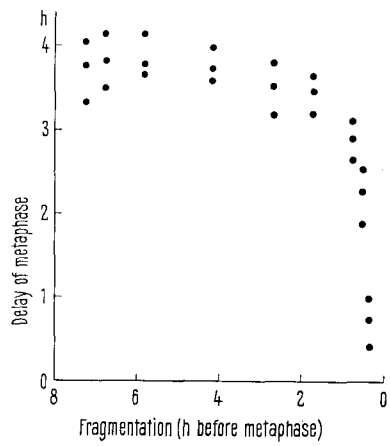

Fig. 1. Delay of mitosis by fragmentation of plasmodia at different times of mitotic cycle. The fragments of each plasmodium were allowed to coalesce in 3 separate groups in different Petri dishes.

[1] Supported by AEC contract No. COO-1432-8.

[2] F. L. HOWARD, Ann. Botany *46*, 461 (1932).

[3] With this term we denote the time which elapses from any stage of mitosis to the same stage of the next mitosis.

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[5] E. GUTTES, S. GUTTES and H. P. RUSCH, Devl. Biol. *3*, 588 (1961).

[6] E. GUTTES and S. GUTTES, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT, Academic Press, New York 1964), vol. 1, p. 43.

[7] E. GUTTES and S. GUTTES, Experientia *19*, 13 (1963).

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at prophase at the time of fragmentation they proceeded through mitosis without appreciable delay. Fragmentation at a time more than approximately 1.0 h prior to metaphase resulted in delay of mitosis of approximately 4 h.

Figure 2A shows the result of an experiment in which the premitotic plasmodia at the time of fusion were at a stage of the mitotic cycle a little more than 3 h prior to mitosis. The nuclei of the composite plasmodia entered mitosis at a time which was half-way between the times at which the nuclei began to divide in the premitotic and postmitotic control fragments.

If the premitotic plasmodia were at a stage approximately 1 h before mitosis at the time of fusion (Figure 2B), division of the nuclei of the composite plasmodia began at a time which was closer to that of the premitotic control fragments than to that of the postmitotic fragments.

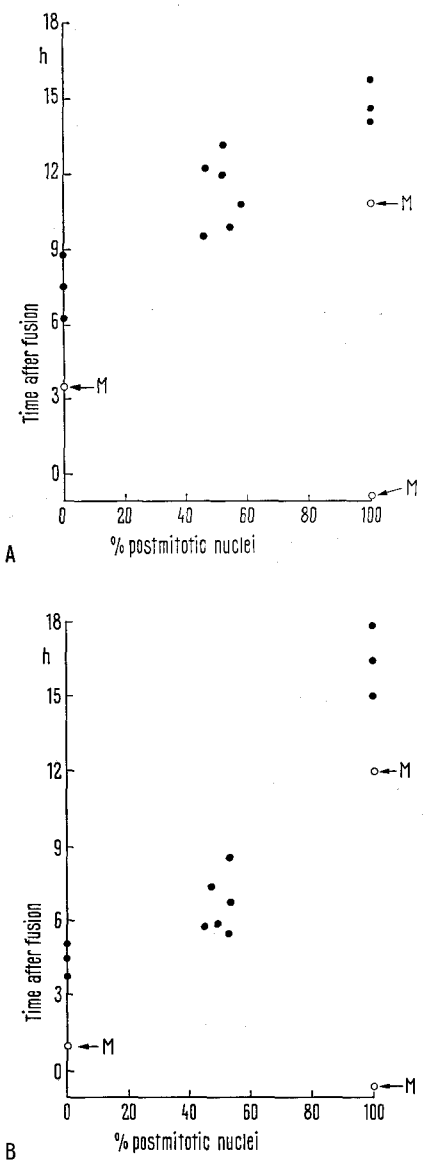

Fig. 2. Time of synchronous mitosis after coalescence of premitotic and postmitotic plasmodial fragments. 'Fusion' refers to the time when the fragments were placed on filter paper. Closed circles, mitosis of postmitotic, premitotic, and mixed fragments, respectively. Open circles, mitosis (M) of non-fragmented plasmodia from first and second batch. (A) At the time of fusion the premitotic fragments were at a stage of the mitotic cycle a little more than 3 h before mitosis. (B) At the time of fusion the premitotic fragments were at a stage approximately 1 h before mitosis.

Discussion. Fusion of premitotic with postmitotic plasmodial fragments in such a proportion that the nuclei of both stages are present in about equal amounts is equivalent to fusion of one uninucleated, premitotic cell with another, also uninucleated, postmitotic cell. The result in our experiments was dependent upon how close to mitosis the premitotic pieces were at the time of fusion. In the combination shown in Figure 2A the length of time by which the premitotic nuclei were set back, and the postmitotic nuclei were advanced, with respect to the next mitosis, was in proportion to the change in the amount of cytoplasm per nucleus. This result confirms the findings of previous experiments using either asynchronous suspensions of large numbers of microplasmodia[4] or whole plasmodia[9] and suggests that, under a given set of environmental conditions, the processes which are directly involved in mitosis are not initiated unless a minimum amount of cytoplasm is present per nucleus. A similar correlation has also been found for *A. proteus*[10]. The significance of this correlation could be that an alteration in energy metabolism favorable to mitosis[11,12] is initiated when the ratio between utilization and production of chemically bound energy reaches a critical value.

The experiment in Figure 2B showed a dominant effect of the premitotic fragments when fusion took place at a time approximately 1 h prior to mitosis of the latter. It might be significant that this is the time at which the previously central nucleolus begins rapidly to increase in size and to assume a slightly eccentric position[5] in the preparation for mitosis. The dominance of the premitotic fragments was not strong enough to suggest the presence of a triggering mechanism. It seems more likely that synthesis of 'mitotic' substances was underway in the premitotic plasmodia at this stage and that the time between fusion and the beginning of the next mitosis in the composite plasmodia was reduced by the time needed to produce the substances present at the time of fusion. This suggestion is supported by the finding[13] that, in *P. polycephalum*, the intermitotic period following upon a mitosis which was delayed by irradiation with UV-light, is considerably shorter than the corresponding period in unirradiated control halves.

Zusammenfassung. Nach Fusion von promitotischen mit postmitotischen Fragmenten des Schleimpilzes *Physarum polycephalum* (Zahlenverhältnis von postmitotischen zu promitotischen Kernen: 1/1) teilten sich die Kerne der so hergestellten Plasmodien synchron.

E. Guttes, Vimala R. Devi and Sophie Guttes

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