phosphate was carried out in Ringer-Krebs bicarbonate buffer without calcium in an atmosphere of 95% CO_2, 5% O_2, for 60 min at 37°C (1.5 μC ^{32}P-phosphate per 1 ml of suspension).

The isolated nuclei were quickly washed with ice-cold 0.1 *M* phosphate buffer (pH 7.4) then extracted by gentle homogenisation with the same buffer and centrifuged. The sediment contained the nuclei, and the supernatant a portion of the ribonucleoproteide from the nuclei. From the supernatant the RNP was precipitated with 5% trichloracetic acid in the cold and was termed sediment I.

The nuclear sediment was treated for 15 min with ice-cold 6% sodium *p*-aminosalicylate solution. Then the same volume of water saturated phenol was added (pH 7) and extraction was continued for another 30 min. After centrifugation the viscose supernatant (the water layer), containing DNA and RNA, was precipitated with alcohol in the presence of potassium acetate buffer at pH 5.2 and left overnight in a refrigerator (Sediment II). All these operations were carried out at 3–4°C.

After an addition of alcohol, sediment III was obtained from the phenol layer and the precipitate at the boundary of the water and phenol layer.

Sediments I–III were subjected to alkaline hydrolysis (0.5 *M* KOH, 18 h, at 37°C) and the hydrolysates were then purified on charcoal after removing of KOH with perchloric acid by a modification of the technique of LEDIG et al.[7]. Fractions I–III, corresponding to sediments I–III, were obtained after elution from the charcoal. Their phosphorus content and the radioactivity were then determined.

Tab. I

Relative radioactivities of nuclear RNA fractions (fraction I = 1.0)

Experiment No.	Fraction No. I	II	III	Remarks
1	1.0	6.3	4.5	*in vivo*, rabbits ♀
2	1.0	4.2	3.8	*in vivo*, rabbits ♀
3	1.0	1.5	1.5	rabbits after partial hepatectomy, spec. activities counted after separation of nucleotides by electrophoresis
4	1.0	2.4	1.4	Incorporation into cell suspensions

Tab. II. Rabbit after partial hepatectomy, RNA composition of fraction II

	AMP	UMP	GMP	CMP	A + U/G + C
(a) Relative RNA base composition	10.00	9.40	16.40	11.90	0.68
(b) Relative radioactivities of RNA nucleotides	10.00	9.00	8.60	7.80	1.16

In some cases the RNA hydrolysates were also subjected to high voltage electrophoresis. The radioactivity of the UV absorbing spots was measured directly on the electrophoreograms. The content of nucleotides in the spots was determined in eluates after elution with 0.01 *M* HCl from their UV absorption values.

Results and Discussion. It has been found that individual RNA fractions, which can be extracted from citrate nuclei of rabbit liver cells differ from one another by their specific activities in the following order: Fraction II > fraction III > fraction I (Table I)[8].

Fraction II, which in most cases is the most active fraction, represents a substantial portion of the nuclear RNA (more than 50%). As to its base composition, this fraction is a marked G–C type RNA (Table II). The incorporation of labelled phosphate at the same time indicates that fraction II also contains another RNA type, i.e. the A–U type, masked by an excess of G–C RNA, but it is revealed owing to its considerably higher specific activity despite its being present in smaler proportion (Table II).

After having compared the results of our experiments with those carried out with microorganisms[9], we may assume that citrate nuclei contain RNA which resembles DNA in its base composition and may be therefore considered to be m-RNA. The extraction of 'phenolic nuclei' from Ehrlich ascitic tumor with *p*-aminosalicylate and phenol gave similar results[10].

The fact that m-RNA is present in the fraction which can be extracted with *p*-aminosalicylate and phenol suggests that it might exist in some bound form in the cell nuclei. We cannot, however, exclude the possibility that divalent metal ions might also be participating in such bonds, in part at least. This may be a similar case to the one assumed by KIRBY for the binding of DNA in the deoxyribonucleoproteid complex[11].

Zusammenfassung. Die in 5%iger Zitronensäure isolierten Kaninchenleberkerne enthalten eine minimale Fraktion von RNS, die sich durch ihren Gehalt an Basen der DNS annähert (A + U/G + C > 1). Diese RNS wird während der Extraktion mit dem 6%igen Natrium-*p*-Amino-salicylat und dem Phenol zusammen mit der DNS und RNS vom G + C-Typ gewonnen.

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Initiation of Mitosis in Post-Mitotic Nuclei of *Physarum polycephalum*[1]

The myxomycete *Physarum polycephalum* can be grown in the form of microplasmodia in submersed agitated culture on semi-defined liquid medium[2]. When the microplasmodia are brought into contact with one another on filter paper, they coalesce readily to form large plasmodia

[1] Supported by NIH Grant No. RG-8495.
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in which the nuclei are mitotically synchronized[3,4]. The first synchronous post-fusion mitosis occurs approximately 6–7 h after coalescence, and from then on the nuclei divide in synchrony approximately every 12–14 h[4]. The time which elapses between the beginning of coalescence and the first synchronous post-fusion mitosis is approximately one-half of the average generation time of the microplasmodia at the time they were removed from the culture flasks[3]. Hence, for those nuclei which had under-gone mitosis just prior to the fusion, the next following intermitotic period is abbreviated by one half. On the other hand, those nuclei which at the time of fusion were preparing for mitosis divide after a period approximately 50% longer than the normal intermitotic period.

