THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Ernest Marshall Johnstone final oral examination for the degree of Master of Science in Surgery. We recommend that the degree of Master of Science in Surgery be conferred upon the candidate.

Minneapolis, Minnesota

May 23 1921

F. C. Mann
Chairman

H. Z. Giffin

C. H. Sanford

Conrad Jacobson.

REPORT

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Ernest Marshall Johnstone for the degree of Master of Science in Surgery. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Surgery.

Frank C. Mann
Chairman

H. Z. Giffin

A. H. Sanford

Conrad Jacobson

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THESIS

A STUDY OF THE RELATION OF THE MARROW TO THE SPLEEN

by

Ernest Marshall Johnstone,
B.S., M.D.

Submitted in partial fulfillment of the requirements for the degree of Master of Science in Surgery in June, 1921.

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INTRODUCTION

Statement of Purpose and Scope of Marrow Study.

In this series of bone marrow investigations, I have had a two-fold purpose; first, to devise a new method of marrow study that would secure a connected picture of marrow changes during the whole course of a disease; second, to pursue such observations of the marrow in connection with post-splenectomy anemia because this blood condition is now a definite and well-defined clinical entity and offers a peculiarly good opportunity for a study of the interrelationships of the hemopoietic organs, particularly the relation of the spleen to the marrow.

Hitherto most observations of marrow function have been limited to single isolated specimens usually secured at autopsy or by etherizing an experimental animal in the laboratory. A single, usually terminal specimen of bone marrow is insufficient evidence. An adequate conception of the behavior of the marrow can be secured only by frequent observations (on the same person or laboratory animal) at regular intervals and over a period of time sufficiently long to give a connected record of marrow changes throughout the whole course of disease. In connection with this problem of technic, one of the most difficult factors is adequate experimental control. I have, therefore, given special attention to the development of a system of rigid experimental control.

In detail the schedule for this investigation of the marrow

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and its relation to the spleen involved the collection of data in such a form as would allow analysis and comparison of ten factors. Of these, four referred to the marrow: namely, erythrogenesis, leukogenesis, marrow vascularity, and density of marrow cells. Four referred to the blood stream: namely, the hemoglobin content, the erythrocyte count, the leukocyte count and a differential study of blood cells. And two factors referred to the experimental animal in a general way; namely the weight at regular intervals and parallel notes on the animal's general condition, such as the healing of the operative wounds and the absence or presence of any influences which might affect the marrow in even a minor way.

All data were collected simultaneously just before taking each specimen of marrow. This parallelism of observations not only justified comparison of data, but also contributed to a much more comprehensive knowledge of what occurs after removal of the spleen than could be secured by studying the marrow alone.

It should be stated at the beginning that my reason for undertaking this marrow study and for giving special attention to the early period after splenectomy is the fact that previous investigators, notably Pearce and Krumbhaar,(1) have reported practically negative findings for the first six months following removal of the spleen. They did, however, note definite marrow changes six months to a year or a year and a half after splenectomy. Their study of the marrow after splenectomy still constitutes the most complete contribution on the subject.

The fact that the most severe stage of anemia and much of the recovery therefrom occurs during the first six months after the removal of the spleen would naturally lead us to expect corresponding marrow changes during the same period. Pearce and Krumbhaar, failing to find such marrow changes during the first six months concluded that the marrow changes observed

later on could not be considered compensatory to an anemia which had already cleared up. These investigators stated their reasoning as follows(2): "If the hyperplasia of the bone marrow is compensatory to increased blood distruction or decreased blood formation one would expect definite hyperplasia during the earlier period, during the first three months after splenectomy at a time when the anemia is evident and repair is taking place and not six months to a year and a year and a half when the blood picture is normal----------it is, therefore, impossible, on account of the late development of the hyperplasis of the marrow, to explain its occurence as compensatory to the anemia following splenectomy."

It is evident that the function of the marrow the first few months after splenectomy is the crux of the problem. If during this period there is hyperplasia and hyperactivity then post-splenectomy anemia is due to no fault of the marrow but is due, rather, to some destructive factor, possibly a hemolysin, which is set into activity by removal of the spleen. This question of the function of the marrow, especially during the period immediately following splenectomy, is of sufficient importance to warrant further investigation. Therefore, I have ventured to continue the study of this problem by devising a new method of marrow investigation and giving more stress to the accomplishment of rigid experimental control.

I wish to acknowledge with great pleasure and appreciation the wise counsel and inspiring personal influence of Dr. F.C. Mann in whose laboratories in the Division of Experimental Surgery and Pathology of the Mayo Clinic, these marrow investigations were conducted. I also gratefully include my thanks to Dr. H.Z. Giffin, Miss Winifred Ashby and Dr. D.C. Balfour, for valuable criticisms and suggestions in connection with the work.

II.

Methods of Experiments.

Five series of experiments were carried through: Series 1, 2, 3 and 4 failed to accomplish exact experimental control. Brief reference, however, will be made to these experimental studies of the first four series of experiments, because they bring out certain points which are important when studied in conjunction with the findings of Series 5. The new method of experimental control for the fifth series of experiments proved satisfactory and the larger part of this report is, therefore, a detailed record of its findings.

Series 1. Studies of the tibia marrow of twenty-five rabbits (thirteen splenectomized and twelve non-splenectomized), covering periods up to fourteen months after splenectomy. In this series the marrow of a splenectomized rabbit was controlled by that of another non-splenectomized rabbit.

Series 2. Study of the femur marrow(central third) of eight splenectomized and six non-splenectomized dogs for a period of over one year after splenectomy. In this series also, the splenectomized animal's marrow was controlled by the non-splenectomized animal's marrow.

Series 3. Study of two goats, of same age, one splenectomized, when three weeks old, and one non-splenectomized, for a period of fifteen months after splenectomy. This series, like 1 and 2, was controlled by a comparison of different animals, the non-splenectomized goat being a control for the splenectomized one.

Series 4. Study of the femur marrows of three splenectomized and three non-splenectomized dogs. In this series each animal's femur marrow (taken from the central third of the left femur at the end of the experiment) was

compared with the animal's own femur marrow taken as a control from the right femur before splenectomy.

Series 5.(a). A study of rib marrow taken at frequent intervals from eight splenectomized dogs and six non-splenectomized dogs operated under ether anesthesia, and employing sterile technic.

Series 5.(b). A study of two splenectomized and two non-splenectomized dog's rib marrow in exactly the same manner as Series 5 (a) except that cocaine anesthesia was used instead of ether.

In this series, (Series 5), a piece of rib $1\frac{1}{2}$ inches long was removed from a point midway between spine and sternum. This specimen, removed before splenectomy, from a healthy and apparently normal dog, constituted the control for subsequent pieces of rib removed from the same animal at regular intervals after splenectomy. The six dogs, not splenectomized and used as additional controls, had exactly the same treatment including the rib resections and abdominal incision except that having delivered the spleen outside the abdomen and having exerted traction and trauma such as was estimated to equal that of splenectomy, the spleen was returned to the abdomen and the wound closed. Thus each specimen of marrow was controlled by a specimen from the same animal before the splenectomy and also controlled by a similar series of rib sections from non-splenectomized animals. It was presumed that specimens removed from similar parts of the ribs (all midway between spine and sternum) would be comparable and test examination of many specimens has confirmed that the marrow from the same relative position of several ribs, is very similar in cell character and activity.

By this method as many as nine specimens of rib marrow from a single animal were secured at intervals of two weeks to several months during a total period of fifty-four weeks observation. Thus in Dog D 32, rib No. 1,

removed before splenectomy, was the control. Rib 2, showed a change two weeks after splenectomy; rib 3, was removed five weeks after splenectomy, rib 4, nine weeks, rib 5, seventeen weeks; rib 6, twenty-nine weeks; rib 7, forty-one weeks, rib 8, fifty-four weeks after splenectomy. In connection with this experiment another non-splenectomized dog was put through exactly the same routine rib resections with the same intervals between resections as an additional control.

Additional data.

1. Before each operation the blood was examined to get the white blood cell count, and the hemoglobin estimation.

2. Similarly before each operation a record was made of the animal's general condition, the healing of former wounds, and the weight.

3. In three experiments, of Series 5 (a) blood smears were also made at frequent intervals to check the types of blood cells and compare them with the cells seen in the marrow of the same animals.

4. At the end of the experiment the animals were etherized and an autopsy done to insure that they were not affected by disease or any factor except absence of the spleen.

Rejected Animals.

Great care was taken to rule out any animals where a complication might introduce an additional factor affecting the marrow. In general, the wounds healed without infection. However, infection did rule out a few animals, especially rabbits. Of a series of thirty-nine rabbits, fourteen were excluded from consideration due to wound infection, "snuffles", pregnancy, etc. The thirteen splenectomized and twelve non-splenectomized rabbits retained, represent therefore, a carefully regulated experiment.

Dogs were much less liable to complications and fewer had to be excluded. Empyemia, pregnancy, a suppurative cystitis, of unknown cause, were cases which required the rejection of three animals. In a few cases the experiments were continued in animals where a very slight and superficial stitch infection occurred. In these cases the presence of such a factor was noted down and clearly marked on the charts where due weight may be attributed to the condition. However, these wounds healed readily and if they did constitue a significant influence on the marrow function it could only be for a brief period of time, and probably at the most, no more than one marrow specimen was affected.

Diet.

The animals were all kept on a uniform diet throughout the experiment except two animals who became so thin and anemic at one period shortly after splenectomy that they were given meat and milk in addition to the regular diet for a short time.

Autopsy.

None of the animals finally retained as satisfactory for marrow study, died due to sickness of any sort. When etherized, every animal except Dog D, 376, appeared to be as healthy as when the experiments began. (This animal (D 376), appeared perfectly healthy up to May 12th, when he suddenly died. (Autopsy showed acute memingitis). Marrow specimens up to rib specimen number five, taken on April 8th, were retained because the animal was healthy for a month after these specimens were taken. The final marrow specimen was not included in the report because it was probably affected by the terminal febrile infection).

At autopsy not only were numerous specimens of femur and rib marrow taken, but also lymph nodes and hemolymph nodes were preserved

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for study. A general search was made for any pathological condition, special attention being given to the chest. In no case was there any sign of empyema or even pleurisy adhesions.

Control.

Thus, in addition to a very rigid system of double control, every effort was made to reject any animals which developed a complication which might act as a subsidiary influence upon the bone marrow. Finally a separate series was studied using cocaine anesthesia in order to investigate whether the anaesthesic is a complicating factor.

III.

Method of Preparing Rib Marrow Sections for Microscopic Study.

Stage 1. Securing Specimen. A piece of rib approximately $1\frac{1}{2}$ inches long is removed midway between the spine and sternum. It is advisable to remove a subsequent specimen of rib marrow from the opposite side of the chest, or at least one or two ribs distant from the previous site of rib resection, in order to insure that there is no local inflammatory reaction persisting in the region from which it is proposed to remove a new marrow specimen.

Stage 2. Preparation of Specimen. The rib section is held very firmly against a block of wood and is cut into five pieces with a sharp, fine saw, taking care not to squeeze out the marrow or injure it, except at the site of actual cutting. The pieces from each end of the specimen are thrown away because at these points the marrow cells are crushed out of their normal relationships, by the bone forceps during removal of the rib specimen. Three pieces of rib approