STIMULATION OF SPLENIC REGENERATION IN RABBITS

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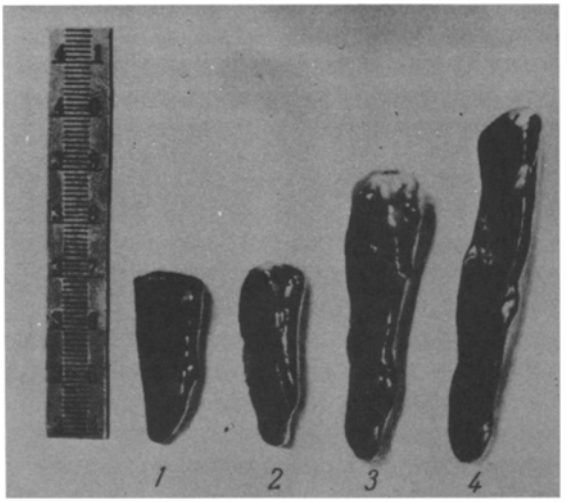
We have studied the possibility of stimulating regeneration in the spleen. The stimuli tried are antireticular cytotoxic serum (ACS) and ethanolamine (cholamine) on the basis of data obtained by a number of authors [1, 3-5] on the stimulating effect of such preparations on agglutinin formation, in which the spleen plays an essential role.

Stimulation of splenic regeneration was studied in rabbits. A number of authors [6, 7] could not detect regeneration in the remaining part of the spleen after partial removal of the organ and even noted its atrophy. On infliction of wounds with loss of substance the defect was filled with scar tissue [2].

METHODS

The stimulating dose of ACS and kolamine was determined by experiments on 24 rabbits by subcutaneous administration of increasing doses of the preparations with subsequent production of Wright's reaction with brucellin antigen and investigation of the blood picture. The intended effect was produced at a dose of 0.005 ml/kg. With this the titer of normal agglutinins rose from 1:5 to 1:40 and was accompanied by a decrease in the peripheral leucocyte count.



Effects of Stimulants on the Length of the spleen: 1) half of a normal spleen; 2) control spleen after 30 days; 3, 4) spleen after stimulation by ethanolamine after 30 days.

TABLE 1. Weight and Size of Regenerated Rabbit Spleen

Condition of experiment	No. of animals	Duration of Expt. (days)	Weight (mg)		Length (mm)		Width (mm)		Thickness (mm)	
			Half removed	Regenerated spleen	Original	At death	Original	At death	Original	At death
Control	5	30	638.6	735.2	22.06	25.52	8.56	9.34	3.88	4.06
ACS stimulation	5	30	709.8	1318.6	22.6	33.3	10.5	12.1	3.62	3.84
Cholamine stimulation	5	30	626.0	1056.0	23.8	40.6	10.6	11.5	3.32	3.75
Control	5	60	751.2	807.5	22.8	23.0	11.5	12.7	5.2	4.9
ACS stimulation	5	60	453.8	1113.4	20.4	39.1	8.6	10.5	5.2	5.4
Cholamine stimulation	5	60	602.8	1158.2	22.4	32.8	10.6	12.3	4.58	5.46

The basic experiments were carried out in 30 rabbits which were divided into groups depending on the type of stimulation and the period of observation. Under ether anesthesia the spleen was exposed and after the length was measured half of the organ was removed. The data on the weight and size of the half removed spleen make it possible to judge the corresponding measurements of the half of the organ which remained in situ.

After the operation one group of animals received ACS with 3 day intervals between fivefold doses from 1-5 mg. The other group received cholamine in the same doses and intervals. For each observation period there was a corresponding number of control animals.

The absorptive function of the reticulo-endothelial system was studied for experimental and control animals by weekly determinations of the Congo Red Index according to Aller and Reiman. Half of the animals were sacrificed at 30 days and the rest at 60 days after the operation. The spleens were weighed and their length, width, and thickness measured; a photograph was made and then they were subjected to histological treatment. Celloidin sections were stained with hematoxylin-eosin, fixed in van Gieson's and impregnated with silver (Foot technique). In the sections the area occupied by red and by white pulp was determined and the average number of Malpighian corpuscles per field of vision was calculated.

RESULTS

In the control animals the Congo red index rose from 87.8 to 98-100, while in the experimental group it fell in one period to 67. Toward the end of the observation period (49 days) the Congo red index in animals which had received stimulation had not reached the original norm and equaled 79-82.

On dissection of sacrificed animals, as a rule, the mesentery was fixed to the injured surface of the spleen. That organ was a light-cherry in color, whereas the normal spleen is usually a dark cherry-red. In nine out of 20 of the animals that had received stimulation a marked increase in the length of the spleen was observed while the width and thickness were not much changed. An example of a spleen significantly increased in length is shown in the picture.

The changes in weight and size of the regenerated spleens may be assessed according to the data in Table 1.

