

THE EFFECT OF THE SECRETION OF THE CUTANEOUS GLANDS OF THE TOAD ON CERTAIN IMMUNOLOGICAL PROPERTIES OF ANIMALS

V. I. Zakharov, N. V. Kolpikov and B. M. Kurtser

Department of General Biology (Head—Prof. V. I. Zakharov) and the Department of Pathological Physiology (Head—Dr. Med. Sci. E. P. Kuchinskii) of the Kishinev Medical Institute (Dir.—Honored Scientist Prof. N. T. Starostenko)

(Presented by Active Member AMN SSSR V. V. Parin)

Translated from *Byulleten' éksperimental'noi biologii i meditsiny* Vol. 49, No. 2, pp. 85-89, February, 1960.

Original article submitted March 19, 1959

Biologically active substances are widely distributed in living organisms. Items of special interest are the animal poisons, which possess high activity. One such product of biological synthesis is the secretion of the cutaneous glands of the toad (SCGT).

The therapeutic and toxic action of the secretion of the cutaneous glands of various toads has been known for a long time. According to E. N. Pavlovskii [10], the Chinese many centuries ago prepared from the poisonous cutaneous glands of the common toad (*Bufo vulgaris*) the therapeutic preparation "Sen-So," which they used as a cardiac drug. The literature [1, 8] contains toxicological and pharmacological information on the poison of the toad. On the basis of N. P. Kravkov's researches, I. P. Pavlov [9] put forward the idea that SCGT possesses a mixed action on the body.

The action of this secretion has been studied [11, 12] on isolated organs and on intact animals.

Systematic investigations of the character of the action of SCGT on animals were made by V. I. Zakharov [2-6]. He studied the effect of SCGT on animals of different species, or of the same species but at different stages of individual development. He also studied the effect of SCGT on warm-blooded animals, in normal conditions and in certain functional disorders reproduced experimentally.

Several authors have shown [7] that SCGT is an effective stimulator of leuko- and thrombopoiesis in white rats after exposure of these animals to x-rays.

These investigations provided the basis for the study of the action of SCGT on certain immunological processes in animals.

Among the protective adaptations developing in man and animals during the process of evolution, an important place is occupied by antibodies and the phagocytic properties of the fixed and mobile cells of the body.

In the present investigation the action of SCGT was studied on antibody formation and on the phagocytic activity of the leukocytes of the blood.

METHOD

Experiments were carried out on white rats and male rabbits. The animals were immunized by the subcutaneous injection of a killed typhoid vaccine. SCGT was injected into the animals subcutaneously—into rats in a dose of 1 ml of a concentration of 1:1000; into rabbits in a dose of 1 ml/kg body weight of a concentration of 1:2000. In all, three series of experiments were performed on 12 rabbits and 46 rats. In the first series of experiments the action of SCGT was studied on the changes in the agglutinin titer in rabbits; in the second series its action on the formation of agglutinins on rats were studied; finally, in the third series, the action of SCGT was studied on the phagocytic activity of the leukocytes in rats.

The agglutinin titer was determined by the agglutination reaction, the intensity of which was read by means of an agglutinoscope. The phagocytic activity of the leukocytes was determined against a 24-hr culture of *Staphylococcus aureus* on agar.

RESULTS

The first series of experiments was carried out on 12 rabbits, of which six were experimental and six control (the latter were immunized but did not receive SCGT). After the initial agglutinin titer had been determined, the animals of the experimental group were injected with SCGT in a dilution of 1:2000 and a dose of 1 ml/kg body weight. Immunization began on the fifth day with a suspension of a typhoid culture (containing 1×10^9 bacterial cells/ml) in a dose of 0.3-0.5 ml. The animals were vaccinated five times at intervals of five days. The rabbits received SCGT four times during the period of immunization, so that the antigen was injected during the time of action of the secretion.

The control group of rabbits received injections of physiological saline (0.85% NaCl) in a dose of 1 ml/kg body weight.

TABLE 1. Agglutinin Titer of the Serum of Rabbits Vaccinated with Typhoid Vaccine and Receiving SCGT

Animal no.	Before vaccination	After vaccination and injection of SCGT			
		after 9 days	after 16 days	after 23 days	after 28 days
2	1:25	1:1 600	1:800	1:400	1:400
3	—	1:1 600	1:400	1:200	1:50
4	—	1:800	1:1 600	1:800	1:100
5	1:25	1:3 200	1:400	1:400	1:200
6	1:25	1:6 400	1:12 800	1:3 200	1:800
7	—	1:1 600	1:3 200	1:800	1:100

TABLE 2. Agglutinin Titer of the Serum of Rabbits Vaccinated with Typhoid Vaccine

Animal no.	Before vaccination	After vaccination			
		after 7 days	after 15 days	after 25 days	after 30 days
1	1:25	1:400	1:800	1:400	1:200
2	1:25	1:1 600	1:800	1:400	1:100
3	—	1:100	1:400	1:400	1:200
5	1:25	1:200	1:50	1:25	—
6	1:25	1:3 200	1:800	1:400	1:200
7	—	1:400	1:200	1:200	1:50

TABLE 3. Agglutinin Titer of the Serum of the Control and Experimental Rats

No. of control animal	On the tenth day after vaccination	No. of experimental animals	On the 11th day after vaccination and injection of SCGT
1	Negative	1	1:3 200
2	»	2	1:3 200
3	»	3	1:1 600
4	1:400	4	1:3 200
5	1:200	5	1:6 400
6	Negative	6	1:400
7	1:400	7	Discarded from the experiment
8	Negative	8	1:800
9	Discarded from the experiment	9	1:6 400
10	1:800	10	1:1 600

Blood for estimation of the agglutinin titer was taken from the experimental animals 9, 16, 23, and 28 days after vaccination, and from the control animals 7, 15, 25, and 30 days after vaccination.

