RELATION OF SPLENIC ACTIVITY TO THAT OF OTHER ORGANS

COMMUNICATION III. EFFECT OF DIATHERMY APPLIED TO SPLEEN AND OF SPLEEN EXTRACTS ON THE LIVER AND HEMOPOIETIC ORGANS DURING PATHOLOGICAL CONDITIONS

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The effect of experimentally induced splenic disorders on the condition of many other organs, particularly the liver, has previously been described [1, 2]. It seemed worth while to investigate the influence of the spleen on experimentally induced disorders of the liver. A humoral regulatory influence exerted by the spleen on metabolic processes in the liver has been reported [3].

METHOD

We applied diathermal treatment to the spleen in the same way as is used in the treatment of endocrine organs by stimulation [8]. At the same time we used splenic extracts. One such extract was obtained from intact rabbits and white rats. To obtain a second kind of extract, rabbits and rats were first joined in pairs, using the method of cutaneous-muscular parabiosis [9] in which, during the operation for joining the animals, the spleen is removed from one of them; 10 days later, the spleen is removed from the second partner, and an extract prepared from it. The spleen of the second parabiont is always larger than that of the first and has hypertrophied to fulfill many humoral functions, and so may serve as a model of the so-called hyperactive spleen; the activity is increased but does not become abnormal, as happens in splenic disorders [16].

The extracts were prepared by a method which has been described in detail by Ungar [17] and by Karjala and his coworkers [15]. For intramuscular injection, a solution of 1mg per ml of the extract obtained in this way was made in peach oil. Diathermy was applied to the spleen with an apparatus giving a wavelength of 200 m at strength of 0.15 amp using electrodes 3 × 3 cm for the rabbit and 1.5 × 1.5 cm for the rat; the treatment was continued for 15 minutes.

The particular liver disorder selected was toxic hepatitis induced by subcutaneous injection of carbon tetrachloride; in rabbits the dose was 0.5 ml per kg 4 times per day, while that for white rats was 0.3 ml per 100 g body weight, given 3 times daily.

Diathermy was started at the same time that the first injection of carbon tetrachloride was given, and was continued daily for 9 days in the case of the rabbits, and for 7 days for the white rats. Injection of 1 ml of an oily solution of splenic extract per kg body weight were given intramuscularly daily for 9 days, beginning at the time of the first carbon tetrachloride injection; the white rats received 0.2 ml per 100 g body weight daily for 7 days, beginning at the same time as the other injection.

The following measurements were made to determine the condition of the liver: changes in the blood sugar induced by adrenalin, total blood serum protein and protein fractions, liver glycogen and fat, uptake of sulfur-labeled methionine by liver protein, and the ability of liver sections to bind phenol and to oxidize tyrosine.

TABLE 1

Effect of Diathermy of the Spleen and Splenic Extracts on the Protein Content and Protein Fractions in the Blood Serum and Figures Showing the Effect of Adrenalin Injections Given to Rabbits Suffering from Toxic Hepatitis

No. of group of rabbits	Description of group	No. of animals	Protein (in g %)	Albumin (in %)	Globulin (in %)			A/Γ	Increase of blood sugar (mg %) during 4-hour test with adrenalin	
					α	β	γ		maximum	average
1	Intact	10	6.7±0.3	60.8±3.1	9.1±0.4	13.9±2 2	16.2±3.0	1.5±0.06	321±24.7	258±14.5
2	Toxic hepatitis	10	7.3±0.3	46.2±4.5	10.2±1.3	20.3±4,1	23.3±4.6	0.9±0.02	192±19.3	157±17.5
3	Toxic hepatitis+ diathermy to spleen	10	7.0±0.2	54.7±2.9	10.3±0.3	15.8±2.2	19.2±1.6	1.2±0.02	253±22.1	193±15.8
4	Toxic hepatitis+ extract of normal rabbit spleen	8	7.1±0.2	50.1±3.2	11.2±0.4	16.9±2.4	21.8±1.8	1.0±0.04	229±17.4	178±11.7
5	Toxic hepatitis + extract of hyperactive rabbit spleen	10	6.9±0.3	56.2±3.5	9.3±0.7	15.6±3.0	18.9±1.9	1.2±0.05	271±15.7	201±13.3
6	Toxic hepatitis + extract of hyperactive white rat spleen	10	7.0±0.4	55.9±3.0	8.8±0.5	16.0±2.8	19.3±2.1	1.2±0.04	262±18.2	196±18.9

In all the tables average values are given and root mean square deviations for each group.

