Splenic Enlargement Induced by Thymus Feeding in Adult Rats.

By

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Spleen is mysterious organ. The intimate relationship of the spleen to hematopoiesis has been recognized for many years. At present the conception expressed in "Hypersplenism" by Dameshek and "Primary Splenic Panhematopenia" by Doan and Wright is stressed. However the pathogenesis of those splenomegalies, so-called "Primary Hypersplenism", that is congenital hemolytic jaundice, idiopathic thrombocytopenic purpura, primary splenic neutropenia and primary splenic panhematopenia - is still remained unknown.

In the present paper we report the investigations that the administration with thymus to rats results in splenomegaly with anemia, leukopenia and thrombocytopenia about 60 days after. Therefore, we assume that such a primary splenomegaly has some what relationship to the primary hypersplenism which Dameshek and Doan described.

METHODS.

Thirty male rats of the Aichi-strain were used when weight ranged 60 to 80g. They were housed in individual wire cages and divided into two groups, which fed two different diets as same as Kjerulf-Jensen's experiment producing thyroid hyperplasia.

Experimental diet contained millet 400 g, soybean flour 400 g, cod liver oil 1 g, ascorbic acid 0.5 g, sodium chloride 20 g and fresh tymus mince of young calves 200 g. On the other hand, control diet contained same materials, but instead of fresh thymus, fresh beef mince used.

Each groups were given 25 g of these diets per Kg body weight every day. The rats were hematologically examined after 20, 40, 60 and 90 days respectively and then sacrificed and their spleens were removed, weighed and submitted to the histological examination. And on the same time, differential counts were performed on bone marrow smears.

RESULTS.

No immediate effects of the feeding were noted in experimental animals, although we examined hematologically. After about 60 days, the animals in experimental
group began to appear enlargement of the abdomen. All animals in experimental group were found to have massively enlarged spleens as compared with control group. The averages of the spleen weight of experimental group reached 625 mg and averaged approximately 45 to 10 g body weight. On the other hand, the average of the spleen weight from control group reached 501 mg and the ratio averaged approximately 29 to 10 g body weight. The average of the spleen weight of experimental group increased more 25% than that of control, and ratio to the 10 g body weight more 55% increased. On the observation according to the course of time, we found nothing particular until 40 days after feeding, but thereafter splenic enlargement gradually increased as shown in Fig 1. As above mentioned, it will be noted that rats fed on experimental diet had relatively and absolutely more enlarged spleen than control.

Fig. 1. Influence of thymus feeding on the spleen weight of rats.

<table>
<thead>
<tr>
<th>Duration</th>
<th>g Bodyweight</th>
<th>mg Spleen Weight</th>
<th>mg S.W./10 g B.W.</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>144</td>
<td>152</td>
<td>639</td>
</tr>
<tr>
<td>60</td>
<td>166</td>
<td>218</td>
<td>625</td>
</tr>
<tr>
<td>90</td>
<td>127</td>
<td>141</td>
<td>414</td>
</tr>
</tbody>
</table>

Erythrocyte count in experimental group, in general, remarkably decreased and leukocyte count were the same, although hematological examinations showed no particular change during feeding in control group. Especially on 90 days after beginning of the feeding, it became 1/4 or 1/5 of control group. So it was noted that remarkable decrease of granulocytes occurred following to the course of time. Platelet count of experimental group showed no particular changes, but in part remarkable decrease. (Fig. 2.)

Fig. 2. Influence of thymus feeding on the count of blood elements.

<table>
<thead>
<tr>
<th>Duration</th>
<th>R. B. C.</th>
<th>W.B.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>5.06</td>
<td>8.52</td>
</tr>
<tr>
<td>60</td>
<td>5.54</td>
<td>7.65</td>
</tr>
<tr>
<td>90</td>
<td>5.95</td>
<td>7.99</td>
</tr>
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Differential count were performed on bone marrow smears in such a cases which reduced peripheral blood elements. However, in controversy of the reduction
of the blood elements, there were hyperplasia in all cases and many increased immature megakaryocytes, that is, maturation asynchronism of megakaryocytic series. In addition to this fact, phagocytic activity of megakaryocytes remarkably increased. And also we observed a number of immature cells both in erythropoietic and granulopoietic series. It was interest for us that although there was anemia, leukopenia and thrombocytopenia in periphery blood, we found hyperplasia and maturation asynchronism in bone marrow as same as Banti's syndrome or primary hypersplenism.

Microscopic examination of the spleens of the experimental animals were performed. Capsule and trabecula was thickened and swelling of intima was seen remarkably in the trabecular artery. Lymphoid follicles did not show any abnormality, but in part reduced and atrophic. The most significant areas of atrophy showed thickened intima in the central artery. The germinal center were prominent and the perifollicular zone of reticulum cells were quite distinct. In the spleen of the rats, which sacrificed on 90 days, sinus was rather extended with thickening of the reticuloendothelial cells, although on 60 days was still narrowed by the congested red pulp. Endothelial cell lining the sinusoids showed an increased phagocytic activity towards erythrocytes with hemosiderosis. Marked increase in the amount of red pulp due to extreme engorgement with erythrocytes and hyperplasia of reticuloendothelial cell of the red pulp were seen in some cases.

