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.

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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).

death from the carotis the following day. The blood was stored at room temperature for several hours, and then in the refrigerator overnight. The sera were collected, centrifuged, filtered in a Seitz-filter apparatus, and stored at 0° until use. The entire procedure was carried out under sterile conditions.

The antigen solutions were obtained by suspending the dried and finely ground material in physiological saline (1:10). The suspensions were shaken vigorously for 2–3 hours and centrifuged 1 hour in an angle-centrifuge at 5,000 rotations per minute. The solutions were entirely clear or somewhat opalescent and generally slightly coloured. After centrifuging, the preparations were dialysed for 24 hours in collodion bags in the cold against large, frequently changed volumes of physiological saline. The p_H of these solutions was about 7·5. Only freshly prepared solutions were used in the serological experiments.

For the purpose of standardization, all solutions were diluted to the same content of undialysable nitrogen. This method of course is not quite correct, as the nature and number of the nitrogen-containing compounds in the solutions are unknown. As a method of standardization, it seems however to be justified since the antigenic titer in the solutions was always proportional to the nitrogen content. This was even true when the solutions were centrifuged for various times at 17,000 g before dialysis, or upon filtration through a Seitz-filter apparatus. Thus, all the standard solutions were of approximately the same antigenic titer when tested against their homologous antisera (see below).

The presence of antigens in the sea-urchin extracts was established by specific precipitation tests. The different antigen dilutions (0·25 ml, after dilution with 0·9% saline) were thoroughly mixed with 0·25 ml of undiluted antiserum in small test tubes. These were kept partially immersed in a water bath at 37° for 2 hours and then in a refrigerator until the following morning. The time of the first visible particulation as well as a particulation after 2 and 24 hours was recorded. In certain cases, ring tests were performed in microtubes; 0·05 ml antigen dilution was carefully placed over the same amount of undiluted antiserum, and a ring formation at the interface was noted after 2 hours at room temperature. In all cases, normal rabbit serum plus antigen, antiserum plus 0·9% saline, and, if necessary, antiserum plus extract from unfertilized eggs of *Strongylocentrotus* and antiserum against *Strongylocentrotus* eggs plus *Paracentrotus*-antigen were used as controls.

Specific absorption experiments were performed by mixing 5–10 ml antiserum with undiluted antigen extract in optimal proportions. These were determined by "optimal proportion titration" as described by BOYD[1] of undiluted antiserum with antigen standard solution. The absorption was carried out with a volume of undiluted extract, containing an amount of nitrogen corresponding to the amount of "antigen"-nitrogen in the optimal tube at titration. The volume of antigen extract added under these conditions was usually equal to or less than $^1/_{10}$ of the volume of the antiserum used. In this way, large changes in volume with the absorptions could be avoided. The mixture of antiserum-antigen was kept at room temperature for 2 hours and then at 4° for 24 hours, after which the precipitates were centrifuged down. After absorption, the antisera were tested by the ring tests with different antigen dilutions and with unabsorbed antisera. In most cases, one absorption was sufficient for complete precipitation of the homologous antibodies. In only a few cases, a second absorption was necessary, at which $^1/_4$ of the original antigen amount was used. For titrations of the absorbed antisera, the same technique was used as described previously. In each tube, a volume of absorbed antiserum calculated to correspond to 0·25 ml of undiluted antiserum was used. The controls were diluted to the same volume with 0·9% saline.

Results

As is frequently found in immunization experiments with embryonic material, the antisera obtained were of rather weak titer. Quite satisfying and rapid (less than 15 minutes) flocculation reactions and strong ring tests could however be obtained with undiluted antisera and antigen standard solutions. These had been prepared so as to cause no precipitations when they were tested in full concentrations ($3.0 \cdot 10^{-5}$ *M N*/ml) against homologous antisera (precipitation inhibition owing to antigen excess). Under these conditions nearly every standard solution gave a precipitation optimum (most rapid reaction) at a dilution of 1/10–1/15, and the highest dilution at which a clear reaction could be obtained in 2 hours was generally at about 1/80. Thus in systematic experiments, dilution series were used starting from the concentrated standard solution over 1/5, 1/10, 1/15, 1/20, 1/30, ... up to 1/100 or 1/150. Undiluted or slightly diluted extracts, i.e., solutions with higher antigen content than the standard solutions, were also used in certain cases, especially after absorption.

