# THE COMBINED ACTION OF THE SPLEEN AND OTHER ORGANS

## COMMUNICATION II. THE RATE OF RENEWAL OF PROTEINS, THE SYNTHESIS OF UREA AND THE CONTENT OF THE ANTIANEMIC FACTOR IN THE LIVER OF WHITE RATS WITH EXPERIMENTAL SPLENOPATHY

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The problem of the splenogenic nature of certain chronic diseases of the liver cannot yet be regarded as finally settled [2-4]. The great practical importance of this problem in clinical practice justifies experimental research in the direction of studying the correlation of liver and spleen in pathological conditions.

Our report is based on the results of investigation of certain indices of liver activity in experimental splenopathy. We determined the rate of renewal of proteins, the formation of urea and the content of antianemic factor in the liver of white rats with splenomegaly caused by methylcellulose.

## EXPERIMENTAL METHOD

The experiment was carried out on white rats weighing 120-160 g kept on a balanced diet. Splenomegaly was induced by intraperitoneal injection by methylcellulose for 15 weeks (2 ml of a 2.5% solution twice a week). In some animals the spleen was removed 10 days before the injections of methylcellulose were started and to prevent bartonelliasis novarsenol was injected in a dose of 0.3 mg per 100 g body weight. As a control intact rats were used on which a spurious splenectomy was performed (laparotomy without removal of the spleen, and injection of novarsenol), followed by injections of methylcellulose, and splenectomized rats not receiving subsequent injections of methylcellulose but receiving novarsenol.

The rate of renewal of protein was studied by injecting the experimental animals subcutaneously with radioactive methionine (labeled with $S^{35}$, in a dose of 5000 impulses/min per 1 g body weight of the animal) dissolved in physiological saline. The rats were starved for 6 hours before the injection of methionine to prevent dilution of the label with the methionine of the food. At different intervals after injection of the labeled amino acid, animals were killed by decapitation, the liver extracted, ground up finely, the proteins precipitated with a 10% solution of trichloroacetic acid, washed twice in a centrifuge with 5% trichloroacetic acid solution and treated with 10 times its volume of alcohol to extract the lipids; the residue was then washed twice with an alcohol-ether mixture (1:1) and twice with ether, and then dried in an incubator for 24 hours. After drying, the protein was rubbed into a fine powder, and samples of the dried protein weighing 10 mg were taken for estimation of its radioactivity with a Geiger-Müller counter.

In order to determine the urea formation in the liver, sections of the organ were washed with a bicarbonate buffer solution (pH = 7.4) and placed in a vessel containing 6 ml of the same buffer. To this solution was added a solution of ammonium chloride in volume to produce a final concentration of 1/100 M (17 mg% of ammonia). In one series of experiments glucose was also added to the solution to determine its stimulating action on the process of urea formation (200 mg per tube). The vessels were filled with a gas mixture consisting of 5.6% $CO_2$

TABLE 1

The Effect of Experimental Splenomegaly due to Methylcellulose on the Inclusion of Radioactive Methionine in the Liver Proteins in White Rats

| Group No. of rats | Group characteristics | Number of animals | Radioactivity of 10 mg of liver protein as a % of that injected per 1 g body weight |
|---|---|---|---|
| 1 | Intact | 15 | 4.1 ± 0.7 |
| 2 | Splenomegaly (methylcellulose for 15 weeks) | 15 | 2.2 ± 0.5 |
| 3 | Splenectomy + methylcellulose for 15 weeks | 15 | 3.9 ± 0.8 |
| 4 | Spurious splenectomy + methylcellulose for 15 weeks | 15 | 2.5 ± 0.8 |
| 5 | Splenectomy (examination 3 weeks after operation) | 15 | 3.2 ± 0.7 |
| 6 | Splenectomy (examination 3 months after operation) | 10 | 4.2 ± 0.4 |
| 7 | Spurious splenectomy (examination 3 weeks after operation) | 10 | 4.0 ± 0.8 |

Note: In the table are shown mean values and mean square deviations from these for each group.

TABLE 2

The Effect of Experimental Splenomegaly due to Methylcellulose on Urea Formation in the Liver

| Group No. of Rats | Group characteristics | Number of animals | Urea formation in the liver in μM of nitrogen per 1 g of tissue | |
|---|---|---|---|---|
| | | | without addition of glucose | with addition of glucose |
| 1 | Intact | 15 | 62.8 ± 3.5 | 94.0 ± 7.1 |
| 2 | Splenomegaly (methylcellulose for 15 weeks) | 15 | 15.6 ± 5.9 | 18.7 ± 4.4 |
| 3 | Splenectomy + methylcellulose for 15 weeks) | 15 | 58.6 ± 7.2 | 82.7 ± 9.9 |
| 4 | Spurious splenectomy + methylcellulose for 15 weeks) | 8 | 19.7 ± 5.8 | 18.5 ± 7.2 |
| 5 | Splenectomy (examination 3 weeks after operation) | 10 | 66.5 ± 5.2 | 88.1 ± 5.2 |

and 94.4% $O_2$, and were placed in a water agitator for 2 hours at 38°C. After this the proteins were precipitated with a 10% solution of trichloroacetic acid, and the fall in the ammonia content of the filtrate and the amount of urea synthesized estimated by Kreb's method [5].