Prior to the administration of thymus the splenectomized rats showed no significant difference in blood cell counts from normal group.

DISCUSSION.

We observed that the feeding of thymus of young calves in rats over a period of 60 days resulted in a development of a moderate splenomegaly with anemia, leukopenia or thrombocytopenia.

The resemblance of such a syndrome produced in the rats by the feeding of thymus to the clinical syndrome in man to which the term "Hypersplenism" has been applied is observed. This syndrome, both in rats and in man, is characterized by splenomegaly, hyperplasia of the bone marrow, anemia and leukopenia.

The role of the spleen in the pathogenesis of neutropenia has been extensively studied in recent years, and two hypotheses have been advanced. Doan and his associates have observed hyperplasia of the reticuloendothelial cells or clasmatocytes of the spleen with an abnormal phagocytosis of the granulocytes, and account for the neutropenia on this basis. They have also applied this concept of selective destruction of cellular elements of the blood to explain the anemia and thrombocytopenia which frequently associated with the neutropenia. They named such a syndrome "Primary Splenic Neutropenia" or "Primary Splenic..."
Panhematopenia. The second hypothesis, which has been strongly supported by Dameshek, is that of "Hypersplenism", in which the spleen exert an abnormal inhibitory effect, probably by means of a hormone, upon the maturation and release of cells from the bone marrow. Dameshek has emphasized this mechanism particularly in idiopathic thrombocytopenic purpura.

However, the pathogenesis of those splenomegalies has still remained unsolved. Especially as to the relation between splenomegaly and bone marrow in Banti's syndrome, such theory as Dameshek's is still too far from our satisfaction. Even in Wintrobe's classification of this syndrome there is only an added chapter which tells about congestive splenomegaly with an unknown cause.

Rousslet believed that the mechanical interference with the splenic circulation may result in so-called congestive splenomegaly. This in turn may affect the activity of blood formation with depression of the cellular elements in the blood. Menon observed also histopathologically. Again, recently Rousslet introduced experimental congestive splenomegaly using silicon dioxide. Several injections of silicon into splenic vein directly will produce a progressive cirrhosis of the liver. Secondary to this a state of splenic vein hypertension has been produced a concomitant congestive splenomegaly.

Massive splenomegaly has been produced by Hueper in rabbits by repeated injections various nonphysiologic macromolecular polymers such as polyvinyl alcohol, methyl cellulose, acasia, pectin, gelation and ovalbumin. Apparently, these high molecular polymers are not metabolized in the body and stored in the tissue, particularly in the liver, kidney and spleen. A transitory leukopenia and thrombocytopenia, mild anemia, increased sedimentation of erythrocytes and prolongation of the coagulation time were observed. Hueper suggested that the anemia observed in his animals might be the result of inhibition of the bone marrow secondary to the marked proliferation of the reticulum cells observed in the large spleens of these animals. Cartwright stated that rats administrating on methyl cellulose developed a marked hyperplasia of the spleens, anemia and leukopenia.

On the other hand, Doan and his coworkers reported the interesting experiments. Following the intravenous injections of 1 gm of sodium nucleinate into normal rabbits, there developed immediately a leukopenia which lasted for several hours. And the histological evidence from the spleen showed a gradual increase in neutrophilic leukocytes in the parenchym of the spleen and slight splenic enlargement. Osogoe reported the experimental leukopenia following by leukocytosis after injection of lymphocytes in normal rabbits. He assumed that it caused by the disturbance of hematopoietic function, and in this case splenic enlargement is due to hypertrophy of white pulp which causes accumulation of lymphocytes in the spleen parenchym. However in the cases of administration of thymonucleic acid the histological evidence in the spleen showed no particular changes and
spleenomegaly not appeared. Then, megakaryocytes in the bone marrow became to have remarkable phagocytic activity. Having noticed that there were no reticulum hyperplasia in the spleen, such as our experiments, I pursued the cause of such splenomegaly in something other than thymonucleic acid.

According such a point of view, I have stated previously that the spleenomegaly and maturation asynchronism in the bone marrow of so-called Banti's syndrome or "Primary Hypersplenism" are thought to be due to the selective dysfunction of the hypophysis and this syndrome should be named "Primary Pituitary Panhematopenia" or "Pituitolienal Syndrome".

SUMMARY.

1) The feeding of thymus of calves in rats over a period of 60 days resulted in a development of a moderate splenomegaly.

2) Anemia, leukopenia and thrombocytopenia with splenomegaly were observed in the different combined form in the different rats and hyperplasia and maturation asynchronism of bone marrow were found in all cases.

3) On the histological features of these splenomegaly revealed that reticulum cells lining sinusoid, were remarkably thickened and showed hyperplasia and definite phagocytic activity with hemosiderosis in all cases.

