THE EFFECT OF THE NORMAL AND PATHOLOGICALLY CHANGED SPLEEN ON THE CLOTTING SYSTEM OF THE BLOOD

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From the work of Soviet and non-Soviet researchers it seems probable that the spleen affects the formation of blood coagulation factors. A decrease in the prothrombin and thromboplastin activity of the blood has been described in splenectomized rats [3, 4, 13]; partial hepatectomy in such rabbits leads to a greater decrease in the blood prothrombin level than in animals with an intact spleen [16]. When administered to dogs irradiated with roentgen rays, splenic extracts increased the number and adhesive power of the platelets and the clot retraction [12], and they also increased the prothrombin consumption in irradiated rats [5]. Irradiation of the spleen with small doses of roentgen rays caused a marked reduction of the clotting time of the blood, while irradiation with large doses caused a prolongation of the clotting time [14]; after irradiation of the spleen with small doses, the concentration of factors V and VII in the blood of the animals was increased. Spleen homogenate increased the thromboplastic activity of the blood of irradiated rats [6].

We have attempted to analyze the effect of the spleen on the indices of the clotting system of the blood, in relation both to the functional state of the bone marrow (thromboplastic activity of the blood, prothrombin consumption) and to the functional state of the liver (blood prothrombin, proconvertin, and factor V concentrations).

EXPERIMENTAL METHOD

In experiments on rabbits the bone marrow was injured with roentgen rays or benzene and the liver with CCl_4. The effect of preliminary splenectomy (1 month before application of the myelotoxic or hepatotoxic agents), or of diathermy treatment of the spleen as a method of stimulating the humoral activity of the organ [1], on the indices of the clotting system of the blood was studied in these animals. In a group of rabbits the indices of blood clotting were analyzed after splenectomy and without any additional interference.

In a series of experiments we induced a methylcellulose splenopathy – the condition most nearly equivalent to clinical splenopathy in man [7, 10, 11] – in albino rats by the method of Palmer and co-workers [10] in order to compare the effects of the normal and pathological spleen on the clotting system of the blood. Splenopathy was induced in rats by injecting 2 ml of a 2.5% solution of methylcellulose intraperitoneally twice a week for 15 weeks.

In all the experiments involving splenectomy, laparotomy without removal of the spleen was performed at the same time on control animals.

In the series involving irradiation with roentgen rays rabbits were irradiated with a dose of 600 r under the following conditions: voltage 180 kV, current 20 mA, focus distance 60 cm, filters 0.5 mm Cu and 1 mm Al. The benzene injury of the marrow was produced by injecting benzene subcutaneously in a daily dose of 1 ml/kg body weight for 3 days in the experiments involving splenectomy, and for 5 days in the other experiments. Toxic hepatitis was produced by injecting CCl_4 subcutaneously in a dose of 0.5 ml/kg on alternate days for 4 doses. Benzene and CCl_4 were injected in peach oil. Diathermy treatment of the spleen was given by means of a type ULD-200 apparatus, using a current of 0.15 A, electrodes measuring 3 × 3 cm, and daily sessions lasting 15 min for 1 week after induction of the lesion of the marrow or liver.

In the series of experiments involving splenopathy in rats some animals received injections of methylcellulose after preliminary splenectomy (in order to define the effect of the drug itself); in a group of rats the pathologically changed spleen was removed at the end of the course of methylcellulose injections, and the indices of blood clotting were analyzed 2 weeks later.

The thromboplastic activity of the blood was determined by the method of B. A. Kudryashov and P. D. Ulitina [2], the prothrombin consumption by Quick's method [13], the blood prothrombin by Ware and Seegers' method [15], proconvertin by the method of Owren and Aas [9], and factor V as described by Lewis and Ware [8]. In the series of experiments involving toxic hepatitis, in addition to determining the prothrombin and procinvertin levels, we also studied the changes in the concentration of these factors after injection of an anticoagulant (dicumarin, intramuscularly in a dose of 25 mg/kg 24 h before determination of the clotting indices), and also a combination of dicumarin (in accordance with the same scheme) and vitamin K (intravenously in a dose of 50 mg/kg).

In this paper we give the results of experiments on 119 rabbits and 52 albino rats.

EXPERIMENTAL RESULTS

The numerical results were analyzed statistically. They are shown in Tables 1-4 (the mean and standard error of the mean are given for each group).

It is clear from Tables 1-4 that splenectomy in rabbits leads to a transient lowering of the thromboplastic activity of the blood and of the ability to utilize prothrombin. After administration of the myelotropic poison benzene, the difference between these indices in the splenectomized and control animals, undergoing laparotomy alone, was still more marked (Table 1). Diathermy of the spleen had a favorable effect of the blood clotting indices depending on the marrow in rabbits irradiated with roentgen rays or poisoned with benzene (Table 2). Stimulation of the activity of the spleen by this method led also to an improvement in the blood clotting indices depending on the state of the liver in rabbits with experimental CCl_4 hepatitis (Table 3).

