THE EFFECT OF BENZENE ON THE LEUKO-AGGLUTINATING AND RETICULOCYTOGENIC PROPERTIES OF SERA

A. Ya. Yaroshevskii

From the Laboratory of the Physiology of Circulation (Head, Prof. G. P. Konradi) and from the Therapeutic Sector (Head, Prof. A. Ya. Yaroshevskii) of the I. P. Pavlov Institute of Physiology (Dir., Academician V. N. Chernigovskii), AN SSSR, Leningrad

(Presented by Academician V. N. Chernigovskii)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 52, No. 7, pp. 40-44, July, 1961

Original article submitted July 8, 1960

Investigators and clinicians have studied the question of benzene's effect on the composition of the blood over a period of many years [1,8,11,12].

The action mechanism of benzene cannot be considered fully understood, however, since most authors mention only the direct effect of this substance on the bone marrow, and only the works of P. Ya. Mytnik [10] mention the important part played by its action on the sympathetic nervous system in the development of benzene leukopenia. In 1958, L. S. Musikhin found that the osmotic resistance of leukocytes changed in a series of cases under conditions of experimental benzene poisoning [9]. One would expect, by analogy with other types of toxic injury to the blood system, that the leuko-agglutinin type of auto-antibodies would appear in the serum of people and animals poisoned with benzene.

Another advantage of the experimental model of benzene poisoning is that it can be used to trace the reticulocytogenic and erythropoietic properties of blood sera in hypoplastic and aplastic conditions of hemopoiesis.

A similar type of investigation showed that massive x-ray irradiation does not affect hemopoietine [erythropoietic substance found in circulating blood] production [13]. We formed analogous conclusions in our clinical observations on patients with aplastic anemia (1959).

With the above data in mind, we studied both the changes in blood composition caused by poisoning with different doses of benzene and the changes in the leukoagglutinating and reticulocytogenic properties of the poisoned animals' sera, taking into account the character of the poisoning.

METHODS AND RESULTS

Change in the Composition of the Blood Attending Benzene Poisoning. We poisoned a total of 27 rabbits, which we separated into three groups. The ten rabbits of the first group received 0.3 ml benzene per 1 kg weight daily for five days; the ten rabbits of the second group were each injected with 1 ml benzene per 1 kg weight until clearly expressed aleukia developed and the animal died (injections given for 10-12 days), and the seven rabbits of the third group were each injected with 1 ml benzene per 1 kg weight every other day for 10-12 days until the appearance of pronounced changes in the blood composition.

Benzene was dissolved in an equal volume of peach oil and injected subcutaneously. The composition of the blood (erythrocytes, hemoglobin, reticulocytes, leukocytes and leukocyte formula) was studied for two weeks before poisoning, during poisoning and for two weeks after the daily administration of benzene ceased.

The changes in blood composition attending benzene poisoning depended on the dose. For example, in all ten of the rabbits which received the 0.3 ml benzene per 1 kg weight dose (a total of 5-8 g benzene), leukopenia developed on the 5th-7th day (average number of leukocytes constituted 44% of the original); the absolute content of leukocytes varied between 1800 and 6000 per 1 mm^3. In eight cases, erythrocytopenia was also observed (an average decrease of 950,000 per 1 mm^3). The hemoglobin content decreased by 12 units. Reticulocytopenia was also observed in every case (reticulocytes decreased to 30% of original level).

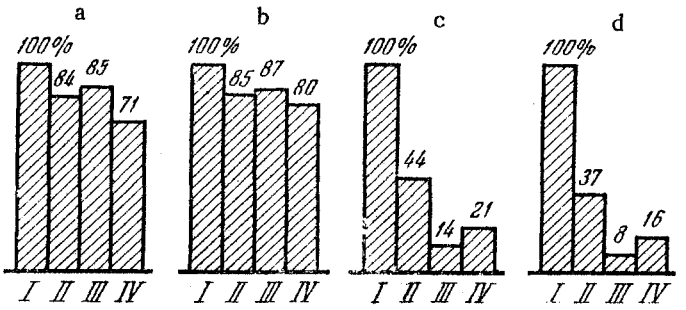

Fig. 1. Change in blood composition attending benzene poisoning: I) original level, II) first group of poisoned rabbits (0.3 ml benzene per 1 kg animal weight daily), III) second group (1 ml benzene per 1 kg weight daily), IV) third group (1 ml benzene per 1 kg weight every other day); a) erythrocytes, b) hemoglobin, c) leukocytes, d) reticulocytes.

