Influence of Spleen on Thrombocytopenia Induced by Humoral Factor(s) of Experimental Hypersplenism

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In a previous communication evidence was presented for the existence of a humoral factor or factors [HF(s)] in rats with experimental methylcellulose-hypersplenism. The HF(s) was shown to be eliminated with the urine of hypersplenic rats since such urine caused slight anemia and thrombocytopenia as low as 65 per cent of normal values when given intragastrically to normal rats. Similar administration of urine from normal rats, from rats with radiation-induced anemia, and from hypersplenic animals after splenectomy, failed to produce the same changes. Although the anemia induced by hypersplenic urine in normal rats disappeared when intragastric administration of HF(s) was discontinued, the thrombocytopenia persisted throughout the period in which the animals received no treatment, and remained unmodified by further introduction of hypersplenic urine into the stomach of the same rats. In order to explain the failure of platelets to return to normal when no hypersplenic urine was given, it was postulated that HF(s) could induce the spleen or other organs to produce and maintain the same type of effect on the platelet count in normal rats, and that once this change was established it did not require further administration of HF(s). The present experiments were undertaken in order to explore the role of the spleen on the thrombocytopenia resulting from intragastric administration of methylcellulose-hypersplenic urine to normal rats.

Material and Methods

Young Wistar rats were used throughout the experiments. Initial weights varied from 100 to 150 Gm. The animals were housed in metal cages and fed rat pellets and water ad libitum. Before any treatment was given, two blood counts were performed in alternate days in each rat, the values obtained were averaged, and the results were used as base line for deviations. Induction of methylcellulose hypersplenism, collection and intragastric administration of urine to normal rats, and weekly hematologic studies, were all carried out according to techniques previously described. Platelet counts were made according to the Rees-Ecker technic. The animals were separated into four groups and each group treated as follows:

Group I: Twenty rats were given intraperitoneal injections of methylcellulose for 17 weeks. These animals became hypersplenic and served as donors of urine for the other experiments. Urine was collected after completion of the methylcellulose treatment.

Group II: Twelve rats were splenectomized and their blood counts followed weekly for four weeks. This group served as control for the effect of splenectomy alone on peripheral blood elements.

Group III: Seventeen rats were splenectomized and the same day were started on daily intragastric administration of 2 cc. of undiluted urine of Group I for four consecutive weeks.

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*Group IV:* Fifteen rats were given 2 cc. of undiluted urine of Group I through a gastric tube daily for four consecutive weeks. At the end of this period, treatment was discontinued for three weeks, and again given for four additional weeks. On the last day of the second period of urine administration, the spleen was removed in 10 rats and the entire group was followed for four more weeks.

Weekly blood counts were made in all animals. All splenectomized rats received 25 mg of Achromycin* intramuscularly on the first and third postoperative day. *Haemobartonella muris* appeared in the blood smears three to four weeks after removal of the spleen in most animals, and at this time observations were discontinued. As in the previous study, graphs have been constructed by averaging all figures available at one specific date and determining the per cent deviation from the normal average obtained as mentioned above, which was not different from the figure derived from 217 blood counts in normal rats and used in the previous paper.1 There were always five or more determinations for each point. Differences in average values were analyzed for statistical significance by means of a t table (p = 0.01).

**Results**

*Group I:* The results in this group were entirely similar to those previously reported.1 In addition to easily palpable splenomegaly, the animals developed thrombocytopenia oscillating between 60 and 70 per cent of normal values; platelet counts lower than 40 per cent were usually accompanied by signs of bleeding and some of the animals died in a few days; autopsies failed to reveal any obvious cause of death. Only those animals which appeared healthy were used for the collection of urine. The thrombocytopenia was not modified by discontinuation of methylcellulose after completion of 10 weeks of treatment.

*Group II:* The effect of splenectomy on the peripheral blood count in our animals appears in figure 1. Throughout the four weeks of observation, there was no change in platelets or red blood cells, while leukocytes showed a marked increase seven days after splenectomy and a tendency to return to normal values in the following three weeks.

*Group III:* Splenectomy followed immediately by a four-week period of intragastric administration of HF(s) resulted in thrombocytopenia of almost 50 per cent of normal values in two weeks, which remained at this level for the last two weeks of observation. Red blood cells showed no significant deviation from normal. There was a marked leucocytosis, different to that observed with intragastric administration of normal urine but similar to the one resulting from splenectomy alone.