4) In these rats, splenectomy seems to prevent the development of anemia and leukopenia.

5) Judging from above findings, it is suggested that this splenomegaly should be related so-called "Primary Hypersplenism".

6) Further experiments are being carried out to determine the actual substance present in the extract of the thymus which causes the enlargement of the spleens, and the influence of splenectomy.

7) It is suggested that the splenomegaly produced by thymus feeding may represent an experimental form of the process to which, in man, the term "Primary Hypersplenism" has applied.

The authors wish to express their deep indebtedness to our respected Prof. G. Kawai of Department of Medicine at Kyoto Pref. University of Medicine for his interest and guidance.

LITERATURES


2) Hueper, W. C.: Macromolecular substances as pathogenic agents.; Arch. Path. 33: 267. '42.


5) **Dameshek, W. & Estern, S.**: The Spleen and Hypersplenism.; Grune & Stratton. 1947, N. Y.


8) **Wintrobe, M. M.**: Clinical Hematology.; Lea & Febiger. 1946. N. Y.

9) **Rousslet, L. M.**: The role of congestion (portal hypertension) is so-called Banti's syndrome.; J. A. M. A. 107:1788. '36.


13) **MASUDA, M.**: Primary pituitary panhematopenia.; J. Kyoto Pref. Med. Univers. 50:488. '52.


16) **OSOGOE, B.**: Influence of the destruction in vivo on transplanted lymphocytes on the phagocytic activity of the megakaryocytes in bone marrow. Separate-Print.

発胸腺授興に伴う発生した大黒鼠の脾腫に就て

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今林 行一

腫質60万至80gの幼若腹検主大黒鼠に於て、腫質100gに及ぶ脾腫を含む食餌を與えた。顕著400g大豆粉400g、発胸腺200g、食餌20g、V.C0.5g及び肝油1gの割合に混じたものを、腫質に就いて連日経口授興した。発胸腺には胸腺の代りに牛肉を用いた。かくして授興糸20日、40日、60日及び90日目に於ける病理組織学的並びに血液学的検査の結果を報告する。

発胸腺10匹の脾腫重量は総平均501g、腫腫との比（脾腫重量mgを腫腫gで除し10倍せるものの）の総平均は29である。発胸腺授興18匹の脾腫重量は総平均626g、腫腫との総平均45で、発胸に比し、重量で25％腫腫との比に於て55％著々増加している。発胸期間別に見ると発胸腺授興で40日目腫腫は発胸と大差はない。60日目に至ると、絶対重量は腫腫と差ははないが、腫腫との比に於て26％増加し、90日目にて、対照比に重量で29％、腫腫との比に於て43％の増加を示し、明らかに有意の脾腫腫の腫大を見めた。腫腫の病變組織学的所見では、皮質及び脾葉の肥厚、脾葉間隔の内膜の腫張、細胞の萎縮を呈するもの等があらわれる。

脾腫は一般に肥厚して居るし、脾腫は40日例では差異あるが、90日例では逆に細薄しているを認める。腫腫組織は著明に增殖肥厚し、旺盛な食肉細胞を認め、血液学的所見としては、特に白血球類の減少が著明で、赤血球も対照に比して減少する。係柱数も又減少するものが多いと、相対的淋巴球増多があるが、係柱白血球類は腫腫に減少の傾向にある。骨髄傷では未成熟粒球の増殖と、その食肉細胞の亢進が認められる。以上我々は大黒鼠に発胸腺授興することにより、著明的に原発性脾腫と末梢血液中の係柱球、赤血球、係柱数の減少をも作り得たので、発胸糖質発胸機能亢進症と何等かの関係を有するものと認め、再報告する。

脾腫の Insulin Test と内分泌学的見地よりの脾腫

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藤木 典生

濱野 辰郎

すでに述べ指摘した様に、Dameshek の所謂 "Hypersplenism" なる概念は、例え病理的に否定しないとしても、その primary hypersplenism に属する症候群に於て病理発生学的問題は未だ定着されていないのである。即ち Primary hypersplenism なる症候群—Congenital hemolytic icterus or anemia, primary hypersplenic thrombocytopenic purpura. Primary splenic neutropenia, primary congenital splenic pahematopoeia, secondary acquired hyperplenic hemolytic anemia,一に就て Dameshek は脾腫機能によって、骨髄傷も末梢血液倉も著しく改善されるので、之等を原発性の脾機能亢進症に由来すると照察のするのであるが、余等の研究では発胸腺5～10年の経過を観察するも、遠隔症例は必ずしも良好とは言いたいものがあり、特に発胸腺授興7年にして遙隔分離したものには、発胸血液倉が正常値近くになっていたにも拘らず、再び著著、高血纖維細胞減少症の如き事が認められ、従ってかかる症候群は単に脾腫の腫張のみによって来るものとは考えられないのである。では一體どんなものか之の症候群の発生原因として観察しているのであるか。之の症候群に於て、血液学的、内分泌学的、病理組織学的的な検