The changes observed the 7th day in the blood composition of the animals of the second group (benzene administered in doses of 1 ml per 1 kg weight) were more pronounced. For example, the number of leukocytes constituted only 14% of the original, and the absolute content varied between 200 and 1000 per 1 mm^3 (the original figures being 9800-12,100 per 1 mm^3). Reticulocytopenia was also more pronounced: the reticulocytes constituted 8% of the original level and, in six cases, had totally disappeared from the blood. At the same time, the changes observed in the number of erythrocytes were about the same as those observed in the first group of rabbits (an average decrease of 980,000 per 1 mm^3), while the hemoglobin decreased 10 units. The changes in the leukocyte formula consisted in lymphopenia and relative neutrophilia (pseudo-eosinophilia).

Leukopenia was recorded in six out of seven cases in the experiments on the third group of animals (1 ml benzene per 1 kg weight injected every other day); the leukocytes decreased to 21% of the original number on the day of the last injection. The reticulocyte content also decreased in six out of seven animals to an average 16% of the original. Anemia was more pronounced; the number of erythrocytes decreased an average of 1,500,000 per 1 mm^3, while the hemoglobin decreased an average of 15 units.

These changes are shown in Fig. 1.

Benzene administration, therefore, not only causes clearly expressed leukopenia, but disturbs erythropoiesis too, as L. S. Musikhin [9] also observed.

Comparison of Number of Leukocytes and Leuko-Agglutination Reaction in Benzene Poisoning

Type of reaction	Number of leukocytes per 1 mm^3		
	Up to 3,500	3,600-4,500	Over 4,500
Positive	12	0	0
Negative	0	3	2

Change in the Leuko-Agglutinating Properties of the Serum. Although various types of leuko-agglutinins have been discovered in many diseases of the blood system associated with leukopenia, this question has as yet received little study in relation to benzene poisoning.

The rabbits' serum was examined for leuko-agglutinating properties before the animals were poisoned and on the last day of the benzene administration. The leuko-agglutination reaction was done according to the usual method. A leukocyte suspension was prepared from the blood of healthy rabbits; 0.4 ml of the experimental serum was then added to 0.2 ml of this suspension, and the mixture was incubated at 38°C for an hour, after which smears were prepared from it. The leuko-agglutination reaction was evaluated immediately, first without and then with staining according to May-Grunwald. The intensity of leuko-agglutination was rated as +, ++ and +++.

Before the poisoning, a weakly positive reaction (+) was observed in only 1 out of 17 healthy rabbits. The reaction became uniformly positive in the animals poisoned with benzene in the dose of 0.3 ml per 1 kg weight, sharply so in the six out of ten animals in which the leukocytes numbered less than 3,500 per 1 mm^3 blood. When

the reaction was conducted with seven rabbits which had received the 1 ml dose of benzene per 1 kg weight, it was positive in every case. In this group of poisoned animals, the leukocytes numbered 2,500 or less per 1 mm^3 (see table).

Therefore, the leukopenia caused by the administration of benzene can, in certain cases, especially in the case of low leukocyte content, be associated with the appearance of the leuko-agglutinin type of antibody as well as with the direct effect of benzene on hemopoiesis.

Study of the Reticulocytogenic Properties of the Sera. As a test of hemopoietic activity, rabbit's serum obtained after coagulation of the blood and clot retraction was subcutaneously injected after 24 hours in a dose of 3 ml into two healthy rabbits, the blood composition of which was studied for 14 days before the injection and 14 days afterwards.

Only two out of the ten sera obtained from healthy animals caused the number of reticulocytes in the rabbits into which they were injected to double and triple on the second and third day after the injections (2.5% and 1.3% to 5% and 4.2% respectively). The variations observed in the other eight cases did not exceed those observed before the experiment. Contrary to the results observed with the administration of human serum, the erythrocyte content increased an average of 558,000 per 1 mm^3 in five cases, while the variations observed in the other five cases did not exceed the normal. Therefore, the serum of healthy rabbits can cause erythrocytosis and reticulocytosis, but more frequently does not possess such properties (Fig. 2). The changes observed persisted for two to three days.

