dingungen Glutamin als Citrullinbildner wirksamer ist als Glutaminsäure plus Ammoniumsalz. Wir werden diese Versuche an anderer Stelle ausführlich besprechen und dort auch die Einzelheiten der Versuchsanordnung und der Bestimmungsmethoden mitteilen.

Im Hinblick auf die elektive Färbbarkeit der Mitochondrien durch Janusgrün (Dimethyl-2,7-phenyl-10-amino-3-[(dimethylamino)-4'-benzolazo]-6-phenazonium-10-chlorid) haben wir untersucht, ob die Citrullinsynthese durch diesen Farbstoff gehemmt wird. Das ist in der Tat schon bei Konzentrationen der Größenordnung $10^{-5}$ bis $10^{-6}$ Mol der Fall. Die Mitochondriensuspension bindet den Farbstoff außerordentlich fest. Nach Fällung der Suspension mit Trichloressigsäure erhält man bei kleinen Farbstoffkonzentrationen (z.B. bis $1{,}6 \cdot 10^{-5}$ Mol) völlig farblose überstehende Lösungen. Es hat sich nun gezeigt, daß die Hemmung durch den Farbstoff praktisch vollständig ist, wenn man so viel Janusgrün zusetzt, daß man nach der Fällung der Suspension durch Trichloressigsäure gerade eine schwachgefärbte überstehende Lösung erhält. Man kann annehmen, daß in diesem Fall die Mitochondrien mit dem Farbstoff «gesättigt» sind. Mit abnehmender Konzentration des Farbstoffs nimmt auch die Hemmung ab. Man erhält, wenn man die Aktivität des Systems gegen den Logarithmus der Farbstoffkonzentration aufträgt, die Kurven in Abb. 3, die den typischen S-förmigen Verlauf von Dissoziationskurven erkennen lassen. Wenn man die Aktivität des Ferments als Maß für den «Sättigungsgrad» der Mitochondrien mit dem Farbstoff annimmt, kann man aus diesen Kurven ablesen, daß die Mitochondrien bei einer Konzentration des Janusgrüns von etwa $5 \cdot 10^{-6}$ Mol zur Hälfte «gesättigt» sind. Wir betrachten das Parallelgehen von Fermenthemmung und elektiver Farbstoffbindung durch die Mitochondrien als weitere Stütze für unsere Annahme, daß die Synthese in den Mitochondrien stattfindet. Die Kurven zeigen, daß die Synthese im glutaminsäurehaltigen System stärker gehemmt wird als im glutaminhaltigen. Auch in dieser Tatsache spiegelt sich eine gewisse Überlegenheit des Glutamins als Citrullinbildner. Über die Wirkungsart des Janusgrüns können wir nichts aussagen.

Unsere Versuche weisen erneut auf die große Bedeutung der Mitochondrien für die chemischen Umsetzungen in der Zelle hin. SCHNEIDER[1] und HOGEBOOM und Mitarbeiter[2,3] haben nachgewiesen, daß wichtige Respirationsfermente, Succinoxydase und Zytochromoxydase, in den Mitochondrien angehäuft sind. Es scheint, daß die gesamte Succinoxydase der Leberzelle sich in den Mitochondrien vorfindet[2]. Die Darstellungsmethode von GREEN, LOOMIS und AUERBACH[4] für die «Zyklophorase» aus Niere, das Fermentsystem, welches Pyruvat nach dem Tricarbonsäurezyklus oxydiert, läßt vermuten, daß auch dieses System Mitochondrien als wesentlichen Bestandteil enthält. Neuerdings teilen auch LEHNINGER und KENNEDY[5] Beobachtungen mit, die darauf schließen lassen, daß die Fermente der Fettsäureoxydation ebenfalls in den Mitochondrien lokalisiert sind.

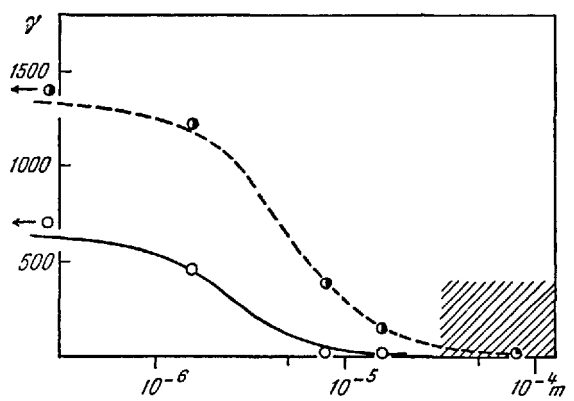


Abb. 3. *Hemmung der Citrullinsynthese durch Janusgrün.* (Versuchsbedingungen siehe Abb. 2). Glutaminsäure, Glutamin: 50 µMol pro Ansatz. Abszisse: Konzentration des Farbstoffes in µMol/cm³. Ordinate: Citrullinbildung in γ. O Glutaminsäure + $NH_4Cl$. ● Glutamin ohne Zusatz. Die Punkte links außen geben die Citrullinbildung ohne Farbstoffzusatz. Die schraffierte Fläche bezeichnet den Konzentrationsbereich des Farbstoffs, in welchem die überstehende Lösung nach Fällung der Suspension mit Trichloressigsäure gefärbt erscheint, in welchem also die Mitochondrien nicht mehr allen Farbstoff zu binden vermögen.

