SUBSTANCES OF THE TYPE OF NORMAL PRECIPITINS TO ANTIGENS OF THE SPONGE Halichondria panicea (P) IN Actinia equina(L), Arenicola marina (L), Mytilus edulis (L), AND Styela rustica (L)

(UDC 576.893.4.097.2:591.111.8-2)

A. A. Yazykov

Institute of Experimental Biology Academy of Medical Sciences of the USSR, Moscow
(Presented by Active Member of the Academy of Medical Sciences of the USSR
N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 6,
pp. 75-77, June, 1966
Original article submitted December 18, 1964

The blood of animals may contain antibodies against antigens never encountered by these animals. Such antibodies have been called normal.

It has been considered [4] that the presence of normal antibodies in a living organism reflects as yet unknown profound phylogenetic relationships between species. This view is confirmed by a number of investigations. Lysins and agglutinins are found in insects [3]. Normal agglutinins with group specificity to blood cells and spermatozoa of 34 species of animals have been found in 12 species of invertebrates [4]. It has been shown by means of the precipitation reaction [1] that substances of the type of normal antibodies are present in the cells of the body-cavity fluids of invertebrates animals, and the antibodies discovered reacted as a rule with antigens of the tissues of animals at lower levels of organization. During attempts to immunize invertebrate animals artificially, an immunological response was obtained only if the antigen used for immunization belonged to a more highly organized animals than that which was immunized [2].

The object of the present investigation was to study the possible existence of substances of the type of normal precipitins in actinians, worms, mollusks, and ascidians to the antigens of a sponge standing at a much lower level in the systematic classification.

EXPERIMENTAL METHOD

The antigen used was an extract of the cell of the sponge Halichondria panicea (P), prepared as follows. Pieces of the sponge weighing 500 mg were ground in a mortar with quartz sand, and 4.5 ml of filtered sea water was then added and the mixture allowed to stand for 24 h at 0°. The homogenate was then centrifuged at 2,500 rpm for 30 min. The supernatant fluid was withdrawn. The extract thus obtained was used as antigen in the ring-precipitation reaction with the tissue fluids of the following invertebrate animals: Actinians—Actinia equina (L), polychaetes—Arenicola marina (L), bivalve mollusks—Mytilus edulis (L), and ascidians—Styela rustica (L), which were used as "sera."

The tissue fluids were obtained by simple surgical operation. A. equina was divided longitudinally and the upper part of the body was removed together with the palpae and the digestive septa. The remainder of the tissue was carefully washed in sea water. The tissue was then cut into small pieces which were placed in a test tube with 5-10 drops of sea water. The pieces were then carefully squeezed with a glass rod. A small incision was made on the surface of the body of the worm A. marina. During a reflex contraction of the worm's body, the tissue fluid appeared,* and was collected in a test tube. To obtain the tissue fluid from the mollusk M. edulis, the adductor muscles

*The tissue fluids of A. marina, M. edulis, and S. rustica obtained ex tempore consisted essentially of the cavity fluid proper and its cells, and also of tissue fluid appearing in the incisions in the animal's body. For simplicity of description, these fluids will be called tissue fluids.

Results of the Ring-Precipitation Reaction between Antigens of the Cells of the Sponge H. panicea and Tissue Fluids of Invertebrate Animals

Dilution of antigens of H. panicea	Tissue fluids of invertebrate animals (1:2)				Nonimmune rabbit serum	Sea water
	A. equina	A. marina	M. edulis	S. rustica		
1: 5	+	+	+	++	—	—
1:10	++	++	—	++	—	—
1:20	++	++	++	++	—	—
1:40	++	++	+	+	—	—
1:80	+	++	—	—	—	—
Control (Rabbit's muscle extract in dilutions of 1:5, 1:10 1:20, 1:40, 1:80)	—	—	—	—	—	—
Sea water	—	—	—	—	—	

of the shell were divided, incisions were made down to the mantle cavity, and the escaping tissue fluid was collected in a test tube. The tissue fluid of the ascidian S. rustica was obtained after making incisions at several places of the tunica, and was collected in a separate test tube. In every case the corresponding tissue fluids were taken from several individual animals. All the tissue fluids were kept at 0° for between 24 and 48 h, which should have been adequate for the passage of normal antibodies from the cellular elements into the medium [1].

The tissue fluids were then centrifuged at 2,500 rpm for 30 min and the supernatant fluid was drawn off. After the operation, and immediately before use, the pH of all the tissue fluids in the experiment was measured by means of a Michaelis indicator apparatus. The tissue fluids thus obtained were diluted twice with filtered sea water (salinity 26-27‰) and used as "sera" in the ring-precipitation reaction with antigens of the sponge H. panicea. The extract from the sponge tissue was poured over the tissue fluid and the tubes were allowed to stand at 12-13°. The results were read after 30 min, and then every successive hour. For control purposes a nonimmune rabbit serum, an extract of rabbit's muscle, and sea water were used.

EXPERIMENTAL RESULTS

The results of five similar experiments using the ring-precipitation reaction between extracts (antigens) from sponge tissue and tissue fluids ("sera") are given in the table.

The results in the table show that when the various dilutions of antigen were poured over the corresponding tissue fluid, a precipitation ring appeared at the boundary between the reagents.

The pH of all the ingredients was between 6.3 and 6.4. The time of appearance of the precipitate depended on the length of time the tissue fluids had been kept at 0°. For example, if the tissue fluids were kept for 24 h, the precipitate appeared only 15-18 h after pouring the antigen over the tissue fluid. When the tissue fluids were kept for 48 h, a precipitation ring appeared 5 to 6 h after pouring. In all cases the rings were very stable and began to disappear several hours after their appearance. The increase in the rate of the reaction when the tissue fluids were kept for 48 h may evidently be explained by the more complete destruction of the cells in the tissue fluids and, consequently, by the more complete release of precipitins into the medium. The duration of the reaction (from 5 to 18 h) was evidently influenced by the temperature of 12-13° at which the reaction was carried out. It is difficult to say on which of the two phases of the reaction this temperature exerted its influence. On the other hand, a temperature of 12-13° is reasonably close to the natural temperature at which the marine animals live.

It may therefore be concluded from the results of these experiments that the tissue fluids of the invertebrate animals A. equina, A. marina, M. edulis, and S. rustica contain substances of the type of normal precipitins, capable of reacting with tissue antigens of the sponge H. panicea. The nature of these substances has not been explained.

LITERATURE CITED

1. O. E. Vyazov, A. I. Mol'kova, B. V. Konyukhov, et al., Transactions of the White Sea Biological Station of Moscow University, 1, Biology of the White Sea [in Russian], Moscow (1962).

2. O. E. Vyazov and L. S. Vol'kova, Byull. éksper. biol., No. 12, 62 (1960).
3. S. I. Metal'nikov, Cited by L. A. Zil'ber. Fundamentals of Immunology [in Russian], Moscow (1958).
4. A. Tyler, Biol. Bull., 90, N 3 (1946), p. 213.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of the first issue of this year.
