Localization of Antibody in Experimental Portal Hypertension Produced by Anti-Dog-Spleen Rabbit Serum

by

SATORU NAKAHAMA

2nd Department of Surgery, Kanazawa University, Medical School

(Director: Prof. Dr. Ichio Honjo)

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I. INTRODUCTION

There have been many attempts to produce portal hypertension experimentally, such *The gist of this article was presented at 96th Meeting of Japanese Surgical Society in Kinki District (Kinki Geka Gakkai).
as constriction or occlusion of the extrahepatic portal system\textsuperscript{2}314351 and protracted sensitization with egg white albumin\textsuperscript{4445}, and discussions have been made variously concerning the mechanism of elevation of portal pressure in these experiments.

ROUSSELOT\textsuperscript{38}, WHIPPLE\textsuperscript{5253} and THOMPSON\textsuperscript{47}48, in Presbyterian Hospital, classified portal hypertension from etiologic stand point into portal hypertension with intrahepatic cause and one with extrahepatic cause, and insisted that Laennec’s cirrhosis is the representative one of the former and Banti’s disease is nothing but congestive splenomegaly secondary to portal hypertension caused by occlusion of the extrahepatic portal system.

It is true that there exist some cases of so-called Banti’s disease in which the occlusion of the extrahepatic portal system is demonstrated. However, not a few cases of portal hypertension are reported to have no occlusive process in the extrahepatic portal system and, in addition, to be deprived of cirrhotic picture in the liver\textsuperscript{26323415}48. On the other hand, it has been unsuccessful to produce long lasting portal hypertension experimentally by constricting or occluding the extrahepatic portal vein, and it has been also unsuccessful, moreover, to produce huge splenomegaly by these procedures\textsuperscript{23109310931115}43581. These above described facts are accepted to indicate that it is very difficult to explain the etiology of so-called Banti’s disease merely from extrahepatic block of the portal vein.

Considering the probability of allergic development of so-called Banti’s disease, SUZUKI\textsuperscript{4445} successfully produced portal hypertension and splenomegaly in rabbits by protracted sensitization with egg white albumin, and he also succeeded in obtaining histological pictures of the liver and spleen which so closely resemble those of Banti’s disease.

The findings of his experiment reveal very important implication in considering the etiology of so-called Banti’s disease and, furthermore, the independency of this disease as a disease unit, and in this respect, the significance of this experiment should be highly estimated.

SUZUKI\textsuperscript{4445} pointed out that there exists a state of splenitis in the process of the protracted sensitization of rabbit in his experiment. As early as in 1898, BANTI\textsuperscript{1} already asserted that Banti’s disease has its etiologic origin in inflammatory change of the spleen. PATRASSI\textsuperscript{44} and SATO\textsuperscript{47} also pointed out from clinical observations that this disease is closely related to splenitis.

Studies on so-called Banti’s disease have hitherto been carried out mainly from the aspect of extrahepatic block of the portal vein.

The present study was carried out from the presumption that some functional disturbance of the spleen caused by such impairment of this organ as splenitis might have close association with so-called Banti’s disease, and as a method of impairing the splenic tissue specifically, rabbits were immunized with homogenate of dog spleen and obtained rabbit serum, i.e. anti-dog-spleen rabbit serum (abbreviated to anti-spleen serum, hereafter) was intravenously injected in dogs, and by this method portal hypertension was able to be successfully produced in dogs.

Furthermore, in the aim of examining localization of anti-spleen antibody in animals of portal hypertension induced by this reverse allergy, tracer experiment was carried out by the use of radioactive \textsuperscript{131}I labelled antibody.
II. MATERIALS AND METHODS

1. Experimental Animals

For the production of anti-spleen serum, rabbits of 2.5 to 4 kg body weight were
used.

As the antigen for immunization of rabbit and for precipitation test, spleen of adult
mongrel dogs was used. Adult mongrel dogs weighing from 6 to 15 kg were used for
the observation of portal pressure and tracer experiment with $^{131}I$ labelled antisypleen
serum.

2. Preparation of Antigen for Immunization of Rabbits and Production of Anti-
Spleen Serum

i. Preparation of Antigen for Immunization of Rabbits

Dogs were anesthetized with intravenous injection of isozol, and the abdomen was
opened with upper median incision. The splenic artery was exposed and the spleen was
extirpated with the splenic pedicle including the splenic artery. A glass canula was inserted
into the cut end of the splenic artery of the extirpated spleen which was connected to a
polyethylene tube. Approximately 10 liters of saline solution was perfused from this tube
and the blood contained in the spleen was washed out as thoroughly as possible until the
fluid streamed out from the cut end of the splenic vein became completely colorless and
clear. Then, the capsule was stripped off, and cut into small pieces, which was well
ground in Waring’s blender and further in glass homogenizer of Potter Ellveihem to make
20 per cent homogenate in saline solution. Marzonine was added to the homogenate to
be 0.01 per cent in final concentration as a preservative and stored at 4°C.

ii. Production of Anti-Spleen Serum

Two cc of the antigen homogenate prepared as in the above was intraperitoneally
injected in rabbits three time a week for 3 weeks. The entire blood of the rabbits was
withdrawn by heart puncture, the thoracic cavity being opened under ether anesthesia, 7
days after the final injection of the antigen homogenate. The blood taken was placed
quietly in refrigerator over a night to separate the serum, and the complement in the
serum was inactivated by heating at 56°C for 30 minutes. Blood corpuscles and minced
muscle of dog well rinsed with saline solution were added to the separated serum, re-
spectively in the same weight as the serum, which was then placed quietly in an incubator
at 37°C for an hour to absorb and remove species specific antibody.