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F. LEUTHARDT und A. F. MÜLLER

Physiologisch-chemisches Institut der Universität Zürich, den 22. September 1948.

*Summary*

By differential centrifugation of rat-liver homogenates prepared in isotonic solutions of mannitol it is possible to obtain mitochondria in suspensions that are apparently free from other cellular components. Our preparation contains all of the enzymes responsible for the conversion of ornithine to citrulline with glutamic acid and ammonium or glutamine. The synthesis is strongly inhibited by Janus green, which is a specific vital stain for mitochondria.

[1] W. C. SCHNEIDER, J. Biol. Chem. *165*, 585 (1946).

[2] G. H. HOGEBOOM, W. C. SCHNEIDER und G. E. PALLADE, J. Biol. Chem. *172*, 619 (1948).

[3] G. H. HOGEBOOM, A. CLAUDE und R. D. HOTCHKISS, J. Biol. Chem. *165*, 615 (1946).

[4] D. E. GREEN, W. F. LOOMIS und V. H. AUERBACH, J. Biol. Chem. *172*, 389 (1948).

[5] A. L. LEHNINGER und E. P. KENNEDY, Fed. Proc. *7*, 166 (1948).

## On the Zoological Specificity of Myosin and Actin

The possibility of union of SZENT-GYÖRGYI's myosin and actin (formation of actomyosin[1]), taken from different zoological species[2], is examined. The present research was done to investigate the zoological specificity of proteins extracted from muscles.

The possibility of combining myosin and actin extracted from different muscles of the same animal had already been demonstrated by RÓZSA's[3] researches. In preliminary researches we united myosin with actin, the latter was stored for more than three weeks in a refrigerator after treatment with acetone and after drying. We extracted also WEBER and EDSALL's myosin (SZENT-GYÖRGYI's actomyosin) from the muscles of *Palinurus vulgaris* L. and from the foot of *Helix pomatia* L. *Helix's* actomyosin was not completely free from mucus. These actomyosins may both be pulled in threads, following WEBER's method[4]. With addition of adenosine triphos-

[1] According to SZENT-GYÖRGYI actomyosin is formed through the union of myosin and actin: A. SZENT-GYÖRGYI, *Chemistry of muscular contraction* (Academic Press, New York, 1947).

[2] The chemical compositions of the actomyosins of different zoological species are reported in: K. BAILEY, Adv. in Prot. Chem. *1*, 289 (1944).

[3] R. RÓZSA, unpublished, quoted after SZENT-GYÖRGYI (1947).

[4] H. H. WEBER, Naturwiss. *27*, 33 (1939).

phate (ATP) all these threads do contract according to SZENT-GYÖRGYI's method.[1]

In our researches we used muscles of mammals: *Cuniculus cuniculus* L.; birds: *Columba livia* BRISS.; Amphibians: *Rana esculenta* L.; bony fishes: *Carassius auratus* L., *C. vulgaris* NISSL.; *Eupomotis gibbosus* L.; crustacea: *Palinurus vulgaris* L. Following GUBA's method[2], we extracted actin from all these animals. The shape of actin particles in watery solutions has been examined with the viscosimetric method described by RANZI[3]; they all appeared fibrillar at $p_H$ 6·5. We extracted SZENT-GYÖRGYI's myosin, soluble in water, from rabbit, pigeon, *Carassius*, *Eupomotis*, langouste.

We mixed watery solutions of myosin (fibrillar particles) with actin solutions at $p_H$ 6·5. We then added $MgCl_2$ to about the concentration of 0·0005 *M*. At this moment CITTERIO read the viscosity. When actin and myosin, both of the same zoological species, are mixed, a sudden rise in specific viscosity is displayed; but the viscosity was, however, increased also in all the combinations in which (see table) we obtained threads.

After viscosity measurement we added KCl to about the concentration of 0·05 *M* and placed the samples in the refrigerator overnight. The following morning we centrifuged and dissolved the precipitate in EDSALL's solution. According to WEBER's method we then tried to obtain a thread from this solution, and we experimented with the ATP action upon this thread.

| Actin | Myosin | | | |
|---|---|---|---|---|
| | Rabbit | Pigeon | Bony fish | Langouste |
| Rabbit | ++ | ++ | + | – |
| Pigeon | | ++ | + | |
| Frog | ++ | ++ | + | – |
| Bony fish | ++ | ++ | + | – |
| Langouste | + | + | + | + |

++ Thread which contracts.
+ Much hydrated thread which contracts.
— It was not yet possible to obtain actomyosin.

