THROMBOCYTOPEN: A CONFIRMATORY REPORT

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Ever since the announcement by Troland and Lee (1) in 1938 of a platelet-reducing substance in the spleens of patients with idiopathic thrombocytopenic purpura, numerous efforts have been made to confirm their findings. Troland and Lee (2) were able to repeat their work at a later date with the same results. Their charts depicting the fall in platelets following the intravenous injection of acetone extracts of spleens from patients with thrombocytopenic purpura into rabbits, a cat, a dog, and a monkey, and the negligible diminution in platelets following injection of a similar extract of normal spleens are indeed impressive. Confirming this initial work, we have been able to find only one article, that of Hobson and Witts (3). Here the decreases in numbers of the circulating platelets were not so great as those of Troland and Lee, but still great enough to seem conclusive.

Far more numerous are those who have failed to demonstrate the presence of thrombocytopen, using a similar experimental technique. Among these are Pohle and Meyer (4), Major and Weber (5), Hodge and Strong (6), Tocantins (7), and Moore (8). Torrioli and Puddu (9) showed that extracts (not acetone) of spleens from patients with thrombocytopenic purpura applied in highly concentrated doses injure the megakaryocytes in cultures *in vitro* of the bone marrow of guinea pigs. They also found that similar extracts of normal spleens possessed like properties, but to a lesser degree. Torrioli and Pusic (10) showed that aqueous extracts of normal spleens, when given to rabbits in large intravenous doses, caused a reduction in circulating platelets. Torrioli and Puddu (9) point out that Troland and Lee in their work used spleens from patients with blood dyscrasias, congenital hemolytic jaundice and Banti's disease. Troland and Lee also used splenic tissue from a patient who died of cardiac failure and from one whose spleen was removed because of a stab wound.

Our interest in this controversial subject was stimulated by the opportunity of obtaining at op-

eration the spleen from a patient whom we had followed for over a year and whose history and findings are summarized below. The other patient with thrombocytopenic purpura died before this work was undertaken, but fortunately the spleen, which had been obtained at autopsy, had been ground up and placed in acetone for possible future examination. The spleens used as controls were taken at autopsy from a patient who died of multiple myeloma and from one who died of moniliasis of the lungs with pulmonary hemorrhage. Platelet counts were done on both of these patients before death and were found to be normal.

Following is a summary of the cases used in this paper:

Case 1, M. M., was a 22-year-old white American college student who was first seen on August 17th, 1938, complaining of bruising easily for the past 8 years. Her family and past histories were not contributory. Her present illness consisted of easy bruising, sometimes without known trauma, severe nose-bleeds, profuse menstrual flow, occasional oozing gums, and profuse bleeding from small cuts over this 8-year period. There was no history of the use of any drugs during this time. There had been no previous treatment prior to entry here except for a diet high in Vitamin C, which gave no relief.

On physical examination, temperature was 36.6° C., pulse 96, respirations 20, and blood pressure 112/58. She was a well developed and well nourished young woman in no distress. Color was good. Several petechiae were present on the feet. There were numerous ecchymotic areas under the tongue and on her body, the largest ones being on her left leg, warm, tender, with raised indurated central portions. The spleen and liver were not felt at the initial examination, but later were palpable. Examination was otherwise not remarkable.

Laboratory work included the following findings: RBC 5,180,000 per cmm., Hgb. 85% (S), WBC 6,720 per cmm., with 67% polymorphonuclear neutrophils, 30% lymphocytes, and 3% eosinophiles; platelets varied between 78,000 per cmm. and "2000 red cells were counted and no platelets found." Ivy bleeding time varied between 13 minutes and over 49 minutes. Clotting time (Lee-White) varied between 5 and 7 minutes. Clot retraction was incomplete at 24 hours. Urine, stool, and blood Wassermann were all negative.

For various reasons splenectomy was not done until

October 10th, 1939. The operation was uneventful and by the 6th post-operative day her platelet count had reached a high point of 1,996,800 per cmm., falling gradually thereafter to around a 500,000 per cmm. level. No further bleeding occurred.

