(Fig. 2). The alternative possibility is increased sensitivity of precursors of secondary CTL compared with those of primary CTL to the action of the amplifiers, more especially because secondary precursors of CTL, unlike primary, can differentiate in the absence of proliferation [15]. Whatever the case, the inability of precursors of primary CTL, unlike precursors of secondary CTL, to adhere specifically to the fixed cells of a monolayer carrying the corresponding H-2 antigens [4] indicates peculiarities of structure of the antigen-binding receptor of MC (precursors of secondary CTL), and their essential differences from precursors of primary CTL. The hypothesis that secondary CTL can differentiate from their precursors without the help of amplifiers requires further study.

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COMPARATIVE STUDY OF METHODS OF PERFRINGENS TYPE A ANTITOXIN ASSAY

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KEY WORDS: perfringens antitoxin; neutralization test; passive hemagglutination test; enzyme-labeled immunosorbent assay.

To determine the level of perfringens antitoxin in human and animal blood sera the toxin neutralization test (TNT), which exists in two variants, is as a rule chosen. One of these is based on neutralization of the test toxin followed by determination of residual α-toxin activity *in vivo* in albino mice. The use of the second variant, namely, titration of antitoxin *in vitro* is associated with the enzymic nature of the α-toxin (phospholipase C, or PLC). After neutralization of the test toxin, residual activity of PLC is determined by the use of hen egg lecithovitellin as the substrate. The use of the passive hemagglutination test (PHT) with an erythrocytic diagnostic serum obtained by sensitizing erythrocytes with highly purified *Clos-*

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TABLE 1. Experimental Doses of Test Toxin in TNT *in Vivo* and *in Vitro*, Determined with Different Dilutions of Standard Antiperfringens Serum

TNT in vivo				TNT in vitro			
Standard serum (in 0.1 ml)	experimental data		deviation of experimental dose from theoretical, %	standard serum (in 0.1 ml)	experimental data		deviation of experimental dose from theoretical, %
	theoretical	experimental			theoretical	experimental	
0,1	—	1:32	—	0,1	—	1:78	—
0,05	1:64	1:42	39,9	0,05	1:156	1:144	7,7
0,02	1:160	1:50	68,8	0,025	1:312	1:250	19,9
0,01	1:320	1:68	78,8	0,0125	1:624	1:450	26,5
Toxin control	—	1:64					

tridium perfringens type A toxoid, in order to determine serum perfringens antitoxin levels in animals has been described [3].

The aim of this investigation was to compare determination of perfringens type A antitoxin levels in human blood sera by the use of the TNT, PHT, and an immunoenzymic method, namely, enzyme-labeled immunosorbent assay (ELISA).

EXPERIMENTAL METHOD

The human blood sera were obtained from persons immunized with *Cl. perfringens* type A toxin, prepared by the standard technology [2]. TNT *in vivo* and *in vitro* was carried out by the usual method. The perfringens antitoxin level was expressed in International Units (IU)/ml serum.

The PHT was carried out with an erythrocytic diagnostic serum. This was prepared by treating formalinized sheep's erythrocytes [4] for 10 min at room temperature with tannin (final concentration 1:40,000), and then by sensitizing them with highly purified *Cl. perfringens* type A toxoid for 20 h at 37°C, in a dose of 7-10 units to 1 ml of 2.5% erythrocyte suspension. Formalin was added 1 h before the end of sensitization up to a concentration of 1%. The diagnostic serum was lyophilized. The sera for testing, in a dilution of 1:32, were heated for 30 min to 56°C and further diluted with physiological saline containing 1% normal rabbit serum. The PHT was set up in a volume of 0.4 ml in polystyrene plates. For the ELISA the same antigen was used as for the PHT, in a concentration of 7-10 IU. The method as used followed the modification described previously [1]. The titer of perfringens antitoxin in the PHT and ELISA was expressed as the negative logarithm to base 2 ($-\log_2$).