The results obtained in the first series of experiments are shown in Tables 1 and 2.

It will be seen from the figures in Table 1 that in three cases the agglutinin titer reached 1:1600-1:3200 on the ninth day; on the 16th day the titer was observed to fall. In the other three cases the titer on the ninth day was 1:800, 1:6400, and 1:1600; on the 16th day it had risen to 1:1600, 1:12,800, and 1:3200, and after 23

TABLE 4. Phagocytic Activity of the Leukocytes in Rats after Subcutaneous Injection of SCGT

Animal no.	Phagocytic activity of leukocytes (in %)						
	Before injection of SCGT	After injection of SCGT					
		after 2 days	after 6 days	after 10 days	after 15 days	after 25 days	after 35 days
1	13	25	24	22	21	—	—
2	16	20	21	18	20	—	—
3	13	19	34	27	26	21	20
4	15	16	30	21	23	25	20
5	21	13	36	25	26	20	—
7	18	14	23	22	27	24	17
8	17	10	22	22	24	15	—
9	17	12	18	16	15	17	18
10	14	19	24	17	16	18	17
11	15	27	31	21	19	20	19
12	11	31	24	24	12	13	14
13	12	20	27	18	11	12	—
14	16	35	32	19	14	18	17
15	18	15	10	13	13	11	10
16	15	18	23	17	16	15	14
17	18	30	31	28	17	20	19

Note. A minus sign (—) means that the phagocytic activity was not determined in these cases.

TABLE 5. Phagocytic Activity of the Leukocytes in the Rats of the Control Group

Animal no.	Phagocytic activity of leukocytes (in %)					
	before injection of physiological saline	after injection of physiological saline				
		after 2 days	after 6 days	after 10 days	after 15 days	after 25 days
1	13	14	12	13	14	12
2	17	16	15	14	17	—
3	12	11	12	10	11	13
4	6	7	8	7	6	—
5	12	13	10	11	12	12
6	10	11	10	9	11	10
7	11	14	10	12	13	—
8	15	12	12	11	13	14
9	10	11	12	9	10	—
10	8	9	7	10	9	10

Note. A minus sign (—) means that the phagocytic activity was not determined in these cases.

days a fall in the agglutinin titer was observed in every case.

In the control group (Table 2) the highest agglutinin titer was observed in four cases on the seventh day after vaccination (1:200, 1:3200, 1:400, and 1:1600); on the 15th day in these cases a fall in the titer was observed; In two cases on the seventh day the titer was 1:400 and 1:100; on the 15th day it reached 1:800 and

1:400. On the 25th day the titer was lowered in every case.

It thus follows from analysis of the results obtained that SCGT considerably increases the antibody-forming (agglutinins) power of animals.

The second series of experiments was conducted on 20 rats (weight 150-200 g), kept on an ordinary diet; 10 of these were used as controls and were only im-

munized, whereas the experimental animals received, in addition, three injections of SCGT (1 ml, 1:1000) during the period of immunization.

The animals were immunized with a suspension of a typhoid culture (1×10^8 bacterial cells/ml), receiving six injections, each of 0.1 ml, at intervals of 3 days.

On the 10th and 11th days after vaccination and injection of SCGT, blood was taken for investigation of the agglutinin titer.

The results obtained in this series of experiments are shown in Table 3. These results show that SCGT caused a sharp increase in the agglutinin titer in the white rats.

In the third series of experiments the effect of SCGT was studied on the phagocytic activity of the leukocytes in rats. The investigation was carried out on 26 rats, ten of which were controls. After the determination of the initial phagocytic activity of the leukocytes, the experimental animals received three injections of SCGT (1 ml, 1:1000) at intervals of 2-3 days. The control animals received subcutaneous injections of 1 ml of physiological saline at the same times.

At intervals of 2, 6, 10, 15, 25, and 35 days, blood was taken from the animals for estimation of the phagocytic activity of the leukocytes. As material for phagocytosis a suspension of Staphylococcus albus in physiological saline was used.

The results of this series of experiments are shown in Tables 4 and 5.

The results of the last series of experiments show that in 14 of the 16 experimental animals there was a considerable increase in the phagocytic activity of the leukocytes. The maximum increase of activity of the leukocytes was observed two and six days after injection of the SCGT. In three cases, a considerable fall in the phagocytic activity was observed two days after the injection of SCGT, but after six days the activity of the leukocytes exceeded the original values.

One animal (No. 15) showed a lowering of the phagocytic activity throughout the whole experiment, and in another case (No. 9) the activity remained within the limits of physiological variation. In the majority of

animals the figures regained their initial values after 25-35 days.

In the control group of animals the phagocytic activity of the leukocytes showed no significant difference from the initial values throughout the entire experiment.

In this series of experiments the SCGT thus had a marked stimulating action on the phagocytic activity of the leukocytes of the blood.

SUMMARY

This work deals with the effect of the secretion of the cutaneous glands of toads on the dynamics of the agglutinin titer in rabbits and rats, as well as on the phagocytic activity of blood leukocytes in rats. The data obtained demonstrate that this substance increases the ability of the animal to form agglutinins, and stimulates the phagocytic activity of the blood leukocytes in rats.

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