Sections of the spleen, which weighed 116 grams, showed the sinuses to be irregularly dilated and the tissue to be infiltrated with well preserved red blood cells. The lymphoid follicles appeared normal, most of them having germinal centers. Around many of them were rings of extravasated red blood cells. The tissue contained considerable brown pigment. Sections of a small accessory spleen showed similar structure.

One hundred grams of ground spleen were used for preparation of the extract to be injected into rabbits.

Case 2, S. N., was a 65-year-old Japanese farmer complaining of bleeding gums for 3 weeks. The family and past histories were not contributory. He had always been well up to the onset of the present illness which started 3 weeks before entry with bleeding from the gums followed by purpuric spots and ecchymoses all over the body, especially over the extremities. There was also oozing from the nose, and on one occasion bleeding for a few minutes from the left ear. Except for slight weakness he felt quite well. He denied taking any drugs prior to the onset of his illness and had had no subsequent treatment except a Vitamin C preparation and yeast.

On physical examination, temperature was 37° C., pulse was 88, respirations 22, and blood pressure 110/74. He looked well. There were fresh and regressing petechiae and ecchymoses all over the skin surface, especially the extremities. The gums were spongy and oozing blood. The cheeks, lips, and tongue were also oozing blood. The edge of the spleen was palpable 3 to 4 cm. below the left costal border. The liver was not felt. Examination was otherwise not remarkable.

The laboratory work included the following findings: RBC 4,450,000 per cmm., Hgb. 80% (S), WBC 9,500 per cmm. with 67% polymorphonuclear neutrophils, and 30% lymphocytes; platelets varied between 80,750 per cmm. and 14,000 per cmm., reticulocytes were 0.6% of erythrocytes. Bleeding time was 10 minutes. Clot retraction was extremely poor at 24 hours. Urine and blood Wassermann were negative. Stool was strongly positive for occult blood (Guaiac).

This patient's course in the hospital was steadily downhill, and in spite of repeated transfusions he died following a large hematemeses 9 days after admission.

After autopsy the anatomic diagnosis was as follows: "Purpura with hemorrhage, stomach, fatal. Pneumonia, bronchial. Pericarditis, subacute, mild. Tuberculosis, lungs, apical, healed with pleuritis, chronic, adhesive. Hyperplasia of prostate, nodular, with hypertrophy of bladder. Arteriosclerosis, general, mild. Gastritis, subacute." The spleen weighed 60 grams. The capsule was markedly wrinkled and of a slate gray color. On cut section, it was softer than normal and the markings were moderately prominent. On microscopic examination, the malpighian bodies were poorly outlined. The trabeculae

and capsule were moderately thickened. The pulp was almost devoid of red blood cells. It was highly cellular. Many of the cells were large and could be identified as reticulum cells. Others were morphologically myeloid cells. One section showed a considerable deposit of golden brown granular pigment.

Marrow from a rib, the sternum, and a vertebral body was moderately hyperplastic, with abundant elements of both the erythrocytic and myelocytic series. Megakaryocytes were slightly increased in counts from smears. Differential count of hematopoietic cells was as follows: Primitive blasts 1%, myelocytes and metamyelocytes 76.5%, polymorphonuclear neutrophils 2%, eosinophils 1.5%, endothelial cells 1%, plasma cells 1.5%, normoblasts 16%, megakaryocytes 0.5%.

Sixty grams of ground spleen were used for preparation of the extract to be injected into rabbits.

Case 3 (Control), L. L., was a 16-year-old Chinese schoolgirl who had been followed in this hospital for 11 years because of recurrent bouts of bronchopneumonia and hypochromic microcytic anemia. Guinea pigs inoculated with the patient's sputum showed lesions from which a *Monilia* was isolated. Her last entry on January 6th, 1940, was because of hemoptysis for 24 hours before entry.