TABLE 2

Effect of Splenic Diathermy and Splenic Extracts on the Uptake of Sulfur Labeled Methionine by Liver Protein and on the Ability of the Liver to Bind Phenols and to Oxidize Tyrosine in White Rats Suffering from Toxic Hepatitis

No. of group of animals	Description of group	Number of animals	Percentage uptake of methionine by liver protein	Relative activity	Binding of phenols (as % of amount injected)	Oxidation of tyrosine (in % of amount injected)
1	Intact	10	8.1 ± 0.4	0.61 ± 0.03	26.4 ± 1.7	42.7 ± 2.5
2	Toxic hepatitis	10	6.5 ± 0.4	0.39 ± 0.07	9.3 ± 1.1	20.2 ± 1.6
3	Toxic hepatitis + diathermy to spleen	10	7.6 ± 0.3	0.51 ± 0.05	15.7 ± 1.3	29.7 ± 2.2
4	Toxic hepatitis + extract of normal rat spleen	10	7.0 ± 0.2	0.45 ± 0.06	12.3 ± 1.0	24.4 ± 2.1
5	Toxic hepatitis + extract of hyperactive rat spleen	10	7.6 ± 0.3	0.54 ± 0.06	15.9 ± 1.8	30.1 ± 1.8
6	Toxic hepatitis + extract of hyperactive rabbit spleen	10	7.8 ± 0.3	0.52 ± 0.03	16.8 ± 1.5	27.1 ± 1.5

TABLE 3

Effect of Diathermy to Spleen and Splenic Extracts on Glycogen and Fat Content of Liver in Rabbits and White Rats Suffering from Toxic Hepatitis

No. of group of animals	Description of group	Rabbits			White rats		
		number of animals	glycogen (in g %)	fat (in g % of dry substance)	number of animals	glycogen (in g %)	fat (in g % of dry substance)
1	Intact	10	2.4 ± 0.5	8.7 ± 0.6	10	2.7 ± 0.3	11.8 ± 0.8
2	Toxic hepatitis	10	0.8 ± 0.2	18.9 ± 0.7	10	0.5 ± 0.1	27.6 ± 1.2
3	Toxic hepatitis + diathermy to spleen	8	1.3 ± 0.3	13.1 ± 0.8	10	1.2 ± 0.2	20.9 ± 0.8
4	Toxic hepatitis + extract of normal rabbit spleen	8	0.9 ± 0.4	15.9 ± 0.5	10	0.7 ± 0.1	23.3 ± 1.6
5	Toxic hepatitis + extract of normal rat spleen	8	1.0 ± 0.2	15.4 ± 0.6	10	0.9 ± 0.1	23.8 ± 1.9
6	Toxic hepatitis + extract of hyperactive rabbit spleen	10	1.4 ± 0.3	12.2 ± 0.7	10	1.1 ± 0.1	18.7 ± 1.2
7	Toxic hepatitis + extract of hyperactive rat spleen	10	1.3 ± 0.6	12.7 ± 0.5	10	1.4 ± 0.91	19.5 ± 1.4

TABLE 4

Effect of Diathermy to the Spleen and Splenic Extracts on Blood Indices in Benzene Poisoning in Rabbits

Blood index	Description of group			
	benzene without therapy (12 rabbits)	benzene + diathermy to spleen (12 rabbits)	benzene + extract of normal spleen (10 rabbits)	benzene + extract no. 1 of hyperactive spleen (12 rabbits)
Leukocytes (in thousands) per mm^3; initial count	9.3 ± 0.4	9.0 ± 0.7	9.6 ± 0.4	9.2 ± 0.2
9-10th day after first benzene injection	2.7 ± 0.4	4.5 ± 0.7	4.2 ± 0.3	4.3 ± 0.6
26-27th day after first benzene injection	5.4 ± 0.8	8.6 ± 0.5	5.9 ± 0.4	8.9 ± 0.6
Granulocytes (in thousands) per mm^3; initial count	3.2 ± 0.3	3.4 ± 0.1	3.5 ± 0.6	3.4 ± 0.5
9-10th day after first benzene injection	1.4 ± 0.1	1.8 ± 0.2	1.3 ± 0.2	2.0 ± 0.4
26-27th day after first benzene injection	2.1 ± 0.2	2.9 ± 0.3	2.2 ± 0.3	3.0 ± 0.3

