The Effect of Orally Administered Bovine Spleen Preparations on Platelet and Leukocyte Counts of Rats

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The intimate relation between spleen and blood cells has been known for over 60 years. For nearly as long, evidence of humoral hemor regulatory substances of splenic origin has been sought. Extensive work with extracts of spleen, usually administered parenterally, has yielded conflicting results.1,2

Effects of orally administered splenic preparations have been studied sporadically since 1915. Lewis and Margot3 noted that, after splenectomy, mice fed sheep spleen became ill. Normal mice were not affected. Schleicher4 fed desiccated cow spleen to patients with pernicious anemia and produced elevations in platelet counts. In the same study, such feedings elevated platelets in normal men but intensified low values in cases of "primary" thrombocytopenia. Cooney and Blatt5 found both platelet elevator and depressor activity in splenic extracts. Russian workers6,7 have reported cases of "hemorrhagic thrombocytosis" fed raw beef spleen. Such treatment induced prompt fall of platelet levels to normal. Thrombocytosis recurred when spleen feedings were stopped. Recently, Monto and coworkers8,9 have described consistent elevations of platelet counts both in rats and human subjects fed a homogenized mixture of beef spleen and water.

This report describes the effect of various beef spleen preparations on the platelet and leukocyte counts of rats. Preliminary studies suggested that a myeloinhibitory factor was present in orally administered spleen.10 Subsequent experiments have failed to fully substantiate the earlier findings.

MATERIALS AND METHODS

Pathogen-free albino male rats, Walter Reed Carworth Farms variety, Wistar strain, 225 to 275 Gm., were used. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed. All were housed in the same
animal room. Splenectomy was performed with nonsterile technic using sodium pentobarbital anesthesia. Blood was collected by lancing the periorbital venous plexus with a heparinized microhematocrit tube while the animal was lightly anesthetized with ether, U.S.P. Each rat was bled a maximum of 1 ml once a week. Five drops of blood were collected in a siliconized test tube containing 1 to 1.5 mg of dried Na EDTA. The tube was then gently agitated and pipettes immediately made. Filled pipettes were continually agitated on an Aloe pipette rotator. Counts were performed within 2 hours.

Platelet counts were determined by the phase microscopy method of Brecher and Cronkite. Leukocytes were counted by a standard technic. Blood smears were stained with Leishman-Giemsa stain. Microhematocrit determinations utilized an International centrifuge which operated at 10,000 g for 4 minutes. Platelet counts were performed in duplicate. The results were averaged. A single hematocrit determination and leukocyte count was made on each blood specimen. White cell differential values were estimated by counting 100 leukocytes. Whenever possible, the pipettes were given code numbers to ensure objectivity.

Beef Spleen

Fresh spleen was obtained from a single government-inspected slaughterhouse. The donor animals were certified free of infectious or toxic disease. Steers and heifers averaged 2 years of age and were bought from individual farmers. Cows averaged 7 years of age and were purchased at auction from cattle dealers. Animals were shipped to the slaughterhouse promptly and were killed within 24 hours after arrival. Specific diet histories of these animals are not known. At the slaughterhouse spleen lots were prepared in one of the following ways: (1) After slaughter, spleens were placed immediately in a cold box containing dry ice and labeled “Fresh Frozen Spleen;” (2) the organs were incubated promptly at 38 C. for 5 hours; and (3) the spleens were hung on offal racks for 3 to 6 hours in a partially open slaughtering shed. They were then kept in a cold meat locker overnight and delivered, still cool, within 24 hours after slaughter. The spleen lots were prepared as above in an effort to discover the reason for our failure to reproduce the original result.

On arrival the spleen lots were packaged and frozen. Packages were allowed to thaw in a standard refrigerator for 24 to 72 hours before use.

Spleen pulp and juice were prepared by passing whole spleen through a kitchen meat grinder. The material was then thoroughly mixed with distilled water and centrifuged at room temperature for 30 minutes at 2000 r.p.m. This was repeated once, decanted, and the pulp squeezed in gauze. The supernatant was strained until cell-free by light microscopy. Thirty-five ml. of the juice was equivalent to 40 Gm. of whole spleen. Forty-five Gm. of wet pulp represented 40 Gm. of whole spleen.

A commercially available rat and mouse food cracker was used for regular diet. All rats received tap water ad lib.

Description of Experiments

Generally, baseline blood studies were performed and splenectomy carried out. Rats were assigned to diet groups by random selection. Weekly blood counts were made thereafter. The animals were fed daily by one of the investigators. Diets were maintained for a minimum of 7 days before being varied. The numerous studies will be arbitrarily grouped to facilitate description.