Comparative experiments with *Paracentrotus* and *Strongylocentrotus* extracts showed a marked species specificity of the antigens in our solutions, although thus far no true comparison of the extracts from the two genera can be made. Antisera against, e.g., unfertilized *Paracentrotus* or *Strongylocentrotus* eggs however reacted slowly and only with slightly diluted heterologous antigen solutions; inhibition phenomena due to antigenic excess were completely lacking. With their homologous antigens, both antisera reacted as described above.

No certain quantitative serological differences between the extracts from different developmental stages of *Paracentrotus* have so far been found when tested with antisera against different stages. Thus the same results were obtained throughout, whether antisera against eggs, 12-hour, or 48-hour stages were tested with standard solutions of unfertilized eggs, 4-, 12-, 48-, and Li-treated 48-hour stages. The antigenic titer and optimal proportion point were about the same in all cases. It must be emphasized however that this matter needs further investigation before more definite conclusions can be drawn.

In a series of experiments on *Paracentrotus* material, different portions of antisera against 48-hour or Li-treated 48-hour stages were absorbed with extracts from either unfertilized eggs, 4-, 12-, 48-hour stages or Li-treated 48-hour stages, and parts of every portion were then titrated with standard solutions of unfertilized eggs, 4-, 12-, 48-, and Li-treated 48-hour stages. Further, undiluted or slightly diluted extracts were used for titration. The results have been assembled in the following table:

Antiserum against 48-hour, or Li-treated 48-hour stages					
Absorbed with	Titrated with				
	unfert. eggs	4-h.	12-h.	48-h.	Li 48-h.
Unfertilized eggs . .	0	0	0	×	×
4-h.	0	0	0	×	×
12-h.	0	0	0	×	×
48-h.	0	0	0	0	0
Li 48-h.	0	0	0	0	0

0 means clearly negative, × clearly positive reaction, according to standard conditions as described above.

The antigenic titer in the solutions was distinctly reduced in the positive reactions after absorption; inhibition due to antigen excess could no longer be observed, and the standard solutions did not react at all at dilutions higher than 1/20 (compared with 1/80 normally).

In an identical manner, the following three series of absorption experiments have furthermore been undertaken. Some of the experiments served only as controls.

(a) Portions of antiserum against 7-hour stages were absorbed with either unfertilized eggs, 4-, 12-, 48-, or Li-treated 48-hour stages.

[1] W. C. BOYD, *Fundamentals of Immunology* (1947, 2nd edition), p. 436 ff.

(b) Antiserum against 12-hour stages: as (a).
(c) Antiserum against unfertilized eggs: as (a).

Each preparation was titrated with extracts and standard solutions of unfertilized eggs, 4-, 12-, 48-, and Li-treated 48-hour stages. The reaction was negative in all these cases.

Discussion

The results of the experiments indicate that one or several molecular species of new specificity occur in 0·9% saline extracts from 48-hour old *Paracentrotus* larvæ. This new type of specificity is serologically fairly distinct from the "egg" antigens which are also present in abundant amounts. These new antigens are not present at all, or perhaps in only undetectable quantities, in the corresponding extracts from unfertilized eggs and earlier developmental stages. In addition, it appears that egg extracts do not contain any precipitin producing antigens besides those in the extracts from the later stages up to 48-hours development. A strong synthetic activity, giving rise to new extractable antigens of probably protein nature, thus seems to start after the blastula stage is passed and the gastrulation is initiated. As will be remembered, suspensions of the entire sea-urchin material were used for immunization. Thus if the new antigens had been present in appreciable amounts from the beginning, but in some fraction not extractable by 0·9% saline, rabbit antibodies against them might have been obtained after injecting earlier stages (The possibility of competition of antigens must however still be kept in mind.). In this connection, it may be of interest to note that the extractability of protein-nitrogen is greater in unfertilized eggs than in later stages. The protein-nitrogen content of 0·9% saline extracts from 48-hour old *Paracentrotus* plutei is thus 19% of the total nitrogen, whereas the corresponding value from unfertilized eggs is 27% (GUSTAFSON, unpublished). The still speculative question of whether single molecular structures or single molecules of the "new" type already exist in the unfertilized egg cannot of course be answered on the basis of an investigation of this kind.

