MECHANISM OF THE EFFECT OF TRICHLOROETHYLENE POISONING ON ANTIBODY FORMATION TO THE O-ANTIGEN OF Salmonella typhi

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In experiments on rats using the method of local passive hemolysis in gel it was shown that acute poisoning with trichloroethylene 24 h before or simultaneously with immunization with antigen from Salmonella typhi causes an increase in the number of antibody-forming cells compared with the control. In the case of subacute poisoning, stimulation at the beginning of poisoning is followed after 20 days by a persistent decrease in the number of these cells. The dynamics of the antibody-forming cells after immunization was the same in the control and experimental rats and the duration of the mitotic cycle was 14 h. Changes in the number of antibody-forming cells in unimmunized rats under the influence of the poison were analogous to those in the immunized animals. The author considers that the effect of trichloroethylene is due to its nonspecific stressor action on immunologically competent precursor cells.

Changes in immunological reactivity are a sensitive test during the action of industrial poisons, and, in particular, the poisoning of animals, even with very small doses of the chlorinated hydrocarbons of the ethane and ethylene series if they are administered for a long period of time, induces a decrease in antibody formation compared with control animals [9]. However, the mechanism of action of these agents has not received adequate study.

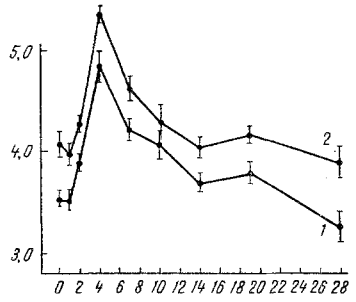

Fig. 1. Dynamics of antibody-forming cells after immunization in control animals and animals poisoned with trichloroethylene (3 mg/liter). Ordinate, log of number of cells; abscissa, time after immunization (in days); 1) control, 2) experiment.

In this investigation an attempt was made to discover the site of action of trichloroethylene by studying the production of isolated antibody-forming cells. By investigations of this kind it has been possible to study the mechanism of action of ionizing radiation and of many immunodepressants and therapeutic agents on the kinetics of these cells [2-4, 6-8].

EXPERIMENTAL METHOD

Experiments were carried out on 295 noninbred albino rats (180-220 g). The animals were immunized by a single subcutaneous injection of Boiven antigen from Salmonella typhi O-901 in a dose of 20 μg and in a volume of 0.1 ml. The number of antibody-forming cells (AFCs) per million nucleated spleen cells and in the spleen as a whole was determined by the method of local hemolysis in gel [10] as modified by Solov'ev et al. [5]. Parallel determinations were made of the weight of the spleen, the number of nucleated cells in the spleen, and the serum antibody titers in the passive hemagglutination test (PHT).

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TABLE 1. Effect of Acute Trichloroethylene Poisoning on Number of AFCs in Spleen of Rats and on Antibody Titers on 4th Day after Immunization

Time of poisoning relative to day of immunization	Number of animals	Number of AFC's per million spleen cells	P	Number of AFCs in spleen	P	Antibody titer
Not poisoned (control)	24	170 (120—250)		133 700 (93 800—190 500)		1:130 (1:110—1:290)
−1	7	380 (260—540)	<0,05	322 100 (207 000—501 200)	<0,05	1:260 (1:125—1:550)
0	14	400 (280—580)	<0,05	253 200 (174 200—367 300)	<0,05	1:160 (1:90—1:280)
0, +1, +2, +3	6	505 (330—780)	<0,05	320 600 (208 000—494 300)	<0,05	1:280 (1:180—1:440)
+1	6	150 (50—280)		85 300 (32 700—222 300)		1:140 (1:80—1:250)
+2		180 (125—260)		144 200 (84 530—246 000)		1:180 (1:110—1:300)

Note: Minus sign indicates day before immunization, plus sign, day after immunization; 0, day of immunization.

In part I of the investigation the rats of series I were poisoned with trichloroethylene in a concentration of 3 mg/liter by a static inhalation method with an exposure of 2 h immediately before and daily after immunization. The animals of series II were immunized only and they acted as the control. The rats of each series were sacrificed, six or seven at a time, 1, 2, 4, 7, 10, 14, 19, and 28 days after immunization and after poisoning for 4 days without immunization. In part II of the investigations five series of animals were subjected to acute trichloroethylene (40 mg/liter) poisoning: 1) once 24 h before immunization, 2) immediately before immunization, 3) before immunization and on the next 3 days, 4) once 24 h after, and 5) once 48 h after immunization. The rats were sacrificed on the 4th day after immunization. In each series control immunized animals which had not been poisoned also were investigated. In part III of the investigation the rats were subjected to subacute poisoning with trichloroethylene in a concentration of 6 mg/liter for 2.5 months with an exposure of 2 h six times a week by a static inhalation method. The animals were immunized six or seven at a time on the 1st, 7th, 20th, 30th, 45th, and 75th days after the beginning of poisoning and they were sacrificed on the 4th day after immunization. In parallel tests rats which had not been poisoned were immunized and sacrificed. Investigations were also carried out on unimmunized rats 4 and 80 days after poisoning and in a parallel series on intact unimmunized animals.