Series 1: This series describes the first group of experiments utilizing cow spleen. (A) Sixteen splenectomized rats were caged in four equal groups. A like number of unoperated animals were similarly grouped. Four diet regimens were evaluated in both the operated and the unoperated rats. These were (1) beef tripe 45 Gm. per rat per day, (2) cooked cow spleen 45 Gm. per rat per day, (3) raw cow spleen 45 Gm. per rat per day, and (4) regular diet, ad lib. No leukocyte differential counts were made. (B) The next study in this series utilized raw cow spleen (labeled Cow Spleen A) from a different lot than that used initially. Four rats were caged individually and 1 week after
Splenectomy were offered 100 Gm. per rat per day of spleen. Four other rats, individually caged, served as controls on a regular diet. This regimen was maintained for 3 weeks. Spleen diet was then discontinued and regular diet substituted. Following this, spleen diet was resumed for a final week.

Series 2: In this group of studies pooled beef spleen was evaluated as well as another lot of cow spleen. (A) Sixteen splenectomized rats were divided into four equal groups. Each rat was caged separately. One group was fed 75 Gm. of raw cow spleen per rat per day as the only diet. Another group received regular diet and 45 Gm. of spleen pulp per rat per day. A third group was given regular diet and 35 ml. of spleen juice per rat per day. The fourth group received only regular diet, ad lib. Spleen preparations were offered in the early morning and, where applicable, regular diet was given at night. On this regimen all offered spleen preparation was eaten as well as varying amounts of regular food. These diets were continued for 4 weeks. The spleen-fed animals were then killed and autopsied. (B) In another experiment, four splenectomized animals were offered 100 Gm. of pooled beef spleen per rat per day as the sole diet. Uneaten spleen in each cage was weighed daily and the daily intake estimated. This was carried out for 2 weeks, after which the rats were killed and autopsied.

Series 3: This series describes studies designed to evaluate diets of spleen from heifers and cows. Effects of quick freezing and incubation were also assessed. (A) Twenty-four splenectomized rats were used. One group of 8 rats and 4 groups of 4 rats were formed. Each rat was caged separately. Diets were begun 1 week after splenectomy and continued for 14 days. The group of 8 rats received 75 Gm. of incubated cow spleen per rat per day. One of the smaller groups was fed 75 Gm. of fresh frozen cow spleen per rat per day. Another group got a similar amount of fresh frozen heifer spleen. One group was given 75 Gm. of cow spleen per rat per day from the same lot that was used in Series 1, labeled Cow Spleen A. The last group ate a regular diet and served as controls. (B) In another experiment, cow spleen obtained by regular shipment from the slaughterhouse was incubated at 38°C for 4 hours. Another lot of cow spleen, frozen for a month, was similarly treated. These spleen preparations were fed in amounts of 75 Gm. per rat per day to different groups of 4 splenectomized rats. A control group was fed regular food. The diets were given for 1 week. (C) Finally, 12 splenectomized rats were divided into 3 equal groups. Each group was caged together. Cow spleen, 50 Gm. per rat per day, was fed to 2 of the groups. The third group acted as a control. The diet was given for 1 week.

Results

In all the test animals there was a postsplenectomy rise in the platelet and leukocyte counts. The peak values were found 1 week after operation. In the control animals, counts fell by the second postoperative week to levels approximately 25 per cent above presplenectomy values. Thereafter counts remained essentially unchanged. Hematocrit values did not differ significantly in any of the groups.

Series 1: (A) In the first experiment raw cow spleen feedings were associated with a dramatic reduction in both platelet and leukocyte counts. These changes were most marked in the splenectomized animals, though reductions were of significance in the unoperated group. Tripe and cooked spleen had no effect. Figures 1 and 2 graph the platelet and leukocyte values of the splenectomized rats. The data are represented as mean values for each group together with the range. The counts rose or fell significantly as cow spleen diet was stopped or started. (B) The second experiment in this series yielded similar but less dramatic changes in platelet levels (fig. 3). No significant changes in leukocyte counts were seen. The reduction in platelet levels exceeded 25 per cent while cow spleen was being fed. This re-
Fig. 1.—Effect of a diet of raw cow spleen on the platelet counts of splenectomized rats. Controls received a regular diet.

duction is significant (p < 0.001). Analysis of variance shows changes in platelet levels to be a function of diet differences. Variance between weeks or between rats within groups was not significant.