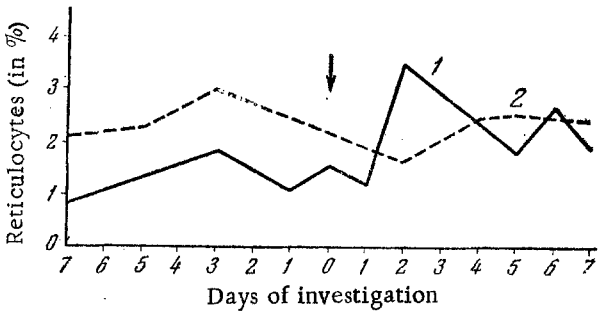

Fig. 2. Change in the reticulocyte content in rabbits following administration of 3 ml of serum from 2 healthy animals (1,2). The arrow shows the day the serum was administered.

The sera of rabbits poisoned with benzene were examined for reticulocytogenic properties on the last day the poison was administered. The 27 experimental sera were injected into 54 healthy rabbits. In the 17 animals of the less severely poisoned first and third groups, these properties were approximately uniform.

As compared with the serum from the healthy rabbits, the serum from the poisoned rabbits did not, in 6 out of 17 cases, cause fluctuations in the reticulocyte content exceeding those observed in the control experiments (an increase from the original 1-1.9% to 2.8-3.3% on the second to fourth day after administration). The hemoglobin content did not change substantially (varying 8 units at most). At the same time, the number of erythrocytes decreased an average of 930,000 per 1 mm^3 in 11 out of 17 cases. We could observe no correlation between the reticulocytogenic and anematizing properties of the sera.

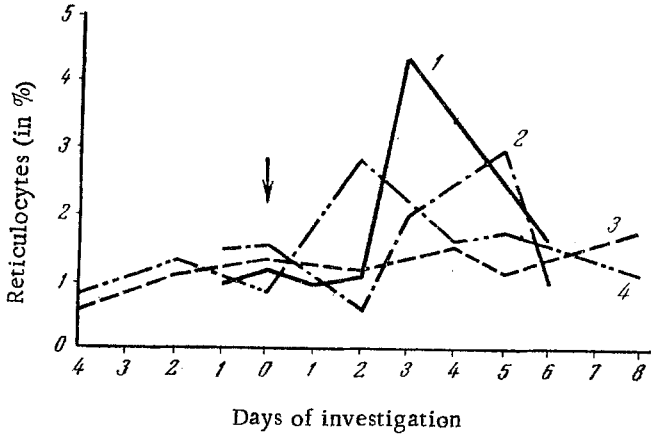

Fig. 3. Change in the reticulocyte content in rabbits following administration of 3 ml of serum from 4 animals poisoned with benzene (1,2,3,4). The arrow shows the day the serum was administered.

Nor was the original content of reticulocytes, hemoglobin and erythrocytes in the blood of the animal donors of any particular significance. As the results of the third group of experiments show, however, the serum from the most severely poisoned animals caused acute reticulocytosis in four out of seven cases (Fig. 3). For example, marked reticulocytosis (an increase of almost $4\frac{1}{2}$ times) was induced by serum taken from rabbit No. 23 when the leukocyte content in this animal constituted a total of only 600 per 1 mm^3 as compared with the original 10,400, the reticulocyte content was 0.1% compared with the original 2.8% and the hemoglobin was 60 units as compared with the original 80. However, the sera of rabbits Nos. 39 and 40, in which the composition of the blood was almost analogous to that of No. 23, did not exhibit such properties.

Therefore, benzene poisoning, even when so acute as to cause the animal's death, does not eliminate the ability of the sera to cause reticulocytosis when administered to healthy rabbits; on the contrary, these properties are more frequently observed, perhaps due to the formation in the serum of substances promoting erythrocyte destruction. Specific proof is required, however, before this hypothesis can be considered conclusive.

SUMMARY

Experimental benzol poisoning of rabbits (in the doses of 0.3 to 1 ml per kg of body weight) provokes, apart from the reduction of the leukocyte count, a marked reticulocytopenia. The appearance of antibody leukoagglutinins, which form at the time of the benzol action, may play a certain role in the mechanism of the effect of the latter on the leukocyte portion of the blood. The serum of healthy animals upon administration provoked reticulocytosis in 2 out of 10 healthy animals. The serum of the benzol-poisoned rabbits (especially in severe cases of poisoning) not only lost these properties, but even provoked reticulocytosis in a greater percentage of cases, in 15 of the 27).

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