Phosphate (ATP) all these threads do contract according to Szent-Györgyi's method.\textsuperscript{1}

In our researches we used muscles of mammals: Cuniculus cuniculus L.; birds: Columba livia Linnaeus; Amphibians: Rana esculenta L.; bony fishes: Carassius auratus L., C. vulgaris Nissl; Eupomotis gibbosus L.; crustacea: Palinurus vulgaris L. Following Guba's method\textsuperscript{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;\textsuperscript{2} they all appeared fibrillar at pH 6.5. We extracted Szent-Györgyi's myosin, soluble in water, from rabbit, pigeon, Carassius, Eupomotis, langoust. We mixed watery solutions of myosin (fibrillar particles) with actin solutions at pH 6.5. We then added MgCl\textsubscript{2} to about the concentration of 0.1 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 Esdahl'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.

"Oh, the behaviour of langouste's myosin. The thread obtained depends upon the myosin rather than the actin mixtures. However, increased also in all the combinations in which (see table) we obtained threads."

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 myosin, united to actins of vertebrates, are always satisfactory.\textsuperscript{4} The threads obtained from myosin of bony fish are always much hydrated. The scarcely hydrated threads contract to $\frac{1}{2}$ of their original length if ATP is added. If the thread is strongly hydrated the reduction is only to $\frac{1}{2}$ or $\frac{1}{3}$. 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\textsubscript{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 akl 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.

**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 occurring 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\textsuperscript{1}, and in the frog by Cooper.\textsuperscript{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.\textsuperscript{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 66 parts seawater and 5 parts lithium chloride solution (iso-tonic with 32 per mille sea-water) during the first 90 hours of development were used (strongly vegetalized larvae). In certain controls, unfertilized eggs and blastulae of Strongylocentrotus droebachiensis 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

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\textsuperscript{1} A. M. Schechtman, J. Exp. Zool. 176, 429 (1947).

\textsuperscript{2} R. S. Cooper, J. Exp. Zool. 101, 143 (1946); 107, 397 (1948).

\textsuperscript{3} V. Burke, N. P. Sullivan, H. Petersen, and R. Weed, J. Infect. Dis. 74, 925 (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 60°C 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 cold, running water against large, frequently changed volumes of physiological saline. The F₄₄ 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 antigen 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 antiserum (see below).

The presence of antigens in the sea-urchin extracts was established by specific precipitation tests. The different antigen dilutions (0-95 ml, after dilution with 0-95% 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°C for 2 hours and then in a refrigerator until the following morning. The time of the first visible precipitation was measured as well as the precipitation of 2 and 24 hours was recorded. In certain cases, ring tests were performed in microtubes. 0-08 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 sera plus antigen serum plus 0-95% saline, and, if necessary, antiserum of rabbit serum plus unfertilized eggs of Strongylocentrotus and antiserum against Strongylocentrotus eggs plus Paracentrotus antiserum 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 Boyce. The diluted antigen extract in optimal proportions. The solution was absorbed 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 1/20 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°C for 24 hours after which the precipitates were centrifuged down. After absorption, the antiserum were tested for the ring tests with different antigen dilutions and with unabsorbed antiserum. 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 1/2 of the original antigen amount was used. For titrations of the absorbed antiserum, 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 0-95% saline.

Results

As is frequently found in immunization experiments with embryonic material, the antiserum 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 antiserum and antigen standard solutions. These had been prepared so as to cause no precipitations when they were tested in full concentrations (3.0 x 10⁻⁹ M N/ml) against homologous antiserum (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 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 antiserum reacted as described above.

No certain qualitative serological differences between the extracts from different developmental stages of Paracentrotus have so far been found when tested with antiserum against different stages. Thus the same results were obtained throughout, whether antiserum 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 antiserum 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:

<table>
<thead>
<tr>
<th>Antiserum against 48-hour, or Li-treated 48-hour stages</th>
<th>Absorbed with</th>
<th>Titrated with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ご利用</td>
<td>48-hour stages</td>
</tr>
<tr>
<td>Unfertilized eggs</td>
<td>4-h.</td>
<td>12-h.</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Li 48-h.</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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.