STUDY OF THE TOXIC AND HEMOLYTIC PROPERTIES OF SERA IN THE MASSIVE TRANSFUSION SYNDROME

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The toxic and hemolytic properties of the sera of animals receiving massive transfusions of homologous blood were studied in experiments on 20 dogs and 432 mice. The toxicity of the recipients' sera was estimated in vivo in mice whose reticuloendothelial system had been blocked by Trypan Blue by calculating the mortality in per cent, and in vitro by the blood culture method. Hemolytic activity was studied by Dacie's principle with the use of erythrocytes labeled with radioactive chromium. The lost blood (45-50 ml/kg) was replaced in a volume 1.5 times greater than the volume of blood removed. The experiments showed that acute blood loss followed by massive transfusion of homologous blood caused the formation of toxic products in the recipient's serum. These toxic properties were found by two biological tests. The maximal toxigenic action was found 2 h and during the first day after blood replacement. On the 17th day no toxic effect of the sera could be found. During a parallel investigation of the hemolytic properties of the serum, a marked increase in its activity was discovered. The highest level of hemolytic activity was reached 24 h after blood replacement. After 3 days the hemolytic properties of the serum no longer differed from what they were initially.

KEY WORDS: massive transfusion syndrome; toxic properties of serum; hemolytic properties of serum.

Many investigations of the changes arising after transfusion of massive doses of homologous blood have recently been published. These complications, known as the "massive transfusion syndrome," cause disturbances of various functions and systems of the body, and the investigation of their pathogenesis is of considerable interest. In some extremal states (hemorrhagic shock, blood loss, burns, trauma, etc.) the blood acquires toxic and hemolytic properties [1, 3, 7, 9, 10, 14, 15].

The object of this investigation was to study the toxic and hemolytic properties of the sera of dogs after massive transfusions of homologous blood.

EXPERIMENTAL METHOD

Experiments were carried out on 20 dogs and 432 mice. Before the experiment the blood of the donor dogs and the recipients was cross-matched for group and individual compatibility. Freshly prepared blood (formula 12^a, Central Institute of Blood Transfusion) was transfused. The volume of blood loss was 40-45 ml/kg body weight. The lost blood was replaced by 1.5 times its volume of transfused blood. The transfusion was started immediately after the blood loss. For the first 40 min the blood was transfused rapidly, but later by the drip method. The blood serum of the dogs was tested 2 h and on the first, second, fourth, sixth, 10th, 14th, and 17th days after the massive transfusions.

Toxicity was determined by biological tests: 1) in mice with a blocked reticuloendothelial system [5]. The block was caused by a single injection of colloidal Trypan Blue, as a freshly prepared 1% solution, in a dose of 2.5 ml/100 g body weight into the caudal vein. This dose is the optimal dose of the dye, as was shown empirically. The test serum (1-2 ml) was injected intraperitoneally 30-40 min after the block. Toxicity was

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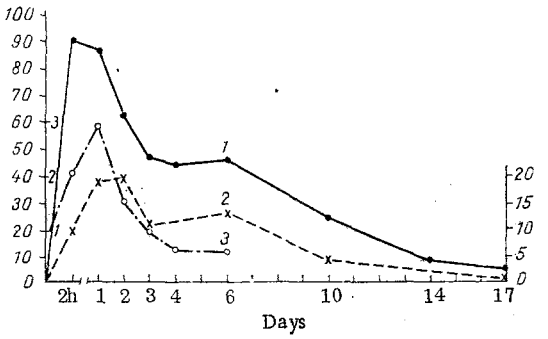

Fig. 1. Toxicity and hemolytic properties of blood serum from dogs with the massive transfusion syndrome. Abscissa, time of taking samples of sera; ordinate, left – mortality among mice (%), hemolysis (in %), on left – toxic effect on blood cultures – inhibition of leukocyte migration (in %); 1) mortality among mice (in %); 2) toxic effect on blood cultures; inhibition of leukocyte migration (in %); 3) hemolysis (in %).

estimated from the percentage of mice dying in the course of 72 h; 2) by the blood culture method, based on determination of leukocyte migration in a leukocyte film culture [4]. The toxic effect was established from the inhibition of migration of leukocytes in the blood culture on the addition of the test material to it and the result was expressed as a percentage of migration of the leukocytes in the control culture, to the nutrient medium of which Ringer's solution was added. The changes in migration were calculated as plus or minus from 100%.

The hemolytic activity of the serum was studied by a method based on Dacie's principle [2, 11] using erythrocytes labeled with radioactive chromium (^{51}Cr). To prevent "autohemolysis," the serum was incubated with the labeled erythrocytes at 4°C. Instead of the optical density of the serum its radioactivity was determined.

EXPERIMENTAL RESULTS

Blood serum taken from the recipient dogs before the experiment had no toxic properties. No mice died after receiving an injection of these sera. In blood cultures they stimulated leukocyte migration (absence of toxicity).

The toxigenic action of the sera reached a maximum 2 h and during the first day after blood replacement (Fig. 1). The mortality among mice receiving injections of sera taken 2 h after blood replacement was 90.5% ($P < 0.001$), falling to 88% by the end of the first day ($P < 0.01$). Toxic effects on blood cultures at these same times were −18 and − 20%, respectively. A definite decrease in the toxic effect was observed from the third day after transfusion: the mortality among mice receiving injections of the sera at this period was 47% ($P < 0.01$). The toxic action on blood cultures was rated at −11%. The toxigenic properties of the sera continued with slight fluctuations until the 10th day, after which their toxic action fell progressively so that on the 17th day after massive transfusion the toxicity of the sera could not be detected by either method.

Incubation of erythrocytes with serum taken before blood loss caused destruction on the average of 0.9% of their number. The hemolytic activity 2 h after blood loss and massive transfusion of homologous blood was doubled to 2% ($P < 0.001$); during the next 24 h it reached its highest level of 3% ($P < 0.001$). Toward the end of the second day the hemolytic activity of the serum was 1.5 times higher than initially ($P < 0.01$), but after 3 days it was the same as when first determined ($P > 0.2$).

The toxemia accompanying the massive transfusion syndrome can evidently be attributed to activation of mechanisms with a role to play in many experimental states (hemorrhagic shock, blood loss, burns, trauma, and so on).

One reason for the development of the toxigenic properties of the blood is injury to liver cells [12]. These observations are in agreement with our own. In previous investigations we found depression of the ingestive function of the reticuloendothelial system or the system of phagocytic macrophages in dogs receiving massive

transfusions of homologous blood [6]. Weakening of the toxigenic properties of the serum during the subsequent days can be explained by restoration of the function of the reticuloendothelial system.

The mechanism of appearance of hemolytic properties in the serum has not yet been explained. There is some evidence that, during unreplaced blood loss and other types of hypoxia the spleen, liver, muscles, and other organs produce an endogenous hemolytic factor, analogous to lysolecithin [13]. Since the nature of the toxic and hemolytic factors is wholly unknown, it is difficult to pronounce on their identity. All that is known for certain is that they are discovered simultaneously. Perhaps the hemolytic factor appears a little earlier, for there is evidence that hemolytic properties can be found as early as 10 min after blood loss [8], as a result of which some erythrocytes and other blood cells may be destroyed. The products of hemolysis block the reticuloendothelial system, as a result of which the total resistance of the body is lowered and the toxigenic properties of the blood are manifested.

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