heated at 56°C for 30 min and absorbed with equal volumes of non-treated erythrocytes for 30 min at room temperature. To serial dilutions of the serum samples (prepared in 0.5 ml volumes in phosphate buffer—1:100 normal rabbit serum diluent) were added 0.05 ml volumes of the hydralazine-red blood cell conjugates. The initial serum specimen (2 weeks) consistently reacted with a titer of 1:240, while the second serum specimen (10 months) had a titer of 1:80 (Table). These titers could be completely inhibited by prior incubation of the sera with free hydralazine hydrochloride (1.0 mg to 10.0 mg) prior to addition of the conjugated red cells, but not by prior addition of bovine serum albumin (1 mg/ml), ragweed pollen extract (0.1 mg/ml) or penicillin (1000 u/ml). Similarly, the serum samples did not agglutinate red cell conjugated by means of BDB to bovine serum albumin, ragweed extract, or penicillin. Several sets of 'control' sera were tested with the hydralazine-red cell preparations as follows: (a) three serum specimens from clinically diagnosed cases of SLE (ranging from 1 to 5 years duration and presumably unrelated to hydralazine therapy) were negative for hemagglutination activity; (b) 27 serum samples, from the clinical serology laboratory obtained from presumably normal blood donors, were negative for anti-hydralazine hemagglutination activity. On the other hand, sera from 4 rabbits, following a series of multiple injections over a period of 6 months with hydralazine (10 mg hydralazine/ml Freund's adjuvant as a water-in-oil emulsion) gave positive hemagglutination reactions with hydralazine-red blood cell suspensions. Pre-injection and early post-injection rabbit sera were negative. Late post injection sera from two rabbits (six months after the initial series of injections) had titers of 1:80, while sera of the other two rabbits gave a titer of 1:20. These titers could be completely inhibited by prior incubation of the sera with hydralazine, but not with the other antigens used above.

Comparative inhibition of hemagglutination of patient's and 'immune' rabbit sera and hydralazine-RBC suspension by prior incubation with buffer, free hydralazine or other antigens

| Preincubation | Hemagglutination titer | | | | | |
|---|---|---|---|---|---|---|
| | Patient's serum | | Rabbit sera (*post*) | | | |
| | A (2 week) | B (10 month) | a | b | c | d |
| None (buffer) | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |
| Hydralazine 0.1 mg | 1:40 | 1:20 | 1:20 | 1:15 | 1:2 | 1:2 |
| Hydralazine 1.0 mg | 1:10 | 1:2 | 1:4 | 1:2 | 0 | 0 |
| Hydralazine 10.0 mg | 0 | 0 | 0 | 0 | 0 | 0 |
| Ragweed Pollen extract 7500 PNU | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |
| Bovine serum albumin 10 mg | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |
| Penicillin 10000 U | 1:240 | 1:80 | 1:80 | 1:80 | 1:20 | 1:20 |

The anti-hydralazine activity of the reactive sera, both from the subject and the rabbits, was associated with $\gamma$-globulin. Serum samples were subjected to zone electrophoresis in 1% agar. Following electrophoresis at 150 volts for 4 h, 0.5 cm agar strips were eluted into phosphate buffer. Anti-hydralazine hemagglutination activity was recovered only in the eluates corresponding to the $\gamma$-globulin region, but not in eluates identified by paper electrophoresis or immunophoresis as containing albumin, $\alpha$- or $\beta$-globulins. The anti-hydralazine activity of the $\gamma$-globulin eluates could be eliminated by prior reaction of the eluate with appropriate goat anti-$\gamma$-globulin sera for 1 h at 37°C.

These observations suggest that an anti-hydralazine factor, presumably antibody, may be found associated with SLE-like symptomatology following long term therapy with the drug. A similar factor can be obtained after long term injection of rabbits with hydralazine. Others have observed a possible SLE like symptomatology (L.E. cell factor, suggestive tissue pathology, etc.) in several experimental animal species following feeding of large quantities of hydralazine over long periods of time[4,5]. It is not known whether such symptomatology, either serological or histological, is causal or incidental. It is presently premature to postulate any immunological relationship between hydralazine, the anti-hydralazine factor described here, and the hydralazine Lupus syndrome or idiopathic SLE. On the other hand, it would be of value to examine anti-hydralazine antibodies in as many individuals as possible who present a similar hydralazine Lupus syndrome. Similarly, it should be of interest to study sera from other individuals who have taken the drug in various quantities, but who have not had the syndrome, as well as sera from more patients with SLE unrelated to hydralazine therapy.

*Résumé.* La présence d'un facteur anti-hydralazine, probablement un anticorps, a été démontrée par l'emploi d'une technique d'hémagglutination dans le sérum d'un malade qui manifestait des symptômes de lupus érythémateux après un long traitement avec de l'hydralazine. Le même facteur anti-hydralazine a été observé dans le sérum des lapins qui avaient reçu des injections répétées d'émulsion d'hydralazine dans un adjuvant.

H. Friedman and W. I. Heine

*Departments of Microbiology and Cardiology, Albert Einstein Medical Center, Philadelphia (Pa., U.S.A.), August 13, 1962.*

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## Über die PAS-Glykogen-Reaktion im Bereich der Ringbinden bei *Amblystoma mexicanum* Cope

Hinweise auf das Auftreten, die Bedeutung und das Schrifttum der Ringbinden brachten bereits frühere Notizen[1-3]. Bisher sind an diesen Strukturen histochemische Untersuchungen kaum ausgeführt worden und zwar vorallem aus zweierlei Gründen: 1. wegen ihres unsteten und seltenen Auftretens und 2. deswegen, weil histochemische Arbeitsweisen die morphologischen Gegebenheiten so verunstalten, dass ihre Erkennung schwierig werden kann. Da es aber nicht klar ist, ob die Ringbinden normale oder pathologische Gebilde sind, erscheint ihre histochemische Bearbeitung sinnvoll.

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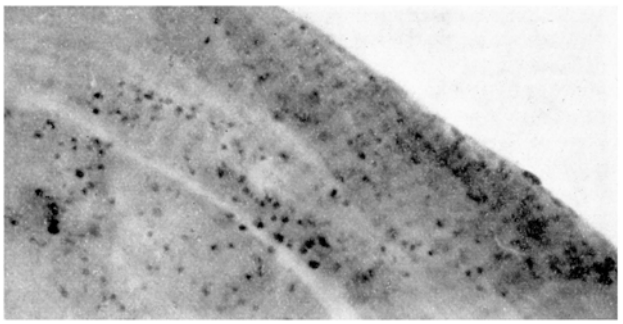
[3] A. Jonecko, Exper. *18*, 166 (1962).

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.

A. JONECKO

*Institut für Histologie und Embryologie, Schlesische Medizinische Akademie, Zabrze-Rokitnica (Polen), 13. Juli, 1962.*

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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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