The experimental induction of methylcellulose splenopathy in albino rats caused a disturbance of the hemostatic activity of the marrow and liver, reflected in a lowering of the thromboplastic activity of the blood, the prothrombin consumption, and the concentrations of prothrombin, proconvertin, and factor V in the blood. The dominant role

TABLE 1. Effect of Splenectomy and Injection of Benzene after Preliminary Splenectomy on the Indices of Blood Clotting in Rabbits Depending on the State of the Marrow

Experimental conditions	No. of rabbits	Thromboplastic activity of the blood (in %)	Prothrombin consumption (in seconds)
Intact animals	20	97.5 ± 2.6	45.7 ± 0.9
Control laparotomy (after 3 weeks)	10	89.0 ± 2.7	45.1 ± 0.8
Splenectomy (after 3 weeks)	10	46.8 ± 2.2	32.8 ± 0.9
Control laparotomy (after 6 weeks)	10	92.7 ± 2.5	42.8 ± 0.7
Splenectomy (after 6 weeks)	10	47.7 ± 2.0	31.0 ± 0.8
Control laparotomy (after 10-11 weeks)	10	93.3 ± 4.0	47.9 ± 1.0
Splenectomy (after 10-11 weeks)	10	80.9 ± 3.2	42.4 ± 0.9
Control laparotomy + benzene for 3 days	9	60.1 ± 2.1	32.8 ± 1.1
Splenectomy + benzene for 3 days	10	36.2 ± 1.4	23.0 ± 1.0

TABLE 2. Effect of Diathermy Treatment of the Spleen on the Indices of Blood Clotting Depending on the State of the Marrow in Rabbits after Irradiation with Roentgen Rays or Poisoning with Benzene

Experimental conditions	No. of rabbits	Thromboplastic activity of the blood (in %)	Prothrombin consumption (in seconds)
Intact animals	20	97.5 ± 2.6	45.7 ± 0.9
Irradiation (after 10-12 days)	10	30.6 ± 2.7	12.5 ± 0.5
Irradiation + diathermy to the spleen	10	49.6 ± 2.1	24.2 ± 0.9
Irradiation (after 18-20 days)	10	21.3 ± 1.6	18.0 ± 0.8
Irradiation + diathermy to the spleen	10	59.8 ± 1.2	34.3 ± 1.1
Irradiation (after 26-28 days)	10	44.1 ± 2.8	27.3 ± 0.9
Irradiation + diathermy to the spleen	10	85.8 ± 2.1	44.3 ± 0.7
Injection of benzene for 5 days	10	43.4 ± 1.9	23.8 ± 0.6
Irradiation + diathermy to the spleen	10	77.7 ± 3.2	37.0 ± 0.9

TABLE 3. Effect of Diathermy Treatment of the Spleen on the Indices of Blood Clotting Depending on the State of the Liver in Rabbits with Hepatitis Caused by Carbon Tetrachloride

Blood clotting indices	Experimental conditions		
	intact (10 rabbits)	toxic hepatitis (10 rabbits)	toxic hepatitis + diathermy to the spleen (10 rabbits)
Prothrombin (in %)	97.4 ± 2.3	50.4 ± 1.7	72.8 ± 1.4
Prothrombin (in %) + dicumarin	52.7 ± 2.1	26.2 ± 2.0	43.7 ± 1.9
Prothrombin (in %) + dicumarin + vitamin K	96.2 ± 2.8	29.6 ± 1.8	61.1 ± 1.4
Proconvertin (in %)	96.8 ± 2.1	42.7 ± 1.2	70.3 ± 2.2
Proconvertin (in %) + dicumarin	56.1 ± 2.5	25.1 ± 0.6	43.4 ± 1.2
Proconvertin (in %) + dicumarin + vitamin K	89.9 ± 1.6	30.6 ± 1.1	61.6 ± 3.1
Factor V (in %)	98.2 ± 2.1	34.4 ± 2.2	59.0 ± 2.1

TABLE 4. Effect of Experimental Splenopathy in Albino Rats on the Indices of the Clotting System of the Blood Depending on the State of the Marrow and Liver

	Experimental conditions			
	intact (12 rats)	methylcellulose after splenopathy (14 rats)	methylcellulose after splenectomy (16 rats)	splenectomy after induction of splenopathy (10 rats)
Thromboplastic activity of the blood (in %)	98.1 ± 1.6	45.2 ± 1.1	91.1 ± 1.5	76.9 ± 2.8
Prothrombin consumption (in seconds)	36.1 ± 0.3	21.7 ± 0.2	34.4 ± 0.3	28.6 ± 0.8
Prothrombin (in %)	96.8 ± 1.1	63.6 ± 1.0	91.0 ± 1.4	92.4 ± 3.2
Proconvertin (in %)	98.7 ± 1.0	50.9 ± 0.9	92.6 ± 1.4	76.1 ± 1.8
Factor V (in %)	99.1 1.2	53.0 1.2	93.6 ± 1.9	85.4 ± 2.2

of splenopathy, and not of methylcellulose, in this phenomenon was proved by the absence of statistically significant changes in the blood clotting indices in animals receiving methylcellulose after preliminary splenectomy (Table 4). This was also demonstrated by the appreciable improvement in the blood clotting indices after removal of the pathologically changed spleen.

The results of our experiments, together with reports in the literature, demonstrate the role of the spleen in the synthesis of blood clotting factors. The spleen evidently exerts this influence through its myelolienal and hepatolienal interrelationships.

Depression of the production of blood clotting factors in splenopathy and the rapid restoration of this process after removal of the pathologically changed spleen may also account for the tendency towards thrombosis encountered in surgical practice after splenectomy for various splenopathies (the changes in the vascular wall in these conditions must also, of course, be taken into consideration together with this mechanism).

SUMMARY

As demonstrated in experiments on 119 rabbits, splenectomy leads to a transitory reduction of blood thromboplastic activity and prothrombin consumption. After the administration of myelotropic poison (benzol) the difference in these indices in splenectomized rabbits and in those subjected to control laparotomy was even more pronounced. Splenic diathermy stimulating the humoral activity of the organ favorably influenced the indices of thromboplastic activity of the blood and prothrombin consumption in X-irradiation and benzol poisoning, as well as the content of prothrombin, proconvertin and Y factor during carbon tetrachloride hepatitis in rabbits. In splenopathy of albino rats caused by methylcellulose, the blood coagulation indices, depending on the state of the bone marrow and liver, showed a marked reduction; excision of pathologically changed spleen normalized these indices (experiments on 52 albino rats).

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