In the extreme, post-mitotic nuclei may divide as early as 45 min after telophase, provided that they coalesce with a large plasmodium in time before the nuclei of the latter enter prophase. This is shown in the following experiment. Microplasmodia from an agitated culture were placed, as described previously[4,5], in circular areas on filter paper supported by glass beads in Petri dishes. 10 min later, growth medium was added to some of the Petri dishes. At this time, only the microplasmodia at the periphery of the microplasmodial aggregates had coalesced, while those in the more central parts were still single. As control, the other Petri dishes received growth medium 1–$^1/_2$ h after the microplasmodia, were placed on the filter paper. At this time almost all microplasmodia in these dishes had coalesced. Since growth medium is inhibitory to coalescence[6], those microplasmodia in the experimental dishes which at the time of its addition were still singular, during the next 12 h coalesced with the rest of the plasmodium at a retarded rate. As they continued their respective mitotic cycles undisturbed during this period, some of them coalesced with the large plasmodium just after their own nuclei had divided. As a result, a few of the nuclei present in the large plasmodia at the beginning of the first post-fusion prophase, were received from microplasmodia in which mitosis had occurred only a short while ago.

Figure A shows nuclei at 45 min after mitosis. Post-mitotic nuclei of that stage contain 2–4 small nucleoli, which gradually fuse with one another and eventually form one central nucleolus (Figure B). At prophase, the chromosomes begin to fill the nucleus more evenly and the nucleolus is displaced toward the nuclear periphery. Figure C shows nuclei of an experimental plasmodium at the beginning of the first post-fusion prophase. The nucleus marked by an arrow is smaller than the others and contains 2 nucleoli indicating that its previous division took place not more than 45 min ago. The percentage of such nuclei was 1.2%, whereas in the control plasmodia only 0.07% of the nuclei entering prophase had multiple nucleoli. No such nuclei were found in either the experimental or the control plasmodia during the second post-fusion prophase. After the first post-fusion mitosis, all except 0.06% of the nuclei in the experimental plasmodia and 0.07% of the nuclei in the control plasmodia were found in telophase. Hence, most if not all of the post-mitotic nuclei entering prophase divided together with the others.

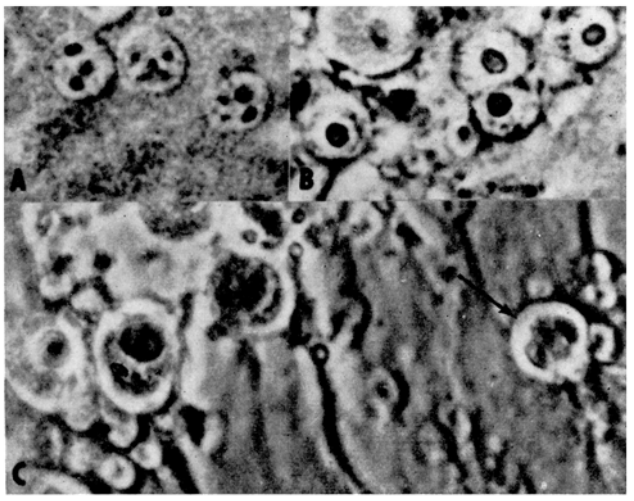

Fig. A–C. Nuclei at various stages of the mitotic cycle. Smear preparations, fixation in 95% ethanol, phase contrast. Magnification: × 1730.—A, approximately 45 min after mitosis.—B, mid-interphase.—C, beginning of the first synchronous post-fusion prophase in an experimental plasmodium. Arrow pointing at a nucleus which has divided not more than 45 min before.

Additional evidence that nuclei can participate in the first post-fusion mitosis without having completed a full intermitotic period, is provided by the ability of a few nuclei to incorporate thymidine-H^3 in pulse experiments during prophase. Sectors from experimental and from control plasmodia were placed, during the first post-fusion prophase, for 10 min, on growth medium containing thymidine-H^3 (concentration 20 µl/ml, spec. activity 6.25 c/m*M*, from Schwarz Bioresearch Inc., Mt. Vernon, N.Y., U.S.A.). For comparison, other sectors from the same plasmodia were supplied with tritiated thymidine during the second post-fusion prophase. Smear preparations from both groups were fixed with 95% ethanol (at 0°C) and processed for autoradiography (stripping film, Kodak AR-10 plates). During the first post-fusion prophase, 25.7% of the nuclei were labelled in the experimental plasmodia, as compared to only 2.7% in the control plasmodia. During the second post-fusion prophase, only 0.08% of the nuclei became labelled in the experimental plasmodia, and 0.11% in the control plasmodia. The almost complete absence of labelled nuclei during the second post-fusion prophase is in agreement with previous findings, by autoradiography[7] and by other methods[5], that in established surface plasmodia of *Ph. polycephalum* DNA synthesis occurs only during a period of a few hours immediately after mitosis. Hence, the labelling of some nuclei in both groups of plasmodia during the first post-fusion prophase suggests that they had divided only a short while before entering prophase.

The finding that mitosis is initiated in post-mitotic nuclei when they are present in pre-mitotic environment is reminiscent of previous observations by HÄMMERLING[8] on Acetabularia, WEISZ[9] on Stentor, and DANIELS[10] on the giant ameba Pelomyxa. This provides further evidence for the hypothesis that nuclear division is initiated by diffusible factors. Apparently the completion of a full mitotic cycle or even of DNA synthesis is not an obligatory prerequisite for a nucleus to respond to such factors.

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Zusammenfassung. Durch Fusion von Mikroplasmodien des Schleimpilzes *Physarum polycephalum* wird der Mitosecyclus ihrer Kerne synchronisiert. Durch künstlich verzögerte Fusion können Tochterkerne einer soeben erfolgten Mitose zur Teilnahme an der ersten synchronen Mitose nach der Fusion gezwungen werden, bevor die morphologische Rekonstitution und die Duplikation ihrer DNS vollendet sind.

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Modifications of Thyroid Activity by Melatonin

In a previous work we have reported that the administration of a pineal extract is capable of preventing thiouracil goitre in rats[1]. These results are in keeping with those of ARON et al.[2], who recently observed exophthalmos in turtles after pinealectomy. On the contrary, YAMADA et al.[3] did not find after treatment with thiouracil any significative weight difference in thyroids from pinealectomized and control rats.

These results moved us to investigate the influence on the thyroid of Melatonin, which is N-acetyl-5-methoxytryptamine, a lightening substance, isolated in the pineal body by LERNER et al.[4].

Material and Methods. These experiments were carried out on albino Wistar male rats (mean weight 150 g), fed during the experimental period of ten days with Purina Fox. Melatonin was supplied by Regis Chem. Co., California, eluted in hydroalcoholic solution (50% water and 50% ethyl alcohol 90°C), and injected subcutaneously.

The animals were divided into six groups: the first group (A) were the controls; the second group (B) drank *ad libitum* a suspension of 0.3 mg/ml of methylthiouracil (MTU); the third group (C) were injected every day with 0.25 ml of hydroalcoholic solution; the fourth group (D) drank *ad libitum* a suspension of 0.3 mg/ml of MTU and were injected every day with 0.25 ml of hydroalcoholic solution; the fifth group (E) received subcutaneously every day 150 γ of melatonin in 0.25 ml of hydroalcoholic solution; the sixth group (F) drank *ad libitum* a suspension of 0.3 mg/ml of MTU and were injected every day with 150 γ of melatonin in 0.25 ml of hydroalcoholic solution. On the 10th day, each rat was given 0.8 μC of carrier-free radioiodine.

After 24 h, the rats were killed and the thyroid glands were immediately removed and weighed on a torsion balance. Thyroid radioactivity was then calculated as % of the dose formerly injected (a standard was specifically prepared).

Histological studies were made on the thyroid tissue stained with hematoxylin-eosin and hematoxylin-orange-phospho-molybdic acid-aniline blue. The cellular height was measured with the help of a Leitz Ortholux Microscope. The cells magnified 2000 times in diameter were drawn on millimeter paper; an average of 100 cells were measured for every tissue slide. Standard deviation of results was evaluated following the formula.

$$\sigma(m) = \sqrt{\frac{Sx^2}{(n-1)\,n}}$$

Parameter t was calculated as follows

$$t = \frac{M_1 - M_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad \text{where } \sigma = \sqrt{\frac{Sx^2_1 + Sx^2_2}{(n_1-1) + (n_2-1)}}$$

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Group and number of animals		Treatment		Thyroid weight mg % of body weight	Cell height in μ	Thyroid ^{131}I uptake % of the dose
A	(10)	Purina Fox diet, H_2O		14.9 ± 1.5	5.8 ± 0.16	42.2 ± 3.4
B	(10)	Purina Fox diet, H_2O + MTU		27.5 ± 1	13 ± 0.11	3.8 ± 0.02
C	(10)	Purina Fox diet, H_2O, hydroalcoholic solution		12.3 ± 1.7	7.6 ± 0.14	35 ± 4.4
D	(10)	Purina Fox diet, H_2O, MTU, hydroalcoholic solution		20.7 ± 1.3	13.5 ± 0.39	11.7 ± 4.1
E	(10)	Purina Fox diet, H_2O, melatonin in hydroalcoholic solution		11.4 ± 0.21	3 ± 0.95	2 ± 0.2
F	(10)	Purina Fox diet, H_2O, MTU, melatonin in hydroalcoholic solution		19.3 ± 1.7	8.7 ± 0.19	3.9 ± 0.03
		t	A–C	1.13	2.8	1.16
			A–E	2.23	15	10.8
			B–D	4.07	0.88	3.3
			B–F	3.56	9.5	13.2
			C–E	0.48	29	7.27
			D–F	1.1	10.9	4.12

theoric t ($P = 0.05$) = 2.3; ($P = 0.01$) = 3.35; ($P = 0.001$) = 5.04