*Group IV:* Results of two four-week periods of intragastric administration of HF(s) separated by a three-week lapse without treatment were again the same as previously reported1 (fig. 2). Thus, thrombocytopenia of 40 per cent or less of normal values developed and remained unmodified by either discontinuation or readministration of HF(s). Red blood cell changes were less marked but followed the same pattern of slight anemia regressing when no HF(s) was being administered and remaining normal despite new intro-

*Achromycin was kindly supplied by Lederle Laboratories Division, American Cyanamid Co.*
Fig. 1.—Effect of splenectomy on peripheral blood counts during four weeks following the operation. There is leukocytosis of almost 60 per cent above normal 7 days after removal of the spleen. Variations in red blood cells and platelets are not significantly different from normal values.

Fig. 2.—Thrombocytopenia and leukocytosis in rats splenectomized and started on intragastric administration of methylcellulose-hypersplenic rat urine. Variations in red blood cells are not significantly different from normal values.
Discussion

Thrombocytopenia induced in normal rats by intragastric administration of methylcellulose-hypersplenic rat urine is a permanent condition, spontaneously irreversible even three weeks after discontinuation of HF(s). Furthermore, it remains unmodified by further treatment with HF(s), maintaining levels 40 per cent or less of normal platelet counts. These effects of HF(s) on normal rats could be due to three possible types of mechanisms: (a) Direct toxic injury of megakaryocytes in bone marrow. This seems unlikely since after discontinuation of HF(s) spared megakaryocytes would proliferate, bringing
the platelet count again to normal values. In addition, during the second period of administration of HF(s) to thrombocytopenic animals, platelet counts are not further depressed, which would be expected from additional damage to previously injured megakaryocytes, or toxic depression of those spared during the first treatment with HF(s). (b) Selective destruction of platelets could also be suggested, but objections similar to those mentioned for the previous hypothetical mechanism would have to be answered. Furthermore, it has been shown that in methylcellulose-hypersplenic rats the life span of platelets is within normal range. (c) We have adopted the hypothesis that HF(s) is capable of inducing a permanent change in the normal rat which will cause and/or perpetuate thrombocytopenia after the administration of HF(s) is discontinued. This hypothesis can be considered as presenting, among many others, two main problems: one is the identification of the tissue or organ where the change is operated, and the other is the nature of the change. The present experiments were designed to test the possibility that the spleen was involved in the thrombocytopenia produced by administration of HF(s). Although in theory the spleen is not the only tissue capable of playing an important role in thrombocytopenia, it was selected as a first approximation to the problem because HF(s) disappears from the urine of methylcellulose-hypersplenic rats after splenectomy. The results of this study seem to establish that the spleen is not required by HF(s) to induce thrombocytopenia in the normal rat, and also that the spleen is not involved in the maintenance of thrombocytopenia after discontinuation of HF(s). The role of other organs, as well as the nature of the persistent thrombocytopenia in animals treated with HF(s), are being studied at present.

Summary

The influence of the spleen on the thrombocytopenia induced in normal rats by intragastric administration of humoral factor or factors [HF(s)] was studied. Splenectomized rats showed the same thrombocytopenia associated with administration of HF(s) as rats with intact spleen. Thrombocytopenia established by two separate and disconnected periods of intragastric administration of HF(s) and followed by splenectomy was not modified. It is concluded that the spleen is not required by HF(s) to induce thrombocytopenia in the normal rat, and also that the spleen does not participate in the maintenance of thrombocytopenia observed after discontinuation of HF(s).

Summario in Interlingua

Esseva studiate le influentia del splen super le thrombocytopenia inducite in rattos normal per le administration intragastric de factor o factores humoral (FH, FFH). Rattos splenectomisate monstrava, in association con le administration de F(F)H, le mesme thrombocytopenia como rattos con splenes intacte. Thrombocytopenia establite per duo separate e non connectite periodos de administration intragastric de F(F)H e sequite per splenectomia non esseva modificate. Es concluindte que le splen non es requirite per le F(F)H que
induce thrombocytopenia in le ratto normal e etiam que le splen non participa in le mantenentia del thrombocytopenia observate post le discontinuation del administration de F(F)H.

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