The table shows the results of the experiments performed up to date. The table shows that the shape of the thread obtained depends upon the myosin rather than upon the actin. The threads, obtained from rabbit or pigeon's myosin, united to different actins of vertebrates, are always satisfactory[4]. The threads obtained from myosin of bony fish are always much hydrated. The scarcely hydrated threads contract to ⅓ of their original length if ATP is added. If the thread is strongly hydrated the reduction is only to ½ or ⅔. Particular attention must be paid to the behaviour of langouste's myosin. The latter, at least all that has been extracted up to date, cannot be combined with actins of vertebrates. We never obtained a precipitate when we mixed langouste's myosin with the actin of rabbit, frog, or bony fish. These mixtures show a specific viscosity equal or inferior to the average of the viscosity of the original solutions. Langouste's actin unites to rabbit, pigeon, and bony fish's myosin. ATP induces small contractions of the much hydrated threads of these actomyosins.

To investigate ATP action on actomyosin solutions, we sometimes took away 1 ml of the solution to which $MgCl_2$ and KCl had been previously added; then 0,5 ml of 1% ATP solution were added to this sample. As a control we used 1 ml of the same myosin and actin mixture with 0·5 ml of distilled water. The precipitate which appeared in the first sample was much more contracted than that of the control. However, no precipitate was to be seen in both, sample and control, when we used a mixture of langouste's myosin and actins of vertebrates.

These researches show, up to date, that it is possible to obtain actomyosin sensible to ATP by mixing myosin and actin of different species of vertebrates. Langouste's myosin does not combine with actins of vertebrates. Myosin of rabbit, pigeon, and bony fish combines with langouste's actin with the formation of actomyosins sensible to ATP.

We are grateful to Hoffmann-La Roche Ltd. of Basle for giving us an ATP solution in the form of the Na-salt and to the Consiglio Nazionale delle Ricerche for the material aid granted for this research.

M. CIGADA, P. CITTERIO, S. RANZI, and L. TOSI

Zoological Laboratory, University of Milan, June 17, 1948.

*Riassunto*

È possibile ottenere actomiosina, sensibile all'ATP, combinando miosine e actine di diverse specie di Vertebrati. La miosina di aragosta, per lo meno quale fin qui estratta; non si combina con le actine dei Vertebrati. Le miosine di coniglio, colombo e teleosteo si combinano con l'actina di aragosta formando un'actomiosina sensibile all'ATP.

[1] A. SZENT-GYÖRGYI, Studies Inst. Medical Chem. Univ. Szeged *1*, 17 (1942).

[2] A. SZENT-GYÖRGYI, *Chemistry of muscular contraction* (Academic Press, New York, 1947).

[3] S. RANZI, Boll. Soc. Ital. Biol. sper. *24* (1948).

[4] Unpublished electron micrographs show that solutions of actomyosin, from rabbit's myosin united with pigeon's actin and from pigeon's myosin united with rabbit's actin, contain fibrillar particles.



## Antigens in the Egg and Early Developmental Stages of the Sea-Urchin

In connection with a more extensive study of the chemical and physiological changes during development of the sea-urchin, a series of serological experiments has also been initiated in order to investigate changes eventually occuring in the serological properties. Specific antigenic structures, more or less identical with those in the adult, were recently demonstrated to be present in the earliest stages of development in the chick by SCHECHTMAN[1], and in the frog by COOPER[2]. A very extensive survey of the literature has been given by the latter author. Ontogenetic changes of organ-specific antigens had earlier been demonstrated by BURKE *et al.*[3], and others.

*Materials and methods*

The present investigation was performed on unfertilized eggs and on the following well-defined developmental stages of *Paracentrotus lividus:* 4 hours (32 and 64 cells), 7 hours (about 1 hour before hatching), 12 hours (early gastrulation), and 48 hours (plutei). Besides these stages, 48-hour stages, treated with a mixture of 95 parts seawater and 5 parts lithium chloride solution (isotonic with 32 per mille sea-water) during the first 20 hours of development were used (strongly vegetalized larvæ). In certain controls, unfertilized eggs and blastulæ of *Strongylocentrotus drœbackiensis* were also used. The material was quickly frozen and dried in this state.

Rabbit antisera were prepared by successively injecting suspensions of the material mentioned above. Three rabbits, whose sera had been tested before injection, were used for each preparation. The suspensions were made of finely ground material in 0,9 p.c. sodium chloride solution (1:10) and were used immediately after shaking vigorously for 2–3 hours. The rabbits received 7 intravenous injections of 3 ml of suspension at 2–3 days intervals. The last 4 injections were preceded on the previous night by injecting the same amount of suspension subcutaneously. All rabbits withstood the whole series of injections. The antisera were tested a week after the last injection, and when found to be sufficiently strong, the rabbits were bled to

[1] A. M. SCHECHTMAN, J. Exp. Zool. *105*, 329 (1947).

[2] R. S. COOPER, J. Exp. Zool. *101*, 143 (1946); *107*, 397 (1948).

[3] V. BURKE, N. P. SULLIVAN, H. PETERSEN, and R. WEED, J. Infect. Dis. *74*, 225 (1944).