Adrenalin was given to rabbits as a subcutaneous injection of 0.3 ml per kg body weight of a 1 : 1000 solution. Total protein was determined using a refractometer, and the protein fractions estimated by paper electrophoresis. Glycogen was determined by Cori's method as modified by Brand, and the total liver lipids by extracting the dried liver tissue with dichlorethane in a Soxhlet apparatus [10]. Sulfur labeled methionine was injected intraperitoneally 24 hours before killing, to give 5000-6000 impulses per minute per g body weight. To eliminate the effect of possible changes in the permeability of liver tissue to the isotope, a determination was made of the activity of the protein relative to that of the protein in 1 g of tissue and to the activity of 1 g of whole tissue. The binding of phenol and the oxidation of tyrosine by the liver sections was measured by the usual methods [6, 7].

RESULTS

The results are shown in tables 1, 2, and 3.

The results shown in the tables indicate that in toxic hepatitis, diathermy applied to the spleen and hyperactive spleen extract exert a definitely favorable influence on the functional condition of the liver in all cases: there is an increased glycemic reaction to adrenalin, liver glycogen is raised, and the fat content reduced; there is a reduction in blood albumen and an increase in the globulin fraction, the antitoxic action of the liver is raised, as is also the ability of the liver to synthesize protein. The effect on these indices of extracts of normal spleen from intact animals is very much smaller.

In addition to the experiments whose results are given in the tables, we also carried out others in which we investigated the effect of splenic diathermy and the action of various splenic extracts on liver function (using the indices of the tests) in intact animals (without hepatitis); it was not possible to determine any consistent changes in these experiments, and the results have therefore not been included in the tables.

It seemed most interesting and important to us that the effect of the hyperactive spleen extract did not depend on whether it was prepared from an animals of the same species as that in which the hepatitis was induced or from the spleen of a different species. The results obtained confirm that the liver and spleen are functionally related, and give certain indications of how to make use of these correlations clinically.

Splenic diathermy and injection of splenic extracts were also used by us during a study of the myelo-lienal correlation under pathological conditions. The related functions of the bone marrow and spleen have frequently been studied by Soviet and foreign workers [5, 11, 12, 13].

TABLE 5

Effect on Rabbits of Diathermy to Spleen and of Splenic Extracts on Blood Indices after General X-ray Irradiation

Blood Index	Description of group				
	irradiation without therapy (14 rabbits)	irradiation + diathermy to spleen (10 rabbits)	irradiation + extract of normal spleen (10 rabbits)	irradiation Extract No. 1 of hyperactive spleen (15 rabbits)	irradiation + Extract No. 1 of hyperactive spleen (15 rabbits)
Leukocytes (in thousands) per mm^3:					
initial count	9.1±0.6	9.4±0.5	9.3±0.8	8.9±0.8	9.3±0.4
10 days after irradiation	1.7±0.4	2.2±0.5	2.3±0.6	2.9±0.3	3.4±0.2
30 days after irradiation	6.2±0.8	8.8±0.4	8.0±0.4	8.9±0.5	9.1±0.6
Thrombocytes (in multiples of one hundred thousand) per mm^3:					
initial count	2.4±0.3	2.7±0.4	2.1±0.2	2.6±0.3	2.4±0.3
10 days after irradiation	0.6±0.8	1.0±0.1	0.8±0.08	1.0±0.1	1.2±0.1
30 days after irradiation	1.4±0.3	2.3±0.2	1.8±0.1	2.1±0.4	2.5±0.2
Young erythroblasts of bone marrow as % of total cells:					
10 days after irradiation	2.3±0.4	5.0±0.6	4.5±0.3	5.2±0.3	6.7±0.6
20 days after irradiation	7.1±0.5	13.2±1.5	10.9±0.8	11.3±1.6	14.8±1.5
Young granulocytes of bone marrow as % of total cells:					
10 days after irradiation	1 3±0.3	4.7±0.6	3.2±0.9	4.5±0.8	5.2±0.5
20 days after irradiation	3.0±0.6	10.8±1.2	6.9±1.1	10.1±0.6	12.1±1.6
Uptake of Fe^{59} per 1 ml erythrocytes (in thousands of impulses per minute):					
10 days after irradiation	0.6±0.05	2.9±0.2	1.3±0.1	2.7±0.2	2.5±0.3
20 days after irradiation	2.7±0.2	6.4±0.2	3.2±0.3	6.1±0.2	7.9±0.4

Our experiments were made on animals with benzene poisoning or suffering from the effects of ionizing radiation.