EXPERIMENTAL RESULTS

The level of perfringens type A antitoxin is determined mainly by the TNT, which consists essentially of determining residual activity of the test toxin after it has reacted with the test serum. This reaction has low sensitivity but high specificity, the only component it detects is the α-antitoxin produced in response to immunization by α-toxoid. The experimental dose of test toxin is generally determined against 0.1 IU of standard antiperfringens horse serum. Under these conditions the sensitivity of the TNT *in vivo* and *in vitro* is 0.5 IU perfringens antitoxin/ml serum. The sensitivity of this test could apparently be increased by diluting the experimental dose of test toxin and then multiplying the results obtained by the coefficient of dilution. However, as will be clear from Table 1, if the quantity of standard serum used in the TNT *in vitro* is reduced by 2, 4, and 8 times, the experimental doses of test toxin titrated against these dilutions of serum are increased by 7.7, 19.9, and 26.5%, respectively. Determination of 0.125 IU/ml during titration of animal blood sera and 0.25 IU/ml when titrating human blood serum is reliable. In the latter case, when determining lower antitoxin levels, nonspecific opalescence develops as a result of the reaction of normal human blood serum with lecithovitellin.

During the TNT *in vivo* deviations of experimental doses of test toxin from the theoretically calculated doses with dilutions of standard serum by 2, 5, and 10 times were found to be 33.9, 68.8, and 78.8%, respectively. The experimental dose of test toxin, titrated against 0.01 IU, was equal to lethality of the experimental dose of test toxin without serum. When determining the perfringens antitoxin level *in vivo*, determination of 0.25 IU/ml is thus reliable

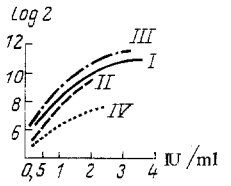

Fig. 1. Comparative titers of type A perfringens antitoxin in human blood sera in PHT, ELISA, and TNT. 1) Human serum (standard), II) rabbit serum (standard), III) human serum (from 96 tests), IV) ELISA with human serum.

provided that, as in experiments *in vitro*, the experimental dose of test toxin is determined against 0.05 IU of standard serum.

Not less than 1 ml of test serum is required for the TNT. The method of analysis for the TNT *in vivo* involves the use of mice, it is expensive in materials, and gives a wide range of variations of results due to individual differences between animals, their conditions of keeping, and the season of the year, and it is time-consuming.

The writers compared determination of the perfringens antitoxin level in 96 human blood sera. The coefficient of correlation of results obtained by determining antitoxin in the TNT *in vivo* and results obtained *in vitro* was 0.88. Under these same conditions the coefficient of correlation between data for the same sera obtained by the PHT and the results of the TNT *in vitro* was 0.64. It will be clear from Fig. 1 that the relationship between antitoxin levels determined in the PHT and TNT *in vitro* was linear between 0.1 and 3 IU/ml. The curve thus obtained, based on analysis of 96 sera, coincided practically completely with the curve plotted on the basis of titration of individual human sera, diluted beforehand to assigned values of serum perfringens antitoxin level (standard human serum). The sensitivity of the test was 0.01-0.005 IU/ml; 0.1-0.05 ml of test serum is sufficient for analysis.

Previously, when studying conditions for the use of PHT to determine the perfringens antitoxin level in blood sera of animals of different species (rabbits, guinea pigs, and mice) the writers showed that the ratio of antitoxin titers in the PHT to their titers in the TNT differed for each species. On this basis it was concluded that when the PHT is used, homologous sera must be adopted as the standards. It will be clear from Fig. 1 that during titration of human blood sera, rabbit antiperfringens serum can evidently be used as the standard. The reaction of perfringens erythrocytic diagnostic serum with anti-oedematiens, anti-histolyticum, and anti-tetanus rabbit sera was negative, but in a dilution of 1:128 ($-\log_2 = 7$) a positive reaction was given by perfringens antisera of types B and D, the culture fluid of which contains PLC. If a highly purified diagnostic serum, revealing only perfringens α-antitoxin in sera, is used the PHT is thus highly specific. The possibility cannot be ruled out that the value of the coefficient of correlation between PHT and TNT *in vitro*, namely 0.64, may be attributable, by analogy with determination of tetanus antitoxin, to the fact that the TNT detects IgG only, whereas antibodies of all classes of immunoglobulins react in the PHT [5].