Li-treated 48-hour stages were used in order to determine if the very strong effect this treatment has on the formation of the organs, on the respiration, etc., would also manifest itself in inhibiting the synthesis of certain antigenic cell compounds. Morphologically, these 48-hour stages are distinguished by a pronounced vegetalization; they chiefly consist of a small ectodermic vesicle, attached to a strongly increased exo-gastrulated entoderm. The results of our absorption experiments however show that no difference between the antigen-constitution of extracts from Li-treated and normal 48-hour stages seems to exist.

A further investigation of the qualitative and quantitative serological properties of our solutions could not be performed owing to lack of sea-urchin material. This was also the chief hindrance for a further study of the functional and structural significance of the antigens described above. Such experiments, which might be of great interest, will be undertaken later.

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Wenner-Gren's Institute for Experimental Biology, University of Stockholm (Sweden), September 11, 1948.

Zusammenfassung

Eier des Seeigels *Paracentrotus lividus* wurden in unbefruchtetem Zustand und in verschiedenen Entwicklungsstadien gefroren, vakuumgetrocknet, fein zerrieben und mit 0,9%iger Kochsalzlösung extrahiert. Diese Produkte wurden als Suspensionen Kaninchen injiziert und damit Immunsera erhalten, in denen spezifische Antikörper mit Hilfe der Präcipitinreaktion nachzuweisen waren. Als Antigenlösungen dienten klare, dialysierte Zentrifugate der Extrakte.

Mit spezifischer Adsorption konnte folgendes festgestellt werden: In den Extrakten von 48 Stunden alten Larven (Plutei) ist eine Antigenfraktion vorhanden, die in früheren Stadien noch fehlt. Sie wird offenbar von der Seeigelzelle erst nach vollendetem Blastulastadium und nach begonnener Gastrulation in größerer Menge gebildet. Extrakte von 48 Stunden alten Larven, die durch Lithiumbehandlung stark vegetativiert waren, unterschieden sich in ihrer serologischen Konstitution anscheinend nicht von denen normaler Plutei.

The Effect of Streptomycin on Tissue Cultures

Streptomycin isolated by WAKSMAN[1] from *Actinomyces griseus* is next to penicillin the most important of the antibiotics thus far investigated. Like penicillin it is administered in large doses and therefore it is of some interest to investigate its direct influence on the cells. Experiments with different preparations of penicillin showed toxic action on mitosis which was due presumably to impurities present in the preparation. (Pure penicillin G was practically harmless for cells[2].) HEILMAN[3] tested several preparations of streptomycin on cultures of rabbit's spleen and found a very low toxicity for wandering cells as well as for fibroblasts. She measured only the growth of the cultures and did not take into account the direct influence on mitosis. BARSKI[4] found no effect in the case of 50–100 S-units/cc of streptomycin in tissue explants of rat's lung.

In our experiments the action of streptomycin was tested on the explants from the heart, aorta, and frontal bone of the chick embryo. The tissue was cultivated by the usual hanging drop method in a medium composed of one drop of fowl-plasma and of one drop of chick-embryo extract. In one series of cultures streptomycin was added directly to the medium. The growth of cultures was measured and after 48 hours the tissue was fixed and stained with Ehrlich hematoxylin. In another series the normal growing cultures were opened after 24 hours' incubation and the hollow in the slide was filled with the solution of streptomycin so that the cells were in direct contact with the solution. After being waxed they were returned to the incubator. After 10 hours' contact the cultures were fixed and stained. Streptomycin used in our experiments was a pure crystalline sample: Streptomycin (sulphate) Cutter Laboratory, California U.S.A. The solutions were diluted with Tyrode and kept in the refrigerator at +2°C. A control with Tyrode was run for each series of streptomycin cultures. In the experiment 180 cultures were investigated and 10,200 mitosis were examined. The mitosis were analysed according to v. MÖLLENDORF[5] and the following results were obtained:

No toxic influence could be seen when 25 S-units/cc

[1] S. WAKSMAN, E. BUGIE und A. SCHATZ, Proc. Staff Meet. Mayo Clin. *19*, 537 (1944).
[2] O. BUCHER, Schweiz. med. Wschr. *76*, 290 (1946); *76*, 375 (1946); *77*, 171 (1947); *77*, 849 (1947).
[3] E. HEILMAN, Proc. Soc. exp. Biol. *60*, 365 (1945).
[4] E. BARSKI, Ann. Inst. Pasteur *74*, 1 (1948).
[5] W. v. MÖLLENDORF, Arch. exp. Zellforsch. *21*, 1 (1937); Z. Zellforsch. *27*, 301 (1937).