3. Serological Certification of Anti-Spleen Serum

Serological certification of anti-spleen serum was performed by precipitation test.

i. Antigen for Precipitation Test

Homogenate of dog spleen used for the immunization of rabbits was centrifuged at
3,000 r.p.m. for 30 minutes, and the supernatant was filtrated with Seitz’s filter using
filter plate No.85 of Toyo Asbestos Co. Obtained filtrate was used as the antigen in
precipitation test. From the liver and kidney also, antigen for precipitation test was pre-
pared in the same way as from the spleen.

ii. Precipitation Test

Precipitation test was carried out following overlying method39). A series of two
times successive dilution of anti-spleen serum was prepared with 1 per cent solution of gum-arabic, and the antigen was similarly diluted with saline solution to become successive dilution of \(10 \times 2^n\) times. Thus diluted antigen solution was overlaid on each test tube of respectively diluted anti-spleen serum solution. After leaving in room temperature for 5 hours, appearance of white ring in the contact surface of the two solutions were examined. The highest dilution number of antigen in the test tube of positive reaction was represented as antigen titer, and the highest dilution number of anti-spleen serum as antibody titer.

4. Injection of Anti-Spleen Serum in Dogs
   Anti-spleen serum was injected in the femoral vein of animals of 3 groups as follows:
   - Group 1: Single injection of anti-spleen serum of 1 cc/kg body weight
   - Group 2: Single injection of anti-spleen serum of 2 cc/kg body weight
   - Group 3: 5 injections of anti-spleen serum of 1 cc/kg body weight with interval of 5 to 7 days
   For the control study, normal rabbit serum was injected as follows in the femoral vein of animals of 2 groups.
   - Group 4: Single injection of normal rabbit serum of 2 cc/kg body weight
   - Group 5: 5 injections of normal rabbit serum of 1 cc/kg body weight with interval of 5 to 6 days

5. Measurement of Portal Pressure
   Prior to the measurement of portal pressure, the animals were kept away from diet for 24 hours. The abdomen of the animals was opened with upper median incision under intravenous anesthesia with isoisol of 15 to 20 mg/kg body weight. A polyethylene tube of 1 mm in caliber was inserted from a tributary of the superior mesenteric vein to the portal vein, and the tube was connected to aqueous manometer. Portal pressure was measured taking zero point in the height of the tip of polyethylene tube in the portal vein.
   In animals of Group 1, 2 and 4, portal pressure was measured before the injection of the serum and several times after the injection with interval of 5 to 7 days. In animals of Group 3 and 5, portal pressure was measured before the injection of the serum and 5 days after the final injection of anti-spleen serum or normal rabbit serum.

6. Radioactive Iodine, \(^{131}\text{I}\)
   Radioactive iodine of Na\(^{131}\text{I}\) in 0.05 per cent Na\(_2\)SO\(_4\) solution from Dainabot Radioisotope Institute was used, and the concentration was adjusted with distilled water to be 1 mC/cc.

7. Determination of Radioactivity
   Radioactivity of tissues was determined by the use of well-type scintillation counter of EA-14 type of Kobe Kogyo Co.

8. Preparation of \(^{131}\text{I}\) Labelled Antibody
   Although various methods of labelling the antibody with \(^{131}\text{I}\) are reported by Dixon\(^{59}\), Pressman\(^{36,37}\), Francis\(^{9}\) and others, in the present experiment, Komukai's method\(^{21}\) was followed, in which \(^{131}\text{I}\) is directly combined to antibody serum and globulin fraction containing antibody is extracted after that.
   Radioactive iodine, \(^{131}\text{I}\) labelled anti-spleen serum for 1 dog was prepared as follows
using 20 cc of anti-spleen serum and 1 mC of radioactive iodine. Two cc of 5N ammonia is added to 20 cc of anti-spleen serum to make it strong alkaline. On the other hand, 1 mC of radioactive iodine is dissolved in 30 cc of 0.1 mol KI solution containing 1 mg of free iodine per 1 cc. Radioactive $^{131}$I-KI solution of a few drops thus prepared is added slowly to the strong alkaline anti-spleen serum and stirred. Leaving the mixture in room temperature for a while, iodination of anti-spleen serum proceeds completely. The mixture is then adjusted to be pH 7.5 with 1 N acetic acid, and anhydrous sodium sulphate is added to be 15 per cent in final concentration and the mixture is left at 37°C for 30 minutes to make $\gamma$-globulin precipitate, which is then centrifuged at 3,500 r.p.m. for 30 minutes. Ten cc of 0.1 mol phosphate buffer of pH 8.0 is added to the sediment, and anhydrous sodium sulphate is further added in a proportion of 15 per cent. The mixture is again left at 37°C for 30 minutes to make $\gamma$-globulin precipitate, and non-globulin-bound iodine is rinsed away. After these procedures are repeated twice, the sediment is wrapped up in a dialysis membrane and dialysed against distilled water for 24 hours. Thus, $\gamma$-globulin becomes soluble in distilled water within the dialysis membrane. Dialysis is further repeated twice, respectively for 2 hours, against saline solution and the supernatant obtained from centrifugation is

1. $^{131}$I labelled $\gamma$-globulin solution (Fig. 1).

For the control study, $^{131}$I labelled $\gamma$-globulin of normal rabbit serum was prepared. Final amount of $^{131}$I labelled $\gamma$-globulin thus prepared was 15 to 20 cc, radioactivity of which being 10 to 20 $\mu$C in total.

9. Certification of $^{131}$I Labelled Globulin

i. Certification by Paper Electrophoresis

It was ascertained by paper electrophoresis if the final supernatant of $^{131}$I labelling was really $\gamma$-globulin.