Series 2: (A) Diets of the juice or pulp from pooled beef spleen had no significant effect on platelet or white cell counts. There was no alteration of the leukocyte differential counts. No effect of raw cow spleen could be demonstrated with the particular lot tested. The platelet count results are depicted in figure 4. (B) Rats offered 100 Gm. of raw pooled spleen per rat per day ate at least 60 Gm. a day for 12 of the 14 days studied. No significant change in blood counts was demonstrated. At the time of autopsy no splenic tissue could be found in any of the rats.

Series 3: (A) Figure 5 graphs the mean values and range for groups fed various cow spleen preparations. No effect on blood cell counts of fresh frozen spleen could be shown. Cow Spleen A from the same lot that produced a platelet lowering effect in Series 1 exerted no such effect after being frozen for 3 months. (B) Cow spleen, incubated at various times, produced no changes. (C) Caging rats within groups together while feeding cow spleen yielded negative results.

In none of the experiments was a rise in platelet level encountered.

DISCUSSION

The initial studies suggested that cow spleen feedings lowered platelet and leukocyte counts. The effect seemed to vary between different lots of cow spleen. Mixtures of beef spleen produced no change. Attempts to duplicate
Fig. 2.—Effect of a diet of raw cow spleen on the leukocyte counts of splenectomized rats. Controls received regular diet.

Fig. 3.—Effect of a diet of raw cow spleen on the platelet counts of splenectomized rats (spleen from a different lot than in fig. 1 and fig. 2.) Controls received a regular diet.
Fig. 4.—Effect of a diet of various beef spleen preparations on the platelet counts of splenectomized rats: raw beef spleen only; beef spleen juice plus regular diet; beef spleen pulp plus regular diet. Controls received regular diet only.

Fig. 5.—Effect of a diet of various cow and heifer spleen preparations on the platelet counts of splenectomized rats: fresh frozen cow spleen; fresh frozen heifer spleen; cow spleen; incubated cow spleen. Controls received regular diet only.

The initial experiments were unsuccessful. Spleen lots obtained at varying times of the year were used. Variation in handling of the organs after slaughter was carried out. None of these measures produced significant alterations in blood counts. Type of test animal, animal housing, and spleen source were not significant variables during these investigations.

It is of interest that no elevation of platelet counts was encountered
whereas Monto and coworkers\textsuperscript{8} regularly found significant rises with beef spleen feedings. Cooney and Blatt\textsuperscript{5} found a platelet elevator fraction in spleen extracts which was active parenterally and orally in mice. A platelet depressor fraction was also demonstrated which was, however, ineffective orally. Splenectomy decreased depressor effect but enhanced that of the elevator component.\textsuperscript{13}

The reasons for such disparate results are obscure. Methodologic discrepancies may be operative, but there is no good evidence for it. The studies here mitigate against age or sex of spleen donor animals as the only significant variables. Seasonal factors seem unlikely from these studies. One cow spleen lot, which initially produced platelet count changes (fig. 3), seemed to lose its effect after deep freeze storage (fig. 5).

There may be some diet or environmental factor in cattle which may trigger latent activity. The positive results reported here occurred only with cow spleen. In other reports, the type of beef spleen was not known.

**Summary**

Several orally administered beef spleen preparations were evaluated for their effect on platelet and leukocyte counts of rats. In some studies, cow spleen exerted a definite though variable count lowering activity. Further studies yielded less dramatic or negative results. Pooled beef spleen, whole or crudely fractionated, had no demonstrable effect on the blood counts.

Other variables, including temperature, feeding methods, seasonal factors, and animal housing did not appear significant.

No consistent, predictable effect of beef spleen feedings could be demonstrated under the various conditions tested.

**Summario in Interlingua**

Diverse oralmente administrate preparatos de splen bovin esseva evallutate quanto a lor effecto super le numeracion plachettal e leucocytic del ratto. In un numero de studios, splen bovin exerceva un definite ben que variabile effecto reductori in le numeracion. Studios additional produceva resultatos minus spectacular o mesmo negative. Collectionate splen bovin—tanto total como etiam crudemente fractionate—habeva nulle demonstrabile effecto super le numerations sanguinee.

Altere variabiles—incluse temperatura, forma de alimentation, factores saisonal, e modo de stabulation—non pareva esser significative.

Nulle uniforme e predicibile effecto del splen bovin in le dieta poteva esser demonstrate sub le varie conditions testate.

**References**

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