On physical examination the temperature was 40° C., the pulse 140, the respirations 64, and the blood pressure 118/45. She was acutely ill, cyanotic, semi-stuporous, and bringing up moderate amounts of bright red blood. Other than this, positive findings were confined to the chest, both lungs being full of coarse râles anteriorly and posteriorly. The liver and spleen were not felt.

Laboratory work was as follows: RBC 4,400,000 per cmm., Hgb. 64% (S), WBC 27,200 per cmm. with 90% polymorphonuclear neutrophils, and 8% lymphocytes; platelets were 312,000 per cmm. No urine was obtained. Stool was negative for occult blood.

Death occurred 3 hours after entry.

The autopsy diagnosis was: Hemorrhage, lung recent, with (a) Hemorrhage, lung old; (b) Siderosis, lung, marked; (c) Fibrosis, lung, marked; (d) Carnification, lung; (e) Pneumonia, bronchial, mild; (f) Fibrosis, lymph node, peribronchial; (g) Siderosis, lymph node, peribronchial; (h) Emphysema, lung. Arteriosclerosis, generalized, mild. Cyst, ovary, unilocular.

The spleen weighed 175 grams. The surface was smooth and the capsule finely wrinkled. On section the pulp was soft but not mushy and could not be scraped off with a knife. The tissue was everywhere dark red save for multiple and well defined malpighian bodies measuring between 1 to 2 mm. in diameter. On microscopic examination, the capsule and trabeculae were thin. There was no fibrosis of parenchymal tissue. The malpighian bodies were large. A few of the sinuses were dilated and engorged with blood; most, however, were collapsed and there were large lakes of coagulated edema fluid as well as much recent hemorrhage in the intersinusoidal spaces of the pulp. Many small brown pigment granules

were scattered about. Polymorphonuclear neutrophils were not frequent.

Bone marrow from the sternum showed about 20 to 30 per cent of the marrow substances to be replaced by fat. Normoblasts and clusters of erythrocytes were common. Myelocytes and megakaryocytes were abundant.

One hundred grams of ground spleen were used for preparation of the extract to be injected into rabbits.

Case 4 (Control), J. McA., was a 73-year-old white farmer who complained of a lump on the breast bone for 16 months and a sore right shoulder for 6 months.

On physical examination, the essential findings were a blood pressure of 186/104, moderate arteriosclerotic changes in the retinal and peripheral vessels, a tender, firm swelling at the upper end of the right humerus, and a firm rubbery mass over and attached to the upper sternum.

Laboratory work was as follows: RBC 4,000,000 per cmm., Hgb. 80% (S), WBC 9000 per cmm. with 75% polymorphonuclear neutrophils and 20% lymphocytes; platelets were 346,000 per cmm. Urine showed Bence-Jones protein. Stool showed no occult blood. Bone survey showed multiple areas of bone destruction.

The patient's course was steadily downhill. Autopsy diagnosis was multiple myeloma, plasma cell type—sternum, clavicle, skull, humerus, ribs, vertebrae, ilia.

The spleen weighed 125 grams and had a smooth, slightly wrinkled capsule. On section, the tissue was soft, dark reddish purple, with the pulp scraping away fairly readily with the knife edge. On microscopic examination, there were large areas of red blood cell extravasation. The follicles were small and without germinal centers.

Sections of the bone marrow free of tumor were normal save for a rather high number of normoblasts.

One hundred grams of ground spleen were used for preparation of the extract to be injected into rabbits.

METHODS

In preparing the extracts from these spleens the same procedure was used throughout, with one exception noted below. The spleens were removed direct from the operating room or morgue and immediately ground by running once through a kitchen meat grinder. The grindings were weighed and placed in five volumes of reagent acetone. The mixture was then placed in the ice-box, was frequently shaken, and was allowed to remain there for a minimum of 3 weeks. The acetone from the spleen of Case 1 was distilled off by heat in a water bath. Evaporation was used with the remaining three. The resulting brownish, sticky residue was then shaken thoroughly with 100 cc. of distilled water and the resulting suspension filtered first through coarse filter paper and then through a Seitz filter into a sterile suction flask.

