EXPERIMENTAL BIOLOGY

THE PRECIPITATION REACTION BETWEEN THE FERTILIZIN OF THE OVA AND THE ANTIFERTILIZIN OF THE SPERMATOZOA IN POLYCHAETA OF THE WHITE SEA

L. L. Lishtvan

From the Laboratory of Immunology of Embryogenesis (Head—Candidate Med. Sci. O. E. Vyazov) of the Institute of Experimental Biology of the AMN SSSR (Director—Prof. I. N. Maiskii) and the White Sea Biological Station of Moscow State University (Director—N. A. Pertsov)

(Received April 3, 1959. Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

The reaction of fertilization is known to possess high species and tissue specificity [5]. In 1919 Lillie [5] put forward a hypothesis according to which the first phase of fertilization—the attachment of the spermatozoon to the homologous ovum—follows a similar course to the antigen—antibody reaction. The role of antigen and corresponding antibody is played by substances connected with the surface of the gametes—the fertilization of the ovum [4] and the antifertilization of the spermatozoon. Many facts have now been collected which tend to show that fertilizin and antifertilizin, which take part in the combination of the gametes, are substances possessing mutually complementary physicochemical properties [8, 9]. The fact that the onset of development (activation) of the ovum is brought about largely by a reaction of antigen-antibody type suggests that immunological reaction play an important part in the course of ontogenesis [1].

Authors who have worked on the problem of the immunology of fertilization have studied mainly the biological role and the chemical nature of fertilizin and antifertilizin [11, 17]. The mechanism of interaction of these substances has not yet been fully studied. We therefore considered it important to investigate the behavior of solutions of fertilizin and antifertilizin by means of a classical immunological reaction—the precipitation reaction.

EXPERIMENTAL METHOD

In the work we used mature gametes of two species of Annelida—Nereis virens and Arenicola marina, belonging to two sub-classes of the class Polychaeta. A solution of fertilizin ("egg water") of N. virens was obtained by allowing a 20% suspension of ova in sea water to stand for 24 hours. After standing, the suspension was centrifuged at a speed of 3000 rpm for 10 minutes. In the reaction we used the transparent, rapidly gelatinizing supernatant fluid.

A solution of fertilizin from A. marina, the ova of which have not such a well-marked gelatinous membrane as the ova of N. virens, was obtained by allowing a 50% suspension of ova in sea water to stand, with periodic shaking, for 24 hours. The supernatant fluid obtained after centrifugation was transparent, colorless, and sometimes was not gelatinized.

Solutions of antifertilizin were prepared from the sperm of the two species of worms in the same way: a 20% suspension of spermatozoa in sea water was heated to 90° three times, after which it was centrifuged at 3000 rmp for 39 minutes. In the reaction we used the transparent supernatant fluid.

TABLE 1

Results of the Ring-Precipitation Test (a) and the Agar Gel Precipitation Test (b) between Fertilizin and Antifertilizin from N. Virens

a)

"Antigen" / "Antibody"	Antifertilizin of N. virens dilutions						
	1:1	1:3	1:10	1:50	1:100	1:500	1:1000
Fertilizin of N. virens (1:3)	+++	++	++	+	+	+	

b)

"Antigen" / "Antibody"	Antifertilizin of N. virens
Fertilizin of N. virens	1 well-marked ring and 1 weak ring

In this and the following tables fertilizin is conventionally called "antibody" and antifertilizin "antigen"

TABLE 2

Results of the Ring-Precipitation Test Between Fertilizin of A. marina and Antifertilizins from Members of Different Types of Invertebrates

"Antigen" / "Antibody"	Antifertilizin of A. Marina	Antifertilizin of N. virens	Antifertilizin of O. Sarsi	Fluid from the cavity of S. Rustica (1:1)	Control-sea water
Fertilizin from A. marina (1:1)	+++	++	—	—	—
Control – sea water	—	—	—	—	

The ring-precipitation test and the agar precipitation test in capillary tubes, as modified by us, were used in the investigation.

TABLE 3

Results of the Ring-Precipitation Test between Fertilizin from N. virens and Antifertilizin from N. virens and A. marina

"Antigen"	"Antibody"	Fertilizin from N. virens (1:1)
Antifertilizin from N. virens	1:1	+++
	1:3	++
	1:10	++
	1:50	+
	1:100	+
	1:500	+
	1:1000	—
Antifertilizin from A. marina	1:1	+
	1:3	+
	1:10	+
	1:50	+
	1:100	—
	1:500	—
	1:1000	—

TABLE 4

Ring-Precipitation Test between Fertilizin and Antifertilizin, and Blood of A. marina

"Antigen"	"Antibody"	Fertilizin from N. virens (1:1)
Antifertilizin from A. marina	1:1	+
	1:3	+
	1:10	+
	1:50	+
	1:100	—
Blood from A. marina	1:1	—
	1:3	—
	1:10	—
	1:50	—
	1:100	—

EXPERIMENTAL RESULTS

The results obtained in the experiments are shown in Tables 1-4.