The apparatus of paper electrophoresis of Kobayashi was used, with veronal buffer of pH 8.6 and No.51 filter paper of Toyo Roshi Co. Normal rabbit serum and $^{131}$I labelled globulin of respectively 0.005 cc was used for electrophoresis in the condition of constant current of 5 mA for 6 hours, until albumin fraction of normal rabbit serum expanded 7 cm in length. After the phoresis, the filter paper was dried at 120°C for 20 minutes, and stained with bromphenol blue solution for 20 minutes, which was then bleached with 1.5 per cent acetic acid and dried in room temperature.

ii. Certification of Binding of Radioactive $^{131}$I with $\gamma$-Globulin

Ten per cent trichlor acetic acid was added to 1 cc of above mentioned $^{131}$I labelled $\gamma$-globulin and protein was precipitated. The mixed solution having precipitate of protein

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1) 20 cc of Anti-Spleen Serum + 2 cc of 5N-NH$_4$OH
2) 30 cc of KI + $^{131}$I 1 mC

$\rightarrow$ Adjusted to pH 7.5 with 1N-CH$_3$COOH

Anhydrous Na$_2$SO$_4$ is added in 15% in final concentration. (37°C, 30 min.)

Centrifugation at 3,500 r.p.m. for 30 min.

Sediment + 0.1 M Phosphate buffer of 10 cc (pH 8.0)

$\rightarrow$ Centrifugation (This procedure is repeated twice.)

Anhydrous Na$_2$SO$_4$ is added in 15% in final concentration. (37°C, 30 min.)

Sediment

$\rightarrow$ Dialysis against distilled water overnight

Dialysed material

$\rightarrow$ Dialysis against saline solution (for 2 hours, twice)

Dialysed material

$\rightarrow$ Centrifugation

Supernatant is $^{131}$I labelled $\gamma$-globulin

* KI solution of 0.1 mol containing 1 mg of isolated iodine per 1 cc.
was centrifuged. Radioactivity of the supernatant and sediment was respectively determined.

iii. Serological Certification of $^{131}$I Labelled $\gamma$-Globulin in Anti-Spleen Serum

Influence of $^{131}$I labelling on antibody titer of anti-spleen serum was examined by mixing precipitation method\(^21\)\(^32\)\(^33\) between $^{131}$I labelled $\gamma$-globulin of anti-spleen serum and corresponding antigen. For the control study, $^{131}$I labelled $\gamma$-globulin of normal rabbit serum was used. One cc of $^{131}$I labelled $\gamma$-globulin solution of anti-spleen serum and antigen solution of 10 times dilution prepared from the various tissues which were to be used for precipitation test were mixed together and incubated at $37^\circ$C for 2 hours and further left in room temperature for 24 hours. The final reading of the precipitation test was made according to the amount of sediment of centrifugation at 500 r.p.m. for 10 minutes.

10. Examination on Localization of $^{131}$I Labelled Anti-Spleen Serum in Vivo

Radioactive $^{131}$I labelled $\gamma$-globulin solution of anti-spleen serum was injected in the femoral vein exposed through a small incision in the inguinal region, of normal dogs weighing from 6 to 8 kg. Six hours, 24 hours and 48 hours respectively after the injection, radioactivity was determined in various organs, the blood being completely washed out by perfusion. Besides, radioactivity in 1 cc of the blood was determined at the same time. Radioactivity of the tissues was represented in the term of ratio of counts per minute in 1 g of tissue and counts per minute in 1 cc of blood (abbreviated to tissue/blood ratio, hereafter) following the method of PRESSMAN\(^6\)\(^7\), and localization of $^{131}$I labelled antibody in various organs was compared with each other.

For the control study, $^{131}$I labelled $\gamma$-globulin of normal rabbit serum was used in the similar way.

The perfusion of the organs for removal of blood was carried out in animal anesthetized with intravenous injection of isozol. The thoracic cavity was opened and the inferior vena cava was cut and left opened for the outlet. Approximately 50 liters of saline solution was perfused from a canula which was inserted into the aortic arch, using the DE BAKEY roller pump\(^8\). After the perfusion, the organs were extirpated and the fluid was well removed. From each organ, specimens of 1 g were taken in duplication for the determination of radioactivity, using torsion balance. Radioactivity was determined for 3 minutes in each material and represented in the term of counts per minutes. For determination of radioactivity of blood, blood was withdrawn before the perfusion, and radioactivity was determined in 1 cc of the blood.

11. Autoradiography

In the femoral vein of dogs, $^{131}$I labelled $\gamma$-globulin was injected. Twenty-four hours after the injection, the animals were slaughtered by withdrawal of blood from the femoral artery and vein, and the spleen, liver, kidney and lymph nodes in the mesentery were taken out. Slices of 5 mm in thickness were taken from various parts of these organs and immediately fixed in a 10 per cent formalin solution over a night. After the fixation of tissues, paraffin blocks of the tissues were prepared following rapid paraffin embedding method\(^33\) which was cut into slices of 10 $\mu$ in thickness. The slice was placed on slide
glass and stained doubly with hematoxylin and eosin, which was then used for autoradiography following stripping emulsion method23) by the use of sensitized plate for stripping autoradiography of ET-2E of Fuji Photo Film Co. Ltd. The sensitized plate was dipped in water of 18 to 20°C for a few minutes in dark room. The emulsion membrane of the plate was gently stripped off and was gently placed on the stained section to cover it, and it was dried by electric fan. After completely dried, the specimen was wrapped with black paper and put in a desiccator containing silica gel for exposure at 4°C for 4 weeks. After the exposure, it was developed by Fuji-Rendol and fixed by Fuji-Fix.

III. RESULTS

1. Precipitation

Precipitation test was performed following overlying method between antigen of the spleen, liver and kidney of dog and anti-spleen serum.