On sensitization of polystyrene plates with the same highly purified antigen as during preparation of the perfringens erythrocytic diagnostic serum, the specificity of the ELISA corresponds to that of the PHT. It will be clear from Fig. 1 that the relationship between antitoxin titers expressed in $-\log_2$ units (ELISA) and obtained in the TNT *in vitro* was linear between 0.01 and 2 IU/ml, the sensitivity of the test was 0.01-0.02 IU/ml, and the coefficient of correlation relative to results obtained in the TNT *in vitro* was 0.39. Between 0.05 and 0.1 ml of test serum was required for the determination. Unlike TNT and PHT, which are equally applicable for analysis of sera of all species of animals, investigation of antiserum in the ELISA requires the presence of enzyme conjugated with anti-IgG against IgG homologous with the test serum. In practice, so far as difficulty of reproduction of the computation and the degree of correlation with the TNT *in vitro* are concerned, ELISA is inferior to the PHT.

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SUPPRESSION OF NORMAL KILLER ACTIVITY IN EMOTIONAL-PAINFUL STRESS AND ITS ABOLITION BY INTERFERON INDUCER

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KEY WORDS: emotional-painful stress; normal killer cells; interferon inducer.

Reactions to stress may have a suppressor effect on the immunogenesis system [6, 9] and, in particular, on antitumor immunity [7, 8]. At the same time it has been shown that activity of the normal killer cell (NK) system, as an essential factor of antitumor resistance, largely depends on synthesis of interferon and its level in the body [3].

The aim of the present investigation was accordingly to study the effect of emotional-painful stress (EPS) on activity of the NK system in animals and to study whether the stress-induced depression of activity of this system can be abolished by means of interferon inducer.

EXPERIMENTAL METHOD

Male August rats weighing 120-160 g were used. The model of EPS was produced in the form of an "anxiety neurosis" by the method in [2].

Activity of NK was tested immediately after exposure to stress for 1.5, 3, and 6 h, and also at various times from 1 to 9 days after the end of a 6-h period of stress.

Spleen cells, adjusted to a final concentration of 20×10^6/ml, were used as the source of NK. These effector cells were transferred to a culture medium prepared from Eagle's medium with 10% fetal calf serum, 2 mM glutamine, 1 mM HEPES buffer, and monomycin in a concentration of 100 U/ml. The targets for NK were human erythroid leukemia cells of line K 562, maintained by serial passages *in vitro*.

Activity of NK (i.e., their ability to cause lysis of target cells) was judged by the outflow of ^{51}Cr from destroyed target cells into the culture medium.

To label the targets, 100 μ Ci of ^{51}Cr (specific activity 350-600 mCi/mg Cr) was added to 5×10^6 K 562 cells in 0.9 ml of culture medium and the sample was incubated for 60 min at 37°C on a water bath. The labeled cells were rinsed vigorously three times to remove free chromium and made up to a concentration of 2×10^5 cells/ml. To 100 μl of the effectors in wells in roundbottomed plates 2×10^4 target cells in 100 μl of medium were added. All experiments were carried out with three different ratios of effectors to targets (100:1, 50:1, and 25:1). The cells were incubated for 14 h at 37°C in a CO_2 incubator, sedimented at 200g for 3 min, after which 100 μl of supernatant was withdrawn from each well. The samples were counted on a gamma-counter. To assess spontaneous liberation of ^{51}Cr, instead of effectors,

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