

# CHANGES IN THE IMMUNOLOGICAL STATE OF THE BODY DURING THE ACTION OF CARCINOGENIC CHEMICAL AGENTS

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It is possible to conclude from results of a number of experimental investigations, that under the influence of a carcinogen there occur in an organism certain immunological changes and that specific antigens are formed long before the appearance of the tumor [1, 3, 7].

The purpose of the present investigation was to determine whether it is possible to utilize the precancerous changes in the immunological state of the organism as an early indicator of a malignant process.

## EXPERIMENTAL METHOD

Fifteen rabbits have been used in the experiment. The animals were immunized twice with typhoid vaccine at two-month intervals. In the course of the first immunization, 0.5 ml of vaccine containing  $1.5 \cdot 10^9$  bacteria per ml was used, while 0.8 ml was utilized for the second injection. Eighteen days after the initial immunization, five animals have been inoculated once subcutaneously with 40 mg of 1% solution of the carcinogen 9, 10 dimethyl - 1,2 benzantracene. One drop of 1% benzol solution of the same carcinogen has been applied to the skin of five other immunized animals. These applications have been continued for four months. Five rabbits received no carcinogen.

As an indication of the altered immunologic reactivity of the animals, changes in the agglutination titer and complement, as well as changes in the content of acetylcholine and cholinesterase, and the total protein and separate protein fractions, have been investigated.

## EXPERIMENTAL RESULTS

The curve showing the change in the agglutination titer of the serum of animals receiving cutaneous application of the carcinogen, is essentially similar to that of the control group, but of somewhat lower value (see figure). The agglutination titer in animals injected subcutaneously with the carcinogen gradually decreased until the second injection, showing considerably lower values than in control. After second injection, it was characterized by higher values, essentially repeating the control curve. Consequently, compared to controls specific reactivity during many periods of investigation was lowered in the experimental animals; apparently, this observation can be correlated with the action of the carcinogen.

After the initial immunization, the titer of the complement of animals receiving cutaneous applications of the carcinogen, as well as that of the control rabbits, is somewhat reduced; after the second immunization irregular fluctuations in titer take place.

As can be seen from Table 1, the titer of the complement is higher in the controls than in the experimental animals; in addition, this difference is significant for rabbits injected subcutaneously with the carcinogen. The latter observation confirms the reduction in reactivity in rabbits inoculated with the carcinogen.

Concentration of acetylcholine in blood has been determined biologically (on eserized muscle of a leech) in all groups of animals on only four occasions, one to two months apart. It has not been possible to demonstrate the presence of acetylcholine in blood of a single experimental animal.

TABLE 1. Titer of Complement in Control and Experimental Animal

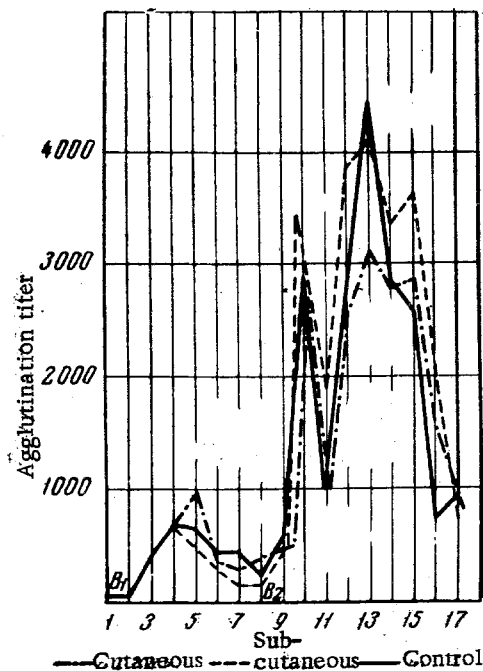
Experimental conditions	Number of observations	Titer of complement	Average of the confidence intervals	At p = 0.05
Cutaneous application of the carcinogen . . . . .	20	$0.09 \pm 0.004$	0.09 (0.082 - 0.098)	1.5
Subcutaneous injection of the carcinogen . . . . .	20	$0.1 \pm 0.007$	0.1 (0.09 - 0.11)	2.0
Control . . . . .	16	$0.08 \pm 0.007$	0.08 (0.07 - 0.09)	-