Antibody titer was from 64 to 256, and antigen titer from 80 to 320, showing the most intense positive reaction in the test between the serum and splenic antigen, which is the corresponding tissue. When hepatic antigen was used, antibody titer was from 8 to 16, and antigen titer from 40 to 80, showing positive reaction of slight degree. When kidney antigen was used positive reaction could not be observed (Tab. 1).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antibody Titer</th>
<th>Antigen Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>64~256</td>
<td>80~320</td>
</tr>
<tr>
<td>Liver</td>
<td>8~16</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>10~80</td>
</tr>
</tbody>
</table>

2. Portal pressure

i. Group of Single Injection of Anti-Spleen Serum of 1 cc/kg Body Weight

In 5 cases out of 8 receiving single injection of anti-spleen serum of 1 cc/kg body weight, elevation of portal pressure of 32 mmH\textsubscript{2}O to 46 mmH\textsubscript{2}O was observed 5 to 7 days after the injection. Ten to 14 days after the injection, 2 animals out of these 5 still maintained elevation of portal pressure of 20 mmH\textsubscript{2}O to 40 mmH\textsubscript{2}O. As late as 20 days after the injection, portal pressure restored to normal in all cases (Tab. 2, Fig. 2).

ii. Group of Single Injection of Anti-Spleen Serum of 2 cc/kg Body Weight

In 3 cases out of 5 receiving single injection of anti-spleen serum of 2 cc/kg body weight, elevation of portal pressure of 45 mmH\textsubscript{2}O to 68 mmH\textsubscript{2}O was observed 5 to 6 days after the injection. Similarly to the former group of animals, portal pressure showed a tendency of gradual fall to restore to normal 18 days after the injection (Tab. 3, Fig. 3).

iii. Group of Repeated 5 Injections of Anti-Spleen Serum of 1 cc/kg Body Weight with Interval of 5 to 7 Days

Portal pressure was measured 5 days after the final injection of 5 times repeated injections of anti-spleen serum of 1 cc/kg body weight with interval of 5 to 7 days. Although in 1 case out of 6, the elevation was slight to be 17 mmH\textsubscript{2}O, in 5 cases of the
Tab. 2 Portal Pressure in Animals with Single Injection of Anti-Spleen Serum of 1 cc/kg Body Weight

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>Portal Pressure (mmH₂O) Before</th>
<th>( ) : Days after Injection</th>
<th>Elevation of Portal Pressure mmH₂O, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>125 → (7) 157 → 124</td>
<td></td>
<td>32, 25</td>
</tr>
<tr>
<td>2</td>
<td>11.5</td>
<td>110 → (7) 125 → (14) 110</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>135 → (7) 175 → (13) 140</td>
<td></td>
<td>46, 33</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>125 → (7) 165 → (13) 127</td>
<td></td>
<td>40, 32</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>123 → (5) 115 → (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>8.5</td>
<td>138 → (5) 125 → (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>120 → (5) 155 → (10) 140 → 107</td>
<td></td>
<td>35, 27</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>115 → (5) 147 → (10) 125</td>
<td></td>
<td>32, 28</td>
</tr>
</tbody>
</table>

Fig. 2 Portal Pressure in Animals with Single Injection of Anti-Spleen Serum of 1cc/kg Body Weight

remainder elevation of portal pressure was marked as 45 mmH₂O to 72 mmH₂O (Tab. 4, Fig. 4).

iv. Group of Single Injection of Normal Rabbit Serum of 2 cc/kg Body Weight

Portal pressure was measured 3 times with interval of 5 to 7 days after single injection of normal rabbit serum of 2 cc/kg body weight. Elevation of portal pressure could not be observed at any stadium (Tab. 5, Fig. 5).
### Tab. 3 Portal Pressure in Animals with Single Injection of Anti-Spleen Serum of 2 cc/kg Body Weight

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>Portal Pressure (mmH₂O)</th>
<th>Elevation of Portal Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>Days after Injection</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>142</td>
<td>210</td>
</tr>
<tr>
<td>12</td>
<td>10</td>
<td>105</td>
<td>(5)</td>
</tr>
<tr>
<td>13</td>
<td>9</td>
<td>120</td>
<td>(5)</td>
</tr>
<tr>
<td>14</td>
<td>10.5</td>
<td>110</td>
<td>(6)</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>108</td>
<td>(6)</td>
</tr>
</tbody>
</table>

**Fig. 3** Portal Pressure in Animals with Single Injection of Anti-Spleen Serum of 2 cc/kg Body Weight

### Tab. 4 Portal Pressure in Animals with 5 Injections of Anti-Spleen Serum of 1 cc/kg Body Weight with Interval of 5 to 7 Days

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>Injection of Anti-Serum</th>
<th>Portal Pressure (mmH₂O)</th>
<th>Elevation of Portal Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Amount (cc/kg)</td>
<td>Interval (Day)</td>
<td>No. of Injection</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>22</td>
<td>8.5</td>
<td>1</td>
<td>5~6</td>
<td>5</td>
</tr>
<tr>
<td>23</td>
<td>13</td>
<td>1</td>
<td>6~7</td>
<td>5</td>
</tr>
<tr>
<td>24</td>
<td>12</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>26</td>
<td>12</td>
<td>1</td>
<td>6~7</td>
<td>5</td>
</tr>
</tbody>
</table>
Fig. 4 Portal Pressure in Animals with 5 Injections of Anti-Spleen Serum of 1 cc/kg Body Weight with Interval of 5 to 7 Days

Tab. 5 Portal Pressure in Animals with Single Injection of Normal Rabbit Serum of 2 cc/kg Body Weight

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>Portal Pressure (mmH₂O) Before</th>
<th>Days after Injection</th>
<th>Elevation of Portal Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>9</td>
<td>132</td>
<td>133 (5) 140 (10) 125 (17)</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>7.5</td>
<td>130</td>
<td>125 (5) 133 (10) 130 (17)</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>6.5</td>
<td>95</td>
<td>91 (7) 103 (14) 105 (21)</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>11.5</td>
<td>110</td>
<td>115 (7) 120 (14) 113 (21)</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>9</td>
<td>128</td>
<td>125 (7) 120 (14) 125 (21)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5 Portal Pressure in Animals with Single Injection of Normal Rabbit Serum of 2 cc/kg Body Weight
v. Group of Repeated 5 Injections of Normal Rabbit Serum of 1 cc·kg Body Weight with Interval of 5 to 6 Days

Portal pressure was measured 5 days after the final injection of 5 times repeated injections of normal rabbit serum of 1 cc/kg with interval of 5 to 6 days. Portal pressure elevated at most 20 mmH₂O, revealing no particular elevation of the pressure (Tab. 6, Fig. 6).