As will be apparent from the results shown in Table 1, fertilizin and antifertilizin react with each other in the ring precipitation test and in the agar gel precipitation test like typical antigen and corresponding antibody.

In order to study the type specificity of the precipitation reaction observed between solutions of fertilizin and antifertilizin, experiments were carried out in which these substances, obtained from members of different types of invertebrates, were combined: fertilizin from A. marina and antifertilizin from A. marina, N. virens (Annelida), Ophiura sarsi (Echinodermata) and fluid from the cavity of the ascidium Styela rustica (Chordata).

The results of this experiment are shown in Table 2.

It can be seen from Table 2 that positive results were obtained only in the reaction with "antigens" of the Annelida type. In all other cases the results were negative.

The experiments results thus showed that the fertilizin-antifertilizin reaction is characterized by type specificity.

It was interesting to find out whether this reaction showed even narrower specificity within the class Polychaeta. In an experiment, the results of which are shown in Table 3, we used the ring-precipitation test between fertilizin from N. virens and antifertilizin from N. virens and A. marina.

It is clear from Table 3 that antifertilizin from N. virens reacted in a dilution of 1:500 with fertilizin of the same species of animal. Antifertilizin from A. marina reacted with fertilizin from N. virens only in a dilution of 1:50. These findings show that the combination of fertilizin and antifertilizin on the gametes of these species possesses a relative species specificity in the precipitation reaction. At the same time, our experiments showed that cross fertilization does not occur between these two species. We never once observed the attachment of the spermatozoa of N. virens with the surface of the ova of A. marina.

The reaction between fertilizin and antifertilizin was thus more specific in those cases when these substances were combined with the surface of the gametes than when they were in solution. These results are in agreement with Tyler's views, based on the study of the agglutination reaction between the sperm of sea-urchins and a solution of fertilizin.

We also tested the tissue specificity of the fertilizin-antifertilizin reaction. We carried out ring-precipitation experiments between a solution of fertilizin from N. virens on the one hand and blood from A. marina on the other.

The results of these experiments are shown in Table 4.

It will be seen from the results in Table 4 that fertilizin from N. virens reacted only with antifertilizin from A. marina. The test with the blood from A. marina was negative. This indicates the tissue specificity of the fertilizin-antifertilizin reaction.

From the experiments described above it was possible to show directly that the reaction between solutions of fertilizin and antifertilizin proceeds along the lines of antigen-antibody reaction.

The reaction between solutions of fertilizin and antifertilizin is characterized by type- and tissue-specificity, as well as by relative species-specificity.

The results described, in our opinion, suggest that the primary immunological reactivity plays a definite role in the process of fertilization.

SUMMARY

The author studied the precipitation reaction between solutions of fertilizin of the ova and antifertilizin of the sperm in Nereis virens and Arenicola marina.

The reaction between fertilizin and antifertilizin solutions is characterized by a type-, tissue-, as well as a relative species-specificity. The reactions between these substances are more specific when they are connected with the gamete surface than when in solution.

LITERATURE CITED

[1] O. E. Vyazov, Uspekhi Sovremennoi Biol., 33, 1, 47 (1952).

[2] N. N. Zhukov-Verezhnikov, Uspekhi Sovremennoi Biol., 18, No. 1, 93 (1944).

[3] N. N. Zhukov-Verezhnikov, Proceedings of the Eighth All-Union Congress of Hygienists, Epidemiologists, Microbiologists and Specialists in Infectious Diseases, 2, P. 55. Moscow, 1956 [In Russian].

[4] F. R. Lillie, Science, 1912, v. 36, p. 527.

[5] F. R. Lillie, Problems of Fertilization. Chicago, 1949.

[6] J. Ronnström, in book: Sovremennye Problemy Tsitiologii (Moscow, 1955) p. 253.

[7] A. Tyler, Proc. Nat. Acad. Sc., 1939, v. 25, p. 317.

[8] A. Tyler, Some Problems in Present-Day Embryophysiology, p. 53. Moscow, 1951 [Russian translation].

[9] A. Tyler, Analysis of Development. Philadelphia – London, 1955, p. 170.

[10] A. Tyler, Exper. Cell. Res., 1956, v. 10, p. 377.