TABLE 2. Concentration of Cholinesterase in the Control and Experimental Rabbits

Experimental conditions	Number of observations	Cholinesterase	Average and the confidence intervals	At p = 0.05
Cutaneous application of the carcinogen . . . . .	20	$25.46 \pm 1.55$	25.46 (22.22 - 28.70)	3.5
Subcutaneous injection of the carcinogen . . . . .	20	$20.42 \pm 1.43$	20.42 (17.43 - 23.41)	2.02
Control . . . . .	15	$16.7 \pm 1.34$	16.7 (13.83 - 19.57)	-

TABLE 3. Concentration of Gamma-Globulins in the Control and Experimental Rabbits

Experimental conditions	Number of observations	$\gamma$ -Globulin	Average and the confidence intervals	At p = 0.05
Cutaneous application of the carcinogen . . . . .	20	$18.4 \pm 1.0$	18.4 (16.3 - 20.5)	1.31
Subcutaneous injection of the carcinogen . . . . .	20	$17.9 \pm 0.94$	17.9 (15.94 - 19.86)	1.65
Control . . . . .	16	$20.7 \pm 1.5$	20.7 (17.5 - 23.9)	-



Variation in rabbit agglutination titer after the first ( $B_1$ ) and the second ( $B_2$ ) injections of typhoid vaccine.

Cholinesterase has been determined chemically according to the method described by Pravdich-Neminskaya. Rabbits injected with the carcinogen had a much higher concentration of cholinesterase than did the control animals. This is particularly applicable to animals receiving the carcinogen cutaneously (Table 2).

Application of the carcinogen led to an increase in the relative concentration of albumins, when compared with the control animals.

Concentration of  $\alpha$ - and  $\beta$ -globulins in the control and experimental animals increased slightly after first immunization, but decreased significantly after the second injection.

As it can be seen from Table 3, there is an indication of a decreasing trend in  $\gamma$ -globulin concentration in the experimental animals.

In accordance with the data of a number of investigators, the concentration of globulins increases with the increasing immunological reactivity.

The interrelation of fractions is altered in the direction of an increase in  $\gamma$ -globulins. A decrease in A/G ratio and an increase in the level of  $\gamma$ -globulins are usually correlated with formation of immune bodies.

However, the contrary was true in our experiments, the relative concentration of  $\gamma$ -globulin in experimental animals decreases and A/G ratio increased. The data pertaining to concentration of albumins in experimental rabbits point to a decrease in reactivity, apparently taking place as a result of action of the carcinogen.

The concentration of destructive acetylcholine cholinesterase in the blood of experimental animals was higher than that of controls and did not decrease towards the end of the investigation. High concentration of cholinesterase correlated in the animals with the absence of acetylcholine, which, in its turn, points out the state of hypergy.

The acquired indicators lead to a conclusion that the decrease in reactivity in immunized rabbits is a result of action of the carcinogen -9, 10 dimethyl -1, 2 benzantracine.

We have been also interested whether it would be possible to disclose with a single biological test the altered reactivity of animals subjected to the action of a carcinogen and remaining, apparently, in a precancerous condition.

One group of animals has been immunized with a typhoid vaccine. Subsequently, some of these animals were treated twice a week with one drop of 1% benzol solution of the carcinogen 9,10 dimethyl -1,2 benzantracine on the skin of the ear. 40 mg of the same carcinogen dissolved in sunflower oil was inoculated subcutaneously into remaining rabbits. The same concentrations of the same solutions of 9,10 dimethyl -1,2 benzantracine as used for group 1, were administered cutaneously or subcutaneously to rabbits of the second group, without previous immunization. After 4 months, 0.2 ml of rabbit serum of the first or the second group was inoculated intravenously every 3 days (a total of 4 injections) into a guinea pig for sensitization. Guinea pigs were bled 8 days after the last injection and 0.2 ml of the serum was introduced intracutaneously into a rabbit previously immunized and subjected to the carcinogen (first group), or into a rabbit immunized previously but subjected to the same carcinogen (group 2). The same serum was injected intracutaneously into an immunized rabbit not treated with the carcinogen and into a rabbit previously neither immunized nor treated with the carcinogen (the control group).

It has been assumed that an effect of carcinogenic activity will be reflected in the organ proteins and in the blood by formation of such corresponding antigens [2, 4, 5, 6]. Introduction of sensitized guinea pig serum (serum with antibodies) into rabbits, which developed specific antigens as a result of carcinogenic action, provided a possibility for reaction between antigen and the antibody. This allergic reaction has been observed also cutaneously.

The first group consisted of 16 animals: 4 donors immunized and then treated with the carcinogen and 12 recipients, of which 4 rabbits were immunized and then treated cutaneously with the carcinogen, 4 rabbits received subcutaneous injection of the carcinogen after immunization and 4 rabbits were only immunized. All animals injected intracutaneously with the serum of sensitized guinea pigs have been observed within 24 h after injection for reaction.

Intracutaneous injections of serum of the sensitized guinea pigs was much more pronounced in the immunized and subsequently treated with the carcinogen recipients than in the only immunized recipients (Table 4).

TABLE 4. Cutaneous Response of Rabbit Treated with the Carcinogen on Intracutaneous Introduction of Guinea Pig Serum, Sensitized with Serum of Rabbits Treated with the Carcinogen

Experimental design		Average size of swelling (in cm <sup>2</sup> ) one day after introduction of serum of the sensitized guinea pig
Donor	Recipient	
Immunization + treatment with the carcinogen	Immunization + subcutaneous carcinogen (4 rabbits) . .	10.1
The same	Immunization + cutaneous carcinogen (4 rabbits) . . . .	8.9
The same	Immunization only (4 rabbits) . . . . .	2.6
Treatment with carcinogen only	Immunization + treatment with carcinogen (3 rabbits) .	6.0
The same	Carcinogenic action only (3 rabbits) . . . . .	1.0

The observed difference is explained, apparently, by formation of an antigen in the recipient-rabbits injected with the carcinogen, and the reaction of the antigen with the intradermally introduced serum antibodies of the sensitized guinea pigs, which determines the corresponding skin reaction. In rabbits, immunized but not treated with the carcinogen, specific antigen was absent from the tissues, thus showing no antigenic interaction with the intradermally introduced serum antibodies of the sensitized guinea pigs. This was also apparent in response of these animals to intracutaneous injection of serum of the sensitized guinea pigs.

Both the donor and the recipient rabbits of group I have been immunized prior to treatment with the carcinogen. It is possible that the immune state of rabbits has been somehow reflected in the reaction following the intracutaneous injection of sensitized guinea pig serum. Therefore, experiments were carried out with animals from the second group, not immunized previously. These studies demonstrated that the recipient rabbits treated with the carcinogen only, responded more intensely to intracutaneous injection of serum of the sensitized guinea pig, than did the control animals without carcinogen. In this manner, immunization prior to treatment with the carcinogen did not have the determining function in response of the recipient rabbits to an intradermal injection of serum of the sensitized guinea pigs.

Our experimental data confirm that the immunological reactivity of organism is decreased after treatment with carcinogen.

It is possible to establish the precancerous stage in rabbits by sensitization of a guinea pig with serum of an animal receiving a carcinogen and subsequent intradermal injection of the sensitized guinea pig serum into an animal receiving the same carcinogen.

#### SUMMARY

To solve the problem on the shifts in the immunological state of the organism during the action of carcinogenic chemical agents the authors carried out the following investigations. Rabbits immunized with the typhoid vaccine were subjected to epidermal and subcutaneous application of 9, 10 dimethyl-1,2 benzanthrane. The agglutination titre, complement, acetylcholine and cholinesterase content and the total content of proteins and individual protein fractions were investigated to the immunological shifts in the animal organism.

The data obtained indicated a reduced immunological reactivity in rabbits as a result of the action of carcinogen 9, 10 dimethyl-1,2 benzanthrane.

An experimental study was also made of the possibility of detecting specific antigens during the precancer state. Rabbits subjected to the action of a carcinogen developed a marked skin reaction (considerably greater than the control) following intradermal administration of blood serum obtained from guinea pig sensitized with the serum of a rabbit to which the carcinogen was applied.

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