**Tab. 6 Portal Pressure in Animals with 5 Injections of Normal Rabbit Serum of 1 cc/kg Body Weight with Interval of 5 to 6 Days**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>Injection of Normal Rabbit Serum</th>
<th>Portal Pressure (mmH₂O)</th>
<th>Elevation of Portal Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>9</td>
<td>1</td>
<td>120</td>
<td>8</td>
</tr>
<tr>
<td>42</td>
<td>7</td>
<td>1</td>
<td>130</td>
<td>12</td>
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<td>43</td>
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<td>45</td>
<td>10.5</td>
<td>1</td>
<td>115</td>
<td>21</td>
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</tbody>
</table>

**Fig. 6** Portal Pressure in Animals with 5 Injections of Normal Rabbit Serum of 1 cc/kg Body Weight with Interval of 5 to 6 Days

3. Certification of ¹³¹I Labelled γ-Globulin

i. Certification by Paper Electrophoresis

Electrophoretic pattern of the supernatant ultimately obtained from the procedure of ¹³¹I labelling had a single peak which corresponded precisely to the fraction of γ-globulin in electrophoretic pattern of normal rabbit serum (Fig. 7).

ii. Certification of Binding of Radioactive ¹³¹I with γ-Globulin

Radioactivity in 1 cc of ¹³¹I labelled γ-globulin solution in anti-spleen serum and ¹³¹I labelled γ-globulin solution in normal rabbit serum was 72817 c.p.m. and 56756 c.p.m., respectively. When protein component in these solutions was precipitated with 10 per cent trichlor acetic acid, the supernatant showed as little radioactivity as 1134 c.p.m. and 1055 c.p.m., respectively, whereas the sediment of γ-globulin fraction showed intense radioactivity of 71329 c.p.m. and 55490 c.p.m., respectively (Tab. 7).
iii. Serological Certification of $^{131}$I Labelled $\gamma$-Globulin in Anti-Spleen Serum

Precipitation test was performed following mixing method between $^{131}$I labelled $\gamma$-globulin in anti-spleen serum and antigens of spleen, liver and kidney. Precipitate was most markedly observed in the test against the corresponding organ, spleen, slightly against liver and none against kidney. In precipitation test between $^{131}$I labelled $\gamma$-globulin in normal rabbit serum and antigens of spleen, liver and kidney, precipitate could not be observed at all (Tab. 8).

| Tab. 8 Precipitation Test of $^{131}$I Labelled Anti-Spleen Serum Using Mix Method |
|----------------------------------|----------------|----------------|
|                                   | Spleen | Liver | Kidney |
| $^{131}$I Labelled $\gamma$-Globulin of Anti-Spleen Serum | #      | +     | -      |
| $^{131}$I Labelled $\gamma$-Globulin of Normal Rabbit Serum | -      | -     | -      |

4. Examination on Localization of $^{131}$I Labelled Anti-Spleen Serum

Radioactivity of the tissues 6 hours after the injection was represented as the mean value of tissue-blood ratio. In control animals injected with $^{131}$I labelled $\gamma$-globulin of normal rabbit serum, the value was 0.51 in spleen and 0.29 in liver, whereas in animals injected with $^{131}$I labelled $\gamma$-globulin of anti-spleen serum, the value was 0.78 in spleen and 0.57 in liver, showing marked increase. In other organs, radioactivity was minute itself and no significant difference could be observed in these two groups (Tab. 9, Fig. 8, a).
Twenty-four hours after the injection, the value was 0.49 in spleen and 0.28 in liver in control animals, while in experimental animals it was 0.75 in spleen and 0.56 in liver. Forty-eight hours after the injection, it was 0.46 in spleen and 0.23 in liver in control animals, and it was 0.71 in spleen and 0.45 in liver in experimental animals. As well as 6 hours after the injection, marked increase could be observed in tissue/blood ratio of spleen and liver 24 hours and 48 hours after the injection in experimental animals compared with those of control. Significant difference could not be observed at any stadium in other organs (Tab. 10, and 11, Fig. 8, b and c).

Since not only antibody globulin but other γ-globulin is contained in 131I labelled γ-globulin fraction of anti-spleen serum, the amount of organ specific antibody is obtained as the result of subtraction of radioactivity of 131I labelled γ-globulin in normal rabbit serum from that of 131I labelled γ-globulin in anti-spleen serum in tissues. Amount of anti-spleen antibody localized in the spleen and liver 24 and 48 hours after the injection ranged from 0.22 to 0.28 in the term of tissue/blood ratio, revealing approximately similar values in these two tissues. In other organs, significant localization of anti-spleen antibody could not be demonstrated (Fig. 9).

5. Autoradiogram

In the findings of autoradiogram of various tissues in dogs receiving the injection of 131I labelled γ-globulin in anti-spleen serum, marked accumulation of radioactive 131I could be demonstrated on the wall of splenic sinuses and in the wall of the trabecular veins.

<table>
<thead>
<tr>
<th>Injection</th>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>Tissue</th>
<th>Blood</th>
<th>Spleen</th>
<th>Liver</th>
<th>Bone</th>
<th>Marrow</th>
<th>Lymph</th>
<th>Node</th>
<th>Kidney</th>
<th>Adrenal</th>
<th>Gland</th>
<th>Muscle</th>
<th>Omentum</th>
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</thead>
<tbody>
<tr>
<td>131I Labelled γ-Globulin of Anti-Spleen Serum</td>
<td>51</td>
<td>6</td>
<td>1493</td>
<td>1008</td>
<td>762</td>
<td>309</td>
<td>255</td>
<td>133</td>
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<td>52</td>
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<td>679</td>
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<td>143</td>
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<td>381</td>
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<tr>
<td>Mean</td>
<td></td>
<td></td>
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<td>745</td>
<td>362</td>
<td>182</td>
<td>125</td>
<td>148</td>
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<td>97</td>
<td>112</td>
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<td></td>
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<tr>
<td>131I Labelled γ-Globulin of Normal Rabbit Serum</td>
<td>55</td>
<td>7</td>
<td>1327</td>
<td>745</td>
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<tr>
<td>56</td>
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<td>191</td>
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<td></td>
<td>1313</td>
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</table>
Tab. 10 Localization of $^{131}I$ Labelled Anti-Spleen Antibody in Vivo; 24 Hours after the Injection

