A NOTE ON SOME ATTEMPTS TO CAUSE THE FORMATION OF CYTOLYSINS AND PRECIPITINS IN CERTAIN INVERTEBRATES.

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THE results obtained in these investigations were negative throughout, but perhaps are worth recording as tending to show that the formation of such 'anti-bodies' as cytolysins and precipitins is not a necessary reaction on the part of any living organism to the presence of foreign cells or substances.

If deductions can be made from such purely negative evidence, it would seem that the production of an 'anti-body' is not essentially a function of protoplasm, but that the power of the formation of such protective substances has been developed by higher types of animals in the course of evolution.

The animals used in the experiments were *Arca ponderosa, Pecten dislocatus, Pecten maximus, Pecten opercularis, Echinus acutus,* and *Echinus miliaris.*

1. *Experiments on the production of cytolysins.*

In one series of experiments a portion of the testis of *Pecten maximus* was removed, washed in a stream of sterile sea water, and a small piece from the interior dissected out and shaken in a little sterile sea water. A suspension of the sperm was thus obtained, and this was injected by means of a hypodermic syringe into the tissues of *Pecten opercularis, Echinus acutus,* and *Echinus miliaris.* In the case of *P. opercularis* the injection was made laterally into the highly vascular adductor muscle: in the case of *E. acutus* and *E. miliaris* a portion of the surface of the shell was sterilised by dropping on a little hydrogen peroxide solution (20 vols.), a small hole was then bored

https://doi.org/10.1017/S0022172400016636 Published online by Cambridge University Press

through the calcareous plates with a drill, and the injection made into the coelomic cavity ; the hole was then sealed by dropping on a little melted shellac. This procedure did not appear to injure the animals, and the seal could always be removed and replaced by fresh shellac when it was wished to make further inoculations into the same animal.

Inoculations of 0·5 c.c., 1 c.c., and 2 c.c. of the suspension of sperm were made at intervals of 2, 4, and 6 days in different series of experiments, and still larger quantities of sperm were used in some of the experiments on the *Echini*. A total of six inoculations were made in each series.

The blood of *P. opercularis* consists of a colourless fluid containing a number of amoeboid corpuscles, which, when the blood is shed, become fixed to each other by their pseudopodial processes, and thus form an agglutinated mass resembling a plasmodium. The clear plasma, which can be obtained by filtering off these corpuscles, is of about the same salinity as the sea water in which the animals live, and contains a coagulable proteid in solution. The coelomic fluid of the *Echini* contains corpuscles of various kinds, some possessing pigment granules, but they can all be removed by shaking the blood, when, as in the case of *Pecten*, they become entangled in a plasmodial mass and settle to the bottom. The plasma contains a rather larger amount of coagulable proteid than that of *Pecten*.

The blood of *P. opercularis*, and the coelomic fluids of the *Echini*, were removed at varying intervals, well shaken to cause the corpuscles to agglutinate, and filtered through glass wool. A little sperm of *P. maximus* was then added to the clear plasma obtained in this way, and 'hanging drop' preparations made of the mixture. Examination under the microscope showed no sign of any cytolytic action, nor did the active movements of the spermatozoa cease sooner than in check preparations made with normal plasma.

Repeated injections of the prepared plasma from the *Echini* and *P. opercularis*, in quantities varying from 1 c.c. to 5 c.c., were made into *P. maximus*, but produced no visible effect on the testis either macro- or microscopically.

Similar but more extensive series of experiments were made by injecting the ripe ova of *P. maximus* into the same animals as were used in the experiments on the injection of sperm, and injections of extracts of the ovary, made by pounding in a mortar with sterile sea water, were also made. Entirely negative results were again obtained.

Further experiments were made with the blood of *Arca ponderosa*, which among invertebrates is peculiarly suited for this work. The blood of this animal is red, the colour being due to the presence of a number of discoidal corpuscles containing haemoglobin. In the fresh condition these corpuscles somewhat resemble those of mammalia, but when fixed and stained they can be seen to possess a three or four lobed nucleus; they also contain a few minute granules of some dark brown substance. They number about 80,000 per c.mm. In addition to these cells a number of colourless amoeboid cells, similar to those found in other Lamellibranchs, are present. The plasma is relatively rich in coagulable proteids, but does not possess the power of clotting. When the blood is withdrawn and allowed to settle, it separates into three layers, at the bottom are the red corpuscles, next is a thin layer of the colourless cells which agglutinate to form a plasmodial mass, and above is a perfectly clear layer of serum having a faint yellowish tinge. If the blood be collected aseptically, and kept in sealed sterile glass tubes at room temperature, the blood cells will remain unaltered, and the masses of white cells retain the power of moving their pseudopodial processes for about six days: after longer intervals (10 to 14 days) under these conditions, haemolysis sets in, the colourless cells disappear, the red corpuscles become much crenated and tend to disintegrate, and a clear solution of their haemoglobin in the serum results. The normal serum, when added to the normal serum of *Pecten dislocatus*, produces a fairly copious precipitate of some proteid substance.

A series of injections of the blood of *Arca ponderosa* was made into the adductor muscle of *Pecten dislocatus* in 0·25 c.c., 0·5 c.c., and 1 c.c. doses, at intervals of 2, 4, and 6 days in different series of experiments. The animals into which 1 c.c. of the blood was injected did not live for more than a few days, and as they became manifestly unhealthy soon after the injection, were discarded: those into which 0·5 c.c. was injected at two day intervals also did not survive more than two or three injections. On the average 1·5 c.c. of blood was about the total maximum that could be given, even in small doses at long intervals.

On withdrawing the blood of the *Pectens* experimented on in this way, and examining it under the microscope, it was seen that most of the injected red corpuscles were very little changed, and many appeared quite normal: some showed a certain amount of crenation, and on fixing and staining showed signs of nuclear degeneration,

and a few were surrounded by white cells which appeared to be exercising a phagocytic action on them. The number of red corpuscles which are destroyed in this way is comparatively small, so that an examination of the blood of a *Pecten* into which 0·25 c.c. of the blood of *Arca* has been injected will show the presence of approximately the same number of red corpuscles after six days as immediately after the injection.

The blood of the *Pectens* into which the injections had been made, was removed at varying intervals, filtered, and added to the red corpuscles of *Arca* which had been washed free from the plasma with sterile sea water. No haemolysis resulted, and examination under the microscope showed that the red corpuscles were quite unaffected by the treatment.

It must therefore be concluded that no cytolysin had been produced.

2. *Experiments on the production of precipitins.*

In these experiments an attempt was made to cause the formation of a precipitin as a reaction to the injection of egg albumen. For this purpose the egg albumen was diluted with four times its bulk of sterile sea water, well shaken, and filtered: this procedure was necessary as egg albumen normally contains a small amount of some substance which gives a slight floccular precipitate when it is diluted with sea water, and which might be a source of error when testing for the precipitin reaction as it also gives the same precipitate with the normal plasma of the blood of marine invertebrates.

This diluted solution of albumen was injected into all the six species of animals mentioned, in doses of 0·5 c.c., 1 c.c. and 3 c.c. at intervals of 2, 4, and 6 days in different series of experiments. The blood of the animals was collected after 2, 3, 4, and 5 injections at the time when the next injection would have been due, and the serum separated from the corpuscles by filtering or allowing them to settle. The clear serum was then drawn up into a glass tube, and after it an equal volume of the diluted egg albumen solution, and the end of the tube sealed in the flame. The fluid in the tube remained perfectly clear in every case, and there was no trace of a precipitate either at the junction of the serum and albumen solution, or on shaking. After standing for twelve hours or more in the case of the serum of the Pectens, a slight cloud was sometimes produced, but this was always

found to be due to bacterial growth, and in cases where the serum and albumen solution were not shaken together, was always confined to the serum and was not more pronounced at the junction of the liquids. In the case of the serum of the coelomic fluid of the *Echini*, this growth was not apparent till much later, and it was not found at all in the case of the serum of *Arca*. If particular care was taken to collect the serum under asceptic precautions the formation of this cloud due to bacterial growth could be entirely prevented.

A further series of experiments was undertaken on the *Echini* in which very much larger doses of albumen were injected. In one series as much as 50 c.c. of the albumen diluted 1 in 5 was injected at 2-day intervals into the coelomic cavity: this had no ill effect on the animals, but rather seemed to act as a stimulant, locomotion appeared to be more rapid, and the tube feet that were unattached to any neighbouring object were usually fully extended, and their waving motion was more pronounced and more rapid than normal. After large doses such as these there was also no trace of a precipitin action. An examination of the coelomic fluid twelve hours after such an injection, showed no perceptible increase in the albuminous contents as roughly measured by precipitation with Esbach's reagent, hence it would seem that the albumen must be rapidly eliminated from the coelomic fluid.

Larger doses, such as 50 c.c. of egg albumen diluted with an equal volume of sterile sea water, had a toxic action, and caused the death of an *Echinus* of average size in about 24 hours.

As the results of these experiments on six different species of animals were so uniformly negative, it did not seem profitable to continue the work, but as a check on the methods some experiments were performed on the production of a precipitin to egg albumen in fish, species of *Pleuronectes* and *Raia* being used for the purpose. A well-marked precipitin reaction was obtained with the serum of these animals after two injections of 1 c.c. of the albumen diluted with four volumes of sea water at four day intervals.

The experiments described in this note were performed partly at the Plymouth Laboratory of the Marine Biological Association of the United Kingdom, and partly at the Laboratory of the United States Fishery Commission at Beaufort, North Carolina. My thanks are due to both these Laboratories for extending to me facilities for work.