In planning the work, we had in mind both the theoretical importance of this correlation and its effects in pathological conditions, as well as the undoubted practical importance from the clinical standpoint. The important part played by the spleen in the pathogenesis of radiation sickness has been demonstrated by the investigations made in recent years [4, 11].

The experiments were carried out on rabbits weighing 1700-2400 g. Total x-ray irradiation was applied as follows: voltage 17 kv, current 20 mamp, focusing distance 90 cm, filters 0.5 mm Cu and 1 mm Al, power of dose 13.7-14.2 r/minute. The rabbits received a dose of 600 r. In the experiments with benzene poisoning 1 ml of this substance per kg weight was injected subcutaneously daily for 5 days. The composition of the blood and bone marrow was measured, the latter being aspirated from a hole trepanned in the femoral bone. In experiments with irradiation, we also determined the uptake of radioactive iron Fe^{59} in the erythrocytes, which gives an indication of the erythropoietic activity of the bone marrow (the isotope was injected intravenously in a dose of 5 microcuries per kg body weight).

Diathermy to the spleen was applied daily, by the method described above, for 2 weeks after irradiating or after the first benzene injection. In the radiation experiments, besides the extracts of hyperactive spleen used previously, which we will refer to in future as "No. 1", we also used another heterogenous splenic extract prepared as follows: the rabbits were joined as parabionts, after which one was irradiated with 800 r of x-rays, while the other was protected by a lead shield; one week later, the spleen was removed from the second (unirradiated) parabiont, and an extract of it made. Usually, the spleen of the unirradiated member of the pair has a far greater weight not only than that of its irradiated partner, but is also heavier than that of a normal rabbit. Our provisional hypothesis was that the spleen of the unirradiated parabiont, as in the case of parabiosis with a splenectomized partner, increases its activity also by way of compensation when joined to an irradiated partner. The extract obtained in this way is referred to as "Extract No. 2". The extracts were injected intramuscularly as 1 ml of an oily solution per kg weight; they were given daily for 2 weeks, starting on the day of the irradiation or of the first benzene injection.

The results of measurements on the changes in the blood indices as affected by benzene and x-ray irradiation are shown in Tables 4 and 5.

From the results shown in the tables it can be seen that in benzene poisoning and in radiation sickness diathermy applied to the spleen, and extracts of hyperactive spleen have a very definitely favorable influence on the blood indices; thus, in the rabbits treated by these methods there was a smaller reduction in the number of formed elements in the peripheral blood, and a smaller reduction in the formation of new granulocytes and erythrocytes in the bone marrow than in the untreated animals, the difference being statistically significant.

The improvement in hemopoiesis effected by splenic diathermy and the injection of hyperactive splenic extract was particularly well shown in the period of early repair of the bone marrow. We were able to observe this phenomenon in both radiation sickness and benzene poisoning.

The results obtained and here presented extend our knowledge of the correlation between bone marrow and spleen and between liver and spleen under pathological conditions, and give real hope of the possibility of using these correlations clinically.

SUMMARY

Studies were made of the glycemic reaction to adrenalin, the protein content and protein fractions in the blood serum, the glycogen and fat contents of the liver, and the ability of the liver to take up sulfur-labeled methionine, to bind phenols, and to oxidize tyrosine. It was shown that diathermy applied to the spleen improves the condition of the liver in rabbits and white rats with hepatitis induced by carbon tetrachloride.

An analogous effect was achieved by an extract from an hyperactive spleen obtained by cutaneous-muscular union of pairs of animals as parabionts.

In a further set of experiments it was shown that splenic diathermy and extracts from a hyperactive spleen cause a marked improvement in peripheral blood and bone marrow indices in rabbits suffering from benzene poisoning or irradiation sickness.

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