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<tr>
<th>Injection</th>
<th>Dog No.</th>
<th>Body Weight (kg)</th>
<th>131I-Labelled $\gamma$-Globulin of Anti-Spleen Serum</th>
<th>Tissue/Blood</th>
<th>Blood</th>
<th>Spleen</th>
<th>Liver</th>
<th>Bone Marrow</th>
<th>Lymph Node</th>
<th>Kidney</th>
<th>Adrenal Gland</th>
<th>Muscle</th>
<th>Omentum</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>cpm/µ</td>
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<td>942</td>
<td>802</td>
<td>174</td>
<td>192</td>
<td>123</td>
<td>143</td>
<td>58</td>
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<tr>
<td>131I</td>
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<td>Tissue/Blood</td>
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<td>0.04</td>
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<td>83</td>
<td>89</td>
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<td>27</td>
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<tr>
<td>of Anti-Spleen Serum</td>
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<td>Tissue/Blood</td>
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<td>0.22</td>
<td>0.14</td>
<td>0.16</td>
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<td>cpm/µ</td>
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<td>Tissue/Blood</td>
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<td>60</td>
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<td>cpm/µ</td>
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<td>Tissue/Blood</td>
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<td>720</td>
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<td>Tissue/Blood</td>
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Tab. 11 Localization of $^{131}I$ Labelled Anti-Spleen Antibody in Vivo; 48 Hours after the Injection

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<th>Tissue/Blood</th>
<th>Blood</th>
<th>Spleen</th>
<th>Liver</th>
<th>Bone Marrow</th>
<th>Lymph Node</th>
<th>Kidney</th>
<th>Adrenal Gland</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td>cpm/µ</td>
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<td>110</td>
<td>144</td>
<td>98</td>
<td>104</td>
<td>34</td>
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<tr>
<td></td>
<td>63</td>
<td>8</td>
<td></td>
<td>Tissue/Blood</td>
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<td>0.50</td>
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<td>0.07</td>
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<td>cpm/µ</td>
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<td>Tissue/Blood</td>
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<td>0.22</td>
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<tr>
<td>of Anti-Spleen Serum</td>
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<td>cpm/µ</td>
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<td>496</td>
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<td>113</td>
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<tr>
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<td>Tissue/Blood</td>
<td>0.52</td>
<td>0.25</td>
<td>0.17</td>
<td>0.25</td>
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<td>0.19</td>
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<td>Tissue/Blood</td>
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</table>
Fig. 8 Localization of 131I Labelled Anti-Spleen Antibody in Vivo
(Tissue/Blood Ratio)
Fig. 9  Organ Specificity of $^{131}$I Labelled Anti-Spleen Antibody
(Tissue/Blood Ratio)
(Fig. 10 and 11). Namely, radioactive $^{131}I$ accumulated in the intrasplenic venous system, and the accumulation could not be demonstrated in the intrasplenic arterial system, reticular tissue of red pulp and white pulp. In the liver, accumulation of radioactive $^{131}I$ could be remarkably observed around Glisson’s sheaths, in the wall of the central vein, in the wall of fine branches of the intrahepatic portal vein and in the wall of the sublobular veins (Fig. 12, 13, 14 and 15).

**Fig. 10** Autoradiographic Finding of the Splenic Sinuses  $\times 100$

**Fig. 11** Autoradiographic Finding of the Trabecular Veins of the Spleen  $\times 100$

**Fig. 12** Autoradiographic Finding of the Glisson’s Sheaths in the Liver  $\times 100$

**Fig. 13** Autoradiographic Finding of the Central Veins in the Liver  $\times 400$

**Fig. 14** Autoradiographic Finding of the Fine Tributaries of the Intrahepatic Portal Venous System  $\times 400$

**Fig. 15** Autoradiographic Finding of the Sublobular Veins  $\times 100$
In the kidney and lymph node, the accumulation could not be observed in any part of the small vessels, glomeruli or lymph sinuses.

In the spleen, liver, kidney and lymph node of animals with injection of $^{131}$I labelled $\gamma$-globulin of normal rabbit serum, accumulation of radioactive $^{131}$I could not be demonstrated.

IV. DISCUSSION

Portal hypertension is a symptom which is almost invariably observed in cases of so-called Banti's disease. There have been many discussions on the mechanism of this portal hypertension. ROUSSELOT, WHIPPLE and THOMPSON considered that portal hypertension is caused by obstructive moment in certain part of the portal system and they classified portal hypertension from the site of its cause into intrahepatic one and extrahepatic one. From extensive clinical observations, they asserted that the cause of so-called Banti's disease is nothing but extrahepatic obstruction of the portal vein. However, it has been difficult to produce portal hypertensive condition and further splenomegaly in experiments by obstructing or constricting the extrahepatic portal vein. For instance, WARTHIN could produce splenomegaly in dogs and rabbits in the early stadium following the ligation of the splenic vein, which was, however, followed by atrophy of this organ. MENON and YAMADA also observed the similar findings in their experiments. Many other experimental studies, such as portal ligation in one stage by CHILD, partial occlusion of the portal vein by DOUGLASS, gradual constriction of the portal vein by STONE et al. and MORRIS et al., constriction of the hepatic vein by KABURAKI, similarly constriction of the inferior vena cava by KERSCHNER and reversed Eck fistula by JÄGER, all indicate difficulty of experimental production of long sustained portal hypertensive condition.