The results were subjected to statistical analysis (calculation of arithmetic mean weights of the spleen and numbers of cells in it, geometric means for the number of AFCs and antibody titers with their errors or confidence limits). The level of significance for differences between the series was $P < 0.05$.

EXPERIMENTAL RESULTS

The results of the first part of the investigation are given in Fig. 1. They show that the number of AFCs was greater in the experimental rats both before and at all times after immunization. The differences were significant (except on the 10th day). The time taken for the number of AFCs in the spleen to double was calculated [6]. It was found to be equal in the control and experimental animals (14 h).

The results of the second part of the investigation are given in Table 1. In acute trichloroethylene poisoning (air concentration two-thirds of the lethal concentration of 50%) 24 h or immediately before immunization the number of AFCs was greater, while in the case of poisoning 1 or 2 days after immunization it was the same as the control level.

The results of the third part of the investigation are given in Fig. 2, which shows that in animals immunized on the 1st day of poisoning the number of AFCs was significantly higher, in those immunized 1 week after the beginning of poisoning it was not significantly different, while starting from the 20th day and until the end of poisoning it was significantly lower in the experimental animals by comparison with the controls. The dynamics of the AFCs at the end of poisoning was the same in the control and experimental rats, and the duration of the mitotic cycle was 14 h. The number of AFCs in the spleen of the experimental, unimmunized rats at the beginning of poisoning was significantly higher, and at the end of poisoning significantly lower than in the unpoisoned, unimmunized animals tested at the same times. The changes in

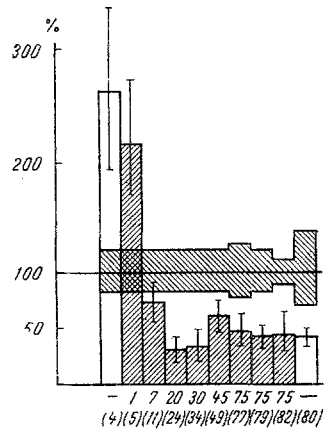

Fig. 2. Effect of subacute trichloroethylene poisoning on number of antibody-forming cells in the spleen of immunized and unimmunized rats. Ordinate, number of cells in percent of control; abscissa, days of poisoning on which rats were immunized (without parentheses) and were sacrificed (in parentheses). Unshaded columns: unimmunized rats.

the antibody titers in the experimental animals were in the same direction as the changes in the number of AFCs, but the differences were only occasionally statistically significant. No significant differences were found in the weight of the spleen and in the number of nucleated cells in it between the control and experimental animals.

Trichloroethylene thus does not affect proliferating AFCs (absence of effect in acute poisoning 1 and 2 days after immunization), and does not alter the rate of mitosis or the number of mitoses in immunologically competent cells (the duration of the mitotic cycle and the dynamics of AFCs production were the same in the control and experimental animals whether there was an increase or a decrease in the number of AFCs in the spleen). Hence, it follows that trichloroethylene affects the number of immunologically competent precursor cells starting differentiation. The changes were in the same direction after injection of the antigen or without it. The effect of trichloroethylene was evidently not connected with any direct cytotoxic action of the poison on the precursor cells, for even large doses of the poison if administered once only produced a stimulant effect, and only the prolonged action of the poison led to a decrease in the number of AFCs.

Trichloroethylene can be considered to act as a nonspecific stressor. The possibility that many factors, including chemical agents, may have a stressor action on immunogenesis through the neuro-hormonal system has been mentioned by Zdrodovskii [1]. Under certain conditions nonspecific stressors themselves induce a plasma-cell response, and if added to antigens they stimulate antibody formation. Hormonal changes taking place in response to the stressor action of trichloroethylene may perhaps bring about partial inhibition of the genetic apparatus of the X-cells, leading to the initiation of differentiation of these transformed cells and to an increase in the number of AFCs without immunization, while in the case of poisoning 24 h or immediately before injection of the antigen they may potentiate its stressor action. During prolonged action of the stressor (trichloroethylene) a phase of exhaustion commences, and it leads to a decrease in the number of spontaneously formed AFCs; immunization against this background leads to a decrease in the number of precursor cells starting differentiation compared with the control, and this results in a decrease in the number of cells producing antibodies.

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