ON THE DURATION OF CIRCULATION OF SARCOLYSIN-C^{14} IN THE BLOOD AND ON ITS EXCRETION IN THE URINE AFTER INTRAVENOUS INJECTION INTO RABBITS

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Sarcolysin [p-di(2-chloroethyl)aminod,l-phenylalanine] represents one of the most active antitumor preparations among the compounds in this group [3]. It is assumed that the power and selectivity of its carcinostatic effect is related to the longer duration of its circulation in the blood compared with other chloroethylamines [2].

G. L. Zhdanov [1,2] and later E. I. Khomchenovskii [6] showed by indirect methods that sarcolysin or active products of its transformation circulate in the blood for about two hours, whereas embichine disappears from the blood stream within 3–5 min after its intravenous injection [1, 7, 8, 9].

According to the findings obtained by one of the authors of the present article [4] the activity of the blood decreases within one hour after intraperitoneal injection of labeled sarcolysin into rats only 2-3 times and persists for about a day on a relatively high level.

It was the aim of the present investigation to study in great detail the circulation of sarcolysin in the blood after its intravenous injection and also to investigate the speed of its excretion in the urine.

The experiments were carried out on six rabbits (males and females) weighing between 2.5 and 3.6 kg. The ureters of the rabbits were isolated under urethane narcosis (in a dose of 1 g per 1 kg weight administered intravenously) and polyvinyl catheters were inserted in one ureter or both ureters near the entrance into the urinary bladder. One of the carotid arteries was isolated and also provided with a polyvinyl catheter which was closed by a clamp. To prevent clotting of the blood, heparin was injected through the same catheter into the carotid artery (in a dose of 100 units per 1 kg weight).

Sarcolysin-C^{14} with a specific activity of 1.62 mC/g in a dose of 10 mg/kg in normal saline was injected into the ear vein of the rabbit. 30 seconds, 1,2,3,5,10,15, 30 minutes and 1,2 and 4 hours after the injection of sarcolysin, blood was taken from the carotid artery and 0.05 ml of the blood was put on to a watchglass. To achieve hemolysis and a more even distribution of the blood a few drops of distilled water were added to each watchglass. The sample was dried in a desiccator and the radioactivity was counted with the aid of a MST-17 counter on a type B apparatus. In addition, 2 ml of blood were repeatedly taken from the carotid artery during the course of the experiment, were hemolyzed with distilled water and precipitated with 75° alcohol. The precipitate was centrifuged four times with 75° alcohol, three times with 96° alcohol, once with a mixture of 96° alcohol and ether and once with ether. The dried proteins were triturated into a fine powder and weighed portions of the powder of 10 mg were put on to watchglasses to count the radioactivity. The supernatant of the first four centrifugations was mixed, evaporated in a porcelain dish, washed off with 1 ml 96° alcohol and brought to a watchglass which was subsequently desiccated after which the radioactivity was counted. The urine from the catheters was collected into tins and every five minutes 0.1 ml from the portions obtained was placed on to a watchglass after which the radioactivity was estimated.

In two experiments the total urine excreted within one or two hours respectively after the injection of sarcolysin-C^{14} was collected, the volume was measured and the average activity was calculated. The latter was expressed in counts/min. The activity of the sarcolysin solution used for the injection was also estimated on the counter.

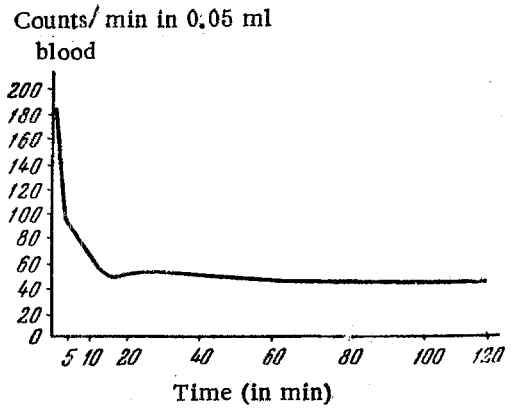

Fig. 1. Curve characterizing the radioactivity of the blood after intravenous injection of sarcolysin-C^{14} into rabbits.