As an intrahepatic factor of portal hypertension, liver cirrhosis is considered to be important. HERRICK considered that hepatic arterial pressure abnormally and subtly influences on portal pressure in cirrhotics by way of the pathologic shunts established between the intrahepatic arteries and portal venous system. SÄEGESSER agreed with the opinion of HERRICK. McINDOE emphasized compression on the portal and hepatic vein due to proliferation of connective tissue in the liver, and maintained that portal pressure elevates in cirrhotics owing to decrease in the intrahepatic vascular bed and particularly owing to increase in vascular resistance in the portal venous system. On the other hand, KELTY and others put emphasis upon intrahepatic compression on the hepatic and portal venous systems due to newly appearing hepatic lobules. KALK and ELIAS insisted that both intrahepatic obstruction of the vascular bed and interference of arterial pressure on portal pressure are the cause of portal hypertension. However, it is widely recognized that portal hypertension is not always observed in cirrhotics and there is not constant correlation between the grade of cirrhotic change and portal pressure, and there exist many problems to be solved concerning the mechanism of elevation of portal pressure in these conditions.

There are many clinical cases of so-called Banti's disease revealing portal hypertension without histological changes of liver cirrhosis and obstructive moment in the portal system. This finding was already pointed out by McMICHAEL, PATRASS and RAVENNA, and ascertained by KIMOTO and others, recently. RAVENNA, SUZUKI and ICHIDA con-
considered that thrombus formation in the portal venous system, frequently observed in cases of so-called Banti's disease, is the secondary change of this disease process. Ito\(^1\) asserted that, in such large vessels as the portal or splenic vein, thrombus is finally formed in such condition as the end stadium, i. e. in the condition of functional devastation of the vascular connective tissue.

As has been reviewed in the above, the mechanism of portal hypertension cannot be explained merely from concept of obstructive moment.

Suzuki\(^1\) produced portal hypertensive condition and splenomegaly in rabbits by protracted sensitization with egg white albumin, and he observed in these animals the picture of Fibroadenie, which has been conceived to be specific to Banti's disease, together with picture of pseudocirrhosis of the liver. From these observations, he postulated splenitic origin of Banti's disease and asserted allergic condition as the etiology. Ito\(^1\) also presumed allergic origin of Banti's disease from his histological investigations on the liver and spleen of patients with this disease. Ichida\(^1\) asserted that the participation of immunological process can be presumed as a cause of Banti's disease. In 1898, Banti\(^1\) described this disease as Splenomegalie mit Leberzirrhose, and insisted independency of this disease and that the disease is caused by inflammatory reaction of the spleen. Patrassi\(^1\) and Di Guglielmo\(^9\) represented agreement with concept of Banti. Sato\(^1\) pointed out the importance of splenic factor in this disease from the observation that blood findings, esophageal varices and histological findings of the liver are all improved in Banti's disease by splenectomy. Kozaka\(^2\) also maintained that the primary etiologic factor should be sought in the spleen. Tokoro\(^9\) considered of splenic origin of Banti's disease and postulated a concept of angiodysplastische Splenopathie.

From the presumption that Banti's disease might have close association with some functional disturbance of the spleen due to such impairment of the splenic tissue as splenitis, in the present experiment anti-spleen serum was intravenously injected in dogs as a method of impairing the splenic tissue selectively, following the analogy of Masugi's nephritis\(^2\).

In the present experiment, marked elevation of portal pressure could be observed 5 to 7 days after the single injection of anti-spleen serum of 1 or 2 cc/kg body weight, which showed the tendency of gradual fall to restore to normal 20 days after the injection. When the injection of the serum was repeated, the degree of portal hypertension was even more remarkable. On the contrary, elevation of portal pressure could not be observed in any case of control animals receiving the injection of normal rabbit serum. Accordingly, it is assumed obviously that portal hypertension observed in the present experiment was caused by certain changes due to antigen-antibody reaction of anti-spleen antibody.

Miyata\(^3\), in our clinic, observed marked elevation of portal pressure following repeated intravenous injections of anti-spleen serum of 1 cc/kg body weight once a week in dogs. In these dogs, he observed histologically intense reactive increase in large mononuclear cells, polymorphnuclear leucocytes and phagocytes in the spleen, which was then replaced by the reaction of plasma cells and reticulum cells, finally leading to atrophic change or that of proliferation of connective tissue. The latter change was particularly remarkable in the trabecle, wall of vessels and splenic sinuses. He also observed thickening of inter-
lobular connective tissue in the liver which resembles the picture of early stage of Banti's disease, although this change did not develop in parallel with that of the spleen. However, he could not find certain organic change acceptable as the cause of portal hypertension at least in the early stadium of the experiment, and he presumed that the cause of portal hypertension observed here consisted in some functional changes due to the injection of anti-spleen serum.

In the present study, tracer experiment was designed to investigate the localization of anti-spleen antibody in portal hypertension caused by reverse allergy. When anti-spleen serum is injected in animals, localization of the antibody can be numerically represented by labelling $\gamma$-globulin of the anti-spleen serum with radioactive $^{131}$I. At the labelling of antibody with radioactive $^{131}$I, the most important thing is that the immunological specificity and titer of the antibody should not be impaired, and it is also very important that radioactive $^{131}$I is tightly bound with the antibody, particularly in experiment in vivo. In this respect, it was ascertained in the present experiment that globulin was chemically and tightly bound with radioactive $^{131}$I, that non-bound radioactive $^{131}$I was not contained in the $^{131}$I labelled globulin of anti-spleen serum and that the result of precipitation test of $^{131}$I labelled anti-spleen serum was not altered from that of anti-spleen serum before the iodination, the specificity of the antibody being unchanged. Accordingly, it is assumed that the method of the labelling of KOMUKAI employed here is well suited to the aim of tracer experiment.