Male rabbits weighing between 2 and 2.5 kilos were used for injection, a new rabbit being used for each injection. Prior to injection platelet counts were done at varying intervals for 3 to 5 days. This was done both

for purposes of standardization and also to ascertain whether there was any marked difference in the count at different times of day or night. We found none. The direct method of Rees and Ecker was used, and each count was checked in a separate counting-chamber. Both chambers were thoroughly searched for clumping, and if any was found the count was repeated. Pipettes were shaken in a mechanical shaker for 10 minutes and the platelets allowed to settle in the counting-chamber for another 10 minutes before the count was started. The diluting solution was passed through a Seitz filter every 24 hours to exclude the possibility of any dust particles or bacteria confusing the count.

FIG. 1. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M. WHO HAD IDIOPATHIC THROMBOCYTOPENIC PURPURA

First rabbit, injected by R.

FIG. 2. INJECTION OF SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M.

Second rabbit, injected by B.

Twenty cubic centimeters of the aqueous solution prepared as stated above were injected into the marginal ear vein of a rabbit after a final control count had been done. These control counts and the counts done at intervals of 5 hours thereafter are recorded in the accompanying charts.

FIG. 3. INJECTION OF THIRD SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M.
Third rabbit, injected by R.

FIG. 4. INJECTION OF FOURTH SAMPLE OF SPLENIC EXTRACT OF PATIENT M. M.
Fourth rabbit, injected by B.

RESULTS

In all the rabbits injected with extract from spleens of thrombopenic purpura patients there was a distinct drop in platelets. Many control counts before injection indicate that these drops are significant. Extracts from control cases uniformly failed to produce any definite fall in the platelet count.

We have almost nothing to add to the comments already set forth in preceding papers. We have carefully studied the published case reports and can find nothing to explain why the platelet reducing substance is sometimes found and more often not. Almost all of the reports concern cases which were undoubtedly instances of idiopathic thrombocytopenic purpura. None of the abstracts of the pathological findings gives any clues as to this discrepancy. The technique used by the different workers was too similar to permit any explanation along this line. Why we failed to obtain any marked drop in the platelet level at 5 hours, as did Troland and Lee, but for the most part

FIG. 5. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT S. N., WHO HAD IDIOPATHIC THROMBOCYTOPENIC PURPURA

Fifth rabbit injected by R.

FIG. 6. INJECTION OF SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT S. N.

Sixth rabbit injected by B.

obtained slower falls and rises, adds still another unanswered question.

SUMMARY

1. Spleens from two patients with idiopathic thrombocytopenic purpura and from two control patients with no evidence of this disease were extracted according to the method of Troland and Lee.

2. The splenic extracts were injected into healthy young male rabbits, the platelets of which

had been counted and repeatedly checked beforehand.

3. In the rabbits injected with extracts from the spleens of the patients with idiopathic thrombocytopenic purpura, the platelets dropped markedly in number, whereas in rabbits injected with control extracts there was no appreciable drop.

4. We consider these data as confirmatory of the work of Troland and Lee.

FIG. 7. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT L. L., WHO DIED OF MONILIASIS AND HAD NO EVIDENCE OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

Seventh rabbit, injected by R.

FIG. 8. INJECTION OF SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT L. L.

Eighth rabbit, injected by B.

FIG. 9. INJECTION OF FIRST SAMPLE OF SPLENIC EXTRACT OF PATIENT J. MCA., WHO DIED OF MULTIPLE MYELOMA AND HAD NO EVIDENCE OF IDIOPATHIC THROMBOCYTOPENIC PURPURA

Ninth rabbit, injected by R.

FIG. 10. INJECTION OF A SECOND SAMPLE OF SPLENIC EXTRACT OF PATIENT J. MCA.

Tenth rabbit, injected by B.

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