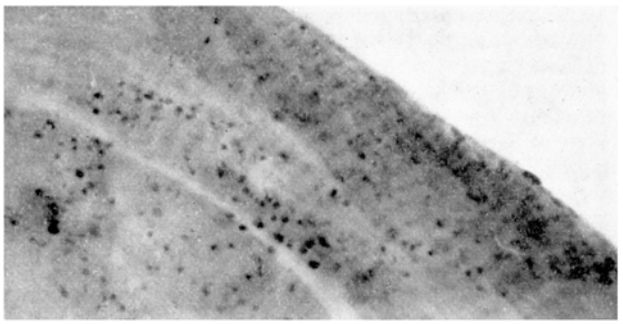
Wie schon erwähnt[2,3], stellt die Skeletmuskulatur der Amphibien ein besonders gutes Untersuchungsobjekt dar. Der histochemische Nachweis von Glykogen durch die PAS-Reaktion gelang an Ringbinden der Rumpfmuskulatur von *Amblystoma mexicanum* Cope. Als besonders geeignet erscheinen ältere Exemplare der larvalen Form, bei denen Ringbinden praktisch immer vorhanden sind, wie schon Voss[4] bemerkt hat. Um das Antreffen von Ringbinden wahrscheinlicher zu gestalten, wurden Ausschnitte von grösserer Fläche in Schafferscher Flüssigkeit fixiert und nach Paraffineinbettung in 6 μ dicke Schnitte zerlegt. An celloidinierten Schnitten wurde die PAS-Reaktion zur Glykogendarstellung gemäss den Anweisungen von HOTCHKISS[5] und PEARSE[6] unter den gebräuchlichen Kontrollbedingungen ausgeführt.

Im Bereich vieler Ringbinden konnte eine purpurrote, zwischen den Myofibrillen liegende, körnige Substanz festgestellt werden, die – heutigen Vorstellungen gemäss – als Glykogen oder glykogenähnliche Substanz angesprochen werden kann (Figur). Im allgemeinen enthalten die mit Ringbinden befallenen Muskelfasern ebensoviel Glykogen wie die normalen Fasern. In schlechter fixierten Abschnitten der Präparate ist innerhalb der Ringbindenfasern, genau wie in gewöhnlichen Muskelfasern, eine typische Polverlagerung der PAS-Reaktion feststellbar. Diese Beobachtung ist ein Beitrag zum Beweis, dass die Ringbinde hypolemmal angeordnet ist. Zwischen dem zentralen Teil der Muskelfaser und der ringartigen Struktur besteht also keine besondere Wand, welche die Polverlagerung des Glykogens aufhalten könnte.

In Hinsicht auf den Ausfall der PAS-Glykogen-Reaktion unterscheiden sich die Myofibrillenkomplexe der Ringbinden also nicht von den normalen Fibrillen. Dieses bezieht sich jedoch nur auf die sogenannten «gewöhnlichen» Ringbinden. Es gibt nämlich Entartungserscheinungen im Bereich der Ringbinden selbst. Diese beruhen auf einer Änderung der Färbbarkeit in der Azan-[7] oder van Gieson-Methode[8]. Ausserdem können die Ringbinden glasig entarten oder auch körnig zerfallen[1]. In diesen Fällen scheint der Ausfall der PAS-Reaktion verändert zu sein.



Körnige Glykogensubstanz im Bereich der Ringbinde und im umschlungenen Muskelfaserteil, Detail eines Querschnittes einer Ringbindenstruktur aus der hypaxonischen Rumpfmuskulatur der larvalen Form von *Amblystoma mexicanum* Cope, 25 cm Länge, Fixierung nach SCHAFFER, PAS-Reaktion am celloidinierten Paraffinschnitt nach HOTCHKISS[5] und PEARSE[6], ohne Gegenfärbung, Ob. 90, Ok. 10.

Summary. Glycogen substance, detected by the PAS-reaction in the so-called 'Ringbinden' of the trunk musculature from the larval form of *Amblystoma mexicanum* Cope, is described. The PAS-glycogen reaction in the myofibril complex of the common 'Ringbinde' does not differ from that found in the normal one.

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The Presence of RNA of the A-U Type in Citrate Nuclei Isolated from Rabbit Liver Cells

SIBATANI et al.[1] have shown that a special type of RNA with an especially high rate of turnover is synthetised in thymocyte nuclei. Its base composition resembles that of DNA. This type of RNA most probably corresponds in its function to the so-called messenger RNA (m-RNA) found in bacteria[2]. It is assumed that his type of RNA also exists in other types of animal cells[3,4].

We tried whether it is possible to use the so-called citrate nuclei, prepared in strong solution of citric acid, for the study of m-RNA. By means of the citrate method, it is possible to prepare very pure nuclei, not contaminated by cytoplasmic RNA, with good yields[5]. This method is easily applicable to a larger quantity of tissue, which would be very suitable for the eventual isolation of m-RNA. It has been known that a certain amount of nuclear RNA is extracted by citric acid but the rest of nuclei is enriched by RNA of a higher turnover[6].

Methods. The initial material for fractionating nuclear RNA were in all cases citrate nuclei isolated from rabbit liver cells in 5% citric acid. Homogenisation was achieved by the Waring Blendor. In our experiments we used rabbits weighing 1140–1425 g, starved for 24 h and given an intravenous injection of ^{32}P-phosphate in physiological saline (250 μC/kg weight). 60 min after the administration of labelled phosphate, the animals were killed by a blow on the head and subsequent bleeding to death. Normal and regenerating rabbit livers were used. In one experiment, the *in vitro* incorporation of ^{32}P-phosphate was followed using a suspension of liver cells. The suspension was prepared by gradually squeezing the tissue through a nylon sieve, gauze and miller's silk. Incubation with ^{32}P-

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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

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