Radioactivity of $^{131}$I labelled $\gamma$-globulin of anti-spleen serum obtained by this method was less than 20 $\mu$C, influence of which on organism can be neglected when injected in dogs, and this is the ideal dosis that can be determined as tracer. Although the iodination was carried out using constant amount of original serum under constant conditions and method, radioactivity of the obtained $^{131}$I labelled $\gamma$-globulin solution was not always similar. Accordingly, it is meaningless to discuss the accumulation of radioactivity in tissues from the comparison of the counts per minute. PRESSMAN$^{36,37}$ examined localization of $^{131}$I labelled anti-organ antibody in the corresponding organs from the ratio of the counts per minute in the tissue and those in the blood. He maintained that this tissue/blood ratio represents the exact value which is not interfered by the differences of injection amount or body weight, and he pointed out that this ratio has its significance during a few days after the injection since radioactivity of blood decreases more than 95 per cent within 7 days. Based on these assertions, localization of anti-spleen antibody was comparatively studied with tissue/blood ratio in the present experiment.

When $^{131}$I labelled $\gamma$-globulin of anti-spleen serum was injected in dogs, localization of the antibody could be obviously observed in the spleen and liver at the determination performed 6, 24 and 48 hours after the injection compared with the occasion of control injection of $^{131}$I labelled $\gamma$-globulin of normal rabbit serum. In addition, the amounts of anti-spleen antibody localized in each 1 g of the spleen and liver were approximately similar. Significant localization of the labelled antibody could not be demonstrated in other organs. These findings are interpreted to indicate that immunological shock was given to the spleen and liver selectively and in the same degree by the injection of anti-spleen serum. PATRASSI$^{40}$ insisted that the liver and spleen are the organs from the same genetic origin and
these two respond similarly against various stimulations, and he postulated a concept of assoziertes splenohepatisches Syndrom. Furthermore, he maintained that disease picture of Banti’s disease in the early stadium is nothing but that of primäre hyperplastische Milzpulritis being accompanied by hepatitis. SUZUKI also asserted that the spleen and liver should be considered to constitute a unit of mesenchymal tissue and it is acceptable that these two similarly show mesenchymal reaction against an identical stimulation.

In the present experiment, localization of anti-spleen antibody was histologically pursued using autoradiography, and marked accumulation of the antibody was demonstrated on the wall of the splenic sinuses and trabecular veins in the spleen and in the liver it was demonstrated around the Glisson’s sheaths, central veins, on the wall of the fine tributaries of the intrahepatic portal venous system and sublobular veins. It is questionable if these findings of the spleen has some direct association with splenitic changes observed by MIYATA. However, in the liver, although MIYATA could not grasp some morphological changes attributable to the cause of portal hypertension, above described accumulation of the antibody in the liver might be accepted to suggest that elevation of portal vein observed in the present experiment was caused by functional changes due to antigen-antibody reaction in the area of the fine venous system such as around the Glisson’s sheaths, central veins, fine tributaries of the intrahepatic portal venous system and sublobular veins. IT0 reported that hemangitic changes take place initially in the small vessels of the spleen and liver in cases of Banti’s disease. IMANAGA also insisted that Banti’s disease can be comprehended as a lesion in the vascular system. Moreover, SUZUKI could observe neither obstructive changes in the hepatic and extrahepatic portal veins nor common changes of the liver attributable to the use of portal hypertension in his experimental portal hypertension produced by protracted sensitization of rabbits with egg white albumin. He presumed the functional mechanism of portal hypertension due to antigen-antibody reaction in the intrahepatic venous system such as the fine tributaries of the portal vein or sinusoids.

In the present experiment, portal hypertension could be produced by impairing mainly the spleen and liver in the process of reverse allergy with the injection of anti-spleen serum and localization of the antibody could be demonstrated by the use of labelled anti-spleen serum and autoradiography. Although the present experiment was designed to use such an experimental procedure of intravenous injection of anti-spleen serum, it is presumable that the disease process like this develops in organisms. In this respect, the findings of the present experiment are accepted to suggest the possibility of allergic etiology of so-called Banti’s disease and some mechanism of portal hypertension observed in this disease.

V. SUMMARY

Anti-dog-spleen rabbit serum, produced by immunization of rabbits with the antigen of dog spleen homogenate, was injected intravenously in dogs, and changes of portal pressure was studied. Moreover, a tracer experiment was carried out using anti-spleen antibody labelled with radioactive in order to investigate the target tissue of the antibody and its localization was pursued by the method of autoradiography. The obtained results are summarized as follows.
1) Portal pressure was measured 5 days after the intravenous injection of anti-spleen serum of 1 or 2 cc/kg body weight of dogs. Slight elevation of portal pressure could be observed, which, however, gradually descended thereafter to restore to normal 20 days after the injection.

2) When the injection of anti-spleen serum was repeated, elevation of portal pressure was more pronounced in 5 cases out of 6, the degree of the elevation being 45 mmH₂O to 72 mmH₂O.

3) It was demonstrated that the target site of the anti-spleen serum is the spleen and liver, by the method of antibody labelling with radioactive ¹³¹I.

4) Marked accumulation of the anti-spleen antibody could be demonstrated, by autoradiography, on the wall of the splenic sinuses and trabecular veins in the spleen, and in the liver it was demonstrated around the Glisson’s sheaths and in the wall of the central veins, fine tributaries of the intrahepatic portal vein and sublobular veins.

5) From these findings of autoradiogram, any association of localization of the antibody with splenitic changes observed in the present experiment could not be ascertained. However, accumulation of the antibody in the portal and hepatic venous systems within the liver makes it possible to presume that the observed portal hypertension was caused by some functional changes in this area owing to antigen-antibody reaction, since there could not be found in the liver any morphological changes attributable to the cause of portal hypertension.

6) It is assumed that the findings of the present experiment reveal some suggestions on the etiology of so-called Banti’s disease and mechanism of portal hypertension observed in this disease.

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VI. REFERENCES


LOCALIZATION OF ANTIBODY IN EXPERIMENTAL PORTAL HYPERTENSION 1327