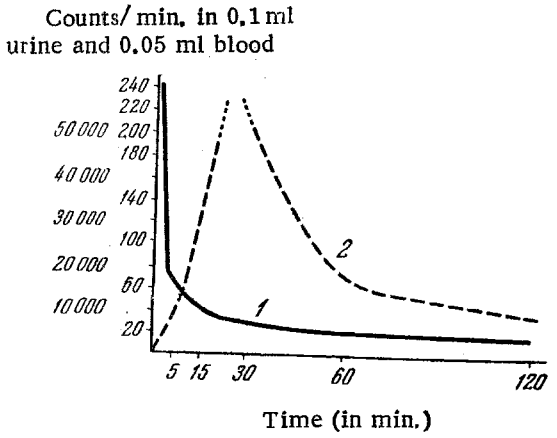

Fig. 2. Curves characterizing the activity of blood and urine after intravenous injection of sarcolysin-C^{14} into rabbits. 1) Blood; 2) urine.

We attempted to decide with the aid of paper-chromatography whether sarcolysin is excreted from the animal body in a free form or whether it is linked to products of metabolism [5]. To obtain chromatograms of the urine, the latter was collected for one-two hours respectively, diluted 10 or 20 times with distilled water and applied to paper which was then placed into the solvent consisting of a mixture of butyl alcohol, acetic acid and water. From the chromatograms autoradiographs were prepared on X-ray films using an exposure time of one week.

In all rabbits the results obtained were more or less identical.

Figure 1 shows the curve characterizing the activity of the blood in one of the rabbits. On the abscissa the time was plotted in minutes and on the ordinate the activity in counts/min for 0.05 ml dried blood. Fig. 1 shows that in these, as in the other rabbits, the activity of the blood falls rapidly in the first five minutes. Then it falls more gradually and after 10-15 min reaches a permanent level which remains more or less unchanged throughout the period of observation (in the case in question for two hours, in some other experiments four hours).

We calculated the mean activity of 1 ml blood for all rabbits used for the experiment for periods of one half to one minute, 10-15 min, and two hours after the injection. It appeared that after one half to one min the mean activity of 1 ml blood was four times higher than the activity injected per g total weight of the rabbit (4000 and 1000 counts/min respectively). After five minutes the activity of 1 ml blood was only $1^1/_2$ times higher than the activity of the injected substance per 1 g weight (1500 counts/min). After the permanent level had been reached the mean activity of 1 ml blood was equal to 580 counts/min which constitutes somewhat more than one half of the activity injected per 1 g weight. If one takes into account that the weight of the blood constitutes approximately 7% of the body weight it appears that even 2-4 hours after the injection of sarcolysin-C^{14} about 4% of the active substance injected still circulates in the blood.

The activity of the protein precipitate two hours after the injection of sarcolysin reaches, on the average, 10 counts/min for 10 mg protein precipitate. By determining the dry weight of the protein precipitate obtained from 2 ml blood (0.35 g), we calculated that the proteins precipitated from 1 ml blood have an activity of 175 counts/min. The activity of the evaporated supernatant corresponding to 1 ml blood also reaches 175 counts/min, i.e., two hours after the injection of sarcolysin the activity was almost equally distributed between the proteins precipitated with alcohol and the soluble part.

In contrast to the activity of the blood which—five minutes after the injection of sarcolysin—shows a marked fall the activity of the urine begins to increase and reaches its maximum value between 10 and 30 minutes after the injection after which it begins to decrease.

Fig. 2 shows the curves characterizing the changes in the activity of the blood and the urine in another rabbit. The striking similarity between the curves characterizing the activity of the blood in both experiments deserves attention. The activity of the urine was so high 30 min after injection of sarcolysin that it could not be plotted on the curve.