In order to estimate the content of antianemic factor in the liver, samples of the organ weighing 250 mg were mixed with 6 ml of Ringer-Locke solution. After standing for 30 minutes in the cold, the extract was treated

TABLE 3

The Effect of Experimental Splenomegaly due to Methylcellulose on the Content of Antianemic Factor in the Liver of White Rats

| Group No. of rats | Group characteristics | Number of animals | % change in the reticulocyte count in recipient rats after injection of ultrafiltrate of liver of experimental rats |
|---|---|---|---|
| 1 | Intact | 15 | + 178.3 ± 19.1 |
| 2 | Splenomegaly (methylcellulose for 15 weeks) | 15 | + 41.5 ± 14.8 |
| 3 | Splenectomy + methylcellulose for 15 weeks) | 15 | + 156.8 ± 20.9 |
| 4 | Spurious splenectomy + methylcellulose for 15 weeks) | 10 | + 34.8 ± 8.2 |
| 5 | Splenectomy (examination 3 weeks after operation) | 10 | + 182.1 ± 25.4 |

by ultrafiltration to remove the proteins; absence of protein from the ultrafiltrate was verified by the reaction with sulfosalicylic acid. The ultrafiltrate was injected subcutaneously in a volume of 2 ml into intact rats in which reticulocyte counts were made on several occasions before and after the injection of the ultrafiltrate of the liver of the experimental animals. The reticulocytes were counted in relation to 1000 red cells. An increase was regarded as specific if it reached 150-200% of the mean value before injection of ultrafiltrate and appeared not later than the 5th day after injection.

This particular method was tried out in rats [1].

## EXPERIMENTAL RESULTS

The experimental results are shown in Tables 1-3.

From the data in Table 1 it can be seen that in experimental splenomegaly due to methylcellulose there is a marked fall in the rate of inclusion of radioactive methionine in the protein of the liver; this shows a fall in the rate of renewal of proteins in the liver. Splenectomy itself, at early periods after removal of the spleen, also leads to some degree of slowing (far less pronounced than after splenectomy, it is true) of the rate of renewal of protein, but this effect of splenectomy is quickly compensated and 3 months after splenectomy the inclusion of radioactive methionine in the liver protein of the splenectomized animals was indistinguishable from that in the controls. The fact that injection of cellulose into splenectomized animals does not change the rate of renewal of protein in the liver is an indication of the importance of splenectomy as such in the development of retardation of the inclusion of radioactive methionine in the liver proteins.

From Table 2 it can be seen that urea formation in the liver during experimental splenomegaly is disturbed and the leading role in this disturbance belongs to the spleen which has undergone pathological changes and not to the methylcellulose itself. It is interesting that the addition of glucose to the liver slices from the animals with splenomegaly does not improve urea formation; this suggests some disturbance of the enzyme systems catalyzing the process of urea formation in the liver. Removal of the spleen, even in early periods after the operation, does not affect the urea-forming function of the liver.

Table 3 shows a clear reduction in the content of antianemic factor in the liver of rats with experimental splenomegaly, as judged by the reticulocyte reaction of recipient rats. In this phenomenon also it is not methylcellulose as such but the splenomegaly which it causes which plays the leading part, as may be seen from Table 3.

The results of this last series of experiments, summarized in Table 3, appear to us to shed light on some of the mechanisms of the so-called splenic cytopenias, and particularly anemia, occurring in various diseases accompanied by enlargement of and pathological changes in the spleen.

The results of our experiments indicate a close link between the spleen and liver in pathological conditions and a marked influence of the pathological spleen on the liver function.

## SUMMARY

In experimental splenomegaly of white rats caused by the intraperitoneal injection of methylcellulose the velocity of protein renovation is found to be decreased in the liver (determined by the test of radioactive methionine incorporation into the liver proteins). The urea-forming function of the liver is also disturbed (tested of the urea synthesis by the liver sections). Experiments demonstrated that the pathological enlargement of the spleen with the disturbance of its functions reduces the antianemic factor content of the liver. This may to a certain extent explain the anemia occurring in various diseases connected with splenomegaly.

## LITERATURE CITED

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