By 30 days after operation the weights of the spleens in the control group had increased by mean of 96.7 mg, and by 60 days by 56.3 mg, that is, quite insignificantly. An entirely different picture is noted in the experimental animals. With ACS stimulation the mean weight of the spleen after a month had increased by 659.6 mg, and with cholamine stimulation, correspondingly, by 430.0 and 545.4 mg. In individual animals the increase was more significant. Thus, in one animal from the group which received ACS, after two months the spleen had increased in weight from 523 to 1987 mg, and in the group which had received cholamine, one animal showed an increase in the same period of time from 452 to 1395 mg. The length of the organ likewise underwent regular changes. As the weight of the regenerated spleens did not fall into the same range as the weights of the controls, statistical treatment of these results cannot be made. The mean increase in length over 30 days in the control animals' spleens was 3.5 mm and

TABLE 2. Number of Malpighian Corpuscles per Microscopic Field and Ratio of Areas of White:Red Pulp in Rabbit Spleen

Condition of experiment	No. of animals	Duration of Expt. (days)	No. of Malpighian corpuscles per microscopic field	Ratio of areas of pulp (%)	
				White	Red
Normal	5	–	26.0	18.0	82.0
Control	5	30	32.0	12.75	87.25
ACS stimulation	5	30	64.0	19.33	80.67
Cholamine stimulation	5	30	67.0	18.74	81.26
Control	5	60	51.0	15.48	84.52
ACS stimulation	5	60	35.0	20.63	79.37
Cholamine stimulation	5	60	37.0	18.16	81.84

over 60 days—only 0.2 mm. During the same period a significant increase in spleen length occurred in the group treated with ACS and cholamine; for the former the greatest increase in length was noted over 60 days, whereas for the latter of cholamine-treated group, the most intensive growth was observed in the first 30 days. The width and thickness of the organ did not change significantly.

The average number of Malpighian corpuscles per field of vision and the ratio of white:red pulp area are of great significance as characteristics of splenic regeneration.

It follows from Table 2, that the average number of Malpighian corpuscles per field increased in the control animals in comparison with normal, but not significantly; it rose somewhat higher after 60 days. In the experimental animals, the number of Malpighian corpuscles rose sharply in 30 days; by the 60th day it decreased approximately two times, however, not reaching normal values. It is important to note that during this sharp fluctuation in the number of Malpighian corpuscles, the ratio of areas of white:red pulp remained essentially unchanged. This phenomenon is explained upon study of the preparations.

In the regenerating spleen, attention is drawn to the significant decrease in diameter of the Malpighian corpuscles and the increase in their number, which is especially marked in the experiments with the stimulation here applied.

Differences in the degree of development of tissue differentiation are especially distinct in the region of wounds. In the control animals, after one month, an abundant, diffuse infiltration of lymphoid elements is noted in the pulp adjacent to the wound. The wound is covered with friable connective tissue regenerate with abundant fat cells and lymphoid elements.

By 2 months, on the whole, a capsule is formed which appears more fatty than the uninjured portion. In animals which have been treated with the stimuli mentioned, a similar picture is observed already 1 month after operation. In 2 months the pulp adjacent to the wound has acquired a characteristically normal splenic structure. The capsule is condensing, but remains thicker than the capsule in noninjured areas of the spleen.

Thus, under ordinary conditions, after removal of half of the spleen in the rabbit there is practically no regeneration. A slight tendency toward decreased weight and length of the organ observed after a 2 month period suggests the possible development of subsequent atrophic processes.

The subcutaneous injection of ACS and cholamine evokes the stimulation to splenic regeneration in the rabbit which takes the form of increased weight and length of this organ. The distinctive type of splenic regeneration in rabbits includes significant elongation of the organ without change in its width or thickness.

Our data confirm the possible use of ACS and ethanolamine (cholamine) as stimulants to regenerative processes in the spleen.

SUMMARY

Antirecticular cytotoxic serum and ethanolamine were used to stimulate splenic regeneration in rabbits (5

subcutaneous injections in a dose of 5 ml per kg of body weight) following resection of half of the spleen. Animals subjected to the action of stimulants and controls were sacrificed in 30 and 60 days.

Experiments demonstrated that the adsorptive function of reticulo-endothelial system decreased in control animals, but following injection of the stimulants, it rose. Thirty and sixty days after the operation no splenic regeneration was noted in control rabbits; conversely by the 60th postoperative day there is a tendency for the organ to reduce in size. Under the effect of the stimulants the spleen grows intensively length-wise without showing any significant increase in the width and thickness. The weight of the spleen rose considerably. The number of Malpighian bodies increased, their diameter shrank whereas the ratio of the white and red pulp areas exhibited no significant changes. Antireticular cytotoxic serum and ethanolamine may be used to stimulate regeneration of splenic processes.

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