Other experiments led to similar results : we were, however, not always able to obtain an even and continued excretion of urine and were consequently not always able to observe the descending part of the curve.

As we said above, in two rabbits we collected the whole urine excreted within one and two hours respectively after the injection of sarcolysin. In the first of these rabbits, we were unable to introduce the catheters into both ureters and the second ureter had to be ligated. In this rabbit one kidney excreted within one hour 6.9% and within the second hour 2.7% of the whole activity injected into the rabbit.

In the other rabbit the catheters were inserted into both ureters and the urine was freely excreted throughout the period of observation.

For one hour the urine was collected separately from each ureter and it appeared that the activity of the urine excreted by both kidneys was almost the same (395,800 and 389,536 counts/min respectively). In the second hour the urine was collected from both ureters into a common container.

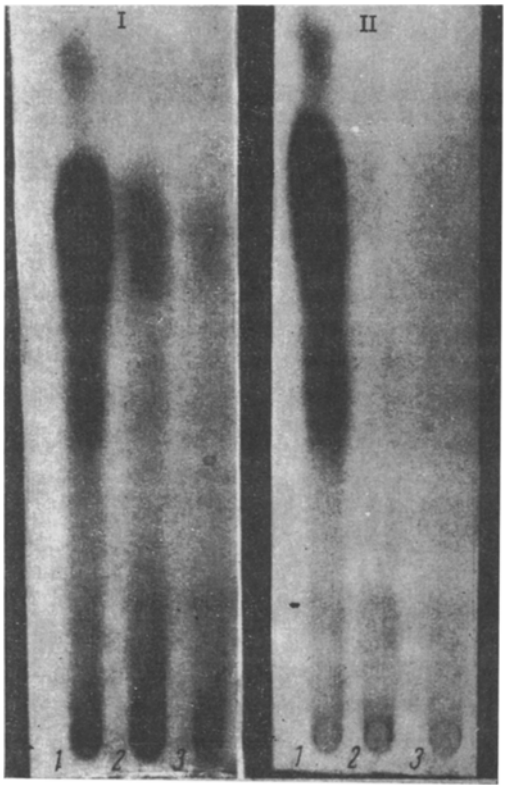

Fig. 3. Autoradiographs produced by chromatograms of the urine in the first (I) and the second (II) hour after intravenous injection of sarcolysin-C^{14} into a rabbit. 1) Aqueous solution of sarcolysin; 2) urine diluted with water 10 times; 3) urine diluted with water 20 times.

This rabbit excreted in the first hour 22.5% and in the second hour 4.5% of the whole activity injected.

Figure 3 shows autoradiographs produced by chromatograms of the urine in one of the three rabbits; The urine was collected for one and two hours respectively. On each of the chromatograms the solution of sarcolysin-C^{14} in distilled water was on the left, the urine diluted ten times with water, was in the middle, and on the right, the urine diluted 20 times. The autoradiograph produced by the chromatogram of the urine collected within one hour clearly shows the spots on the level of the sarcolysin spot and the intensity of the spots proves to be reversely proportional to the dilution of the urine. In the urine collected after two hours from the same rabbit no spots can be seen at the level of the sarcolysin spot.

The above findings warrant the conclusion that at least in the first periods after the injection of sarcolysin this substance is excreted by the kidneys partly in a free form.

Our experiments thus showed that in the first five minutes after intravenous injection of sarcolysin-C^{14} the activity of the blood falls rapidly due to the fact that sarcolysin, as had been shown earlier [3,4], is linked to the tissues, but also due to the fact (shown in the present paper) that the sarcolysin is excreted with the urine, partly in a free form. After this time, however, the activity of the blood remains for a considerable period on a level approximately corresponding to 4% of the activity of the preparation injected, an activity which is almost evenly distributed between the blood proteins precipitated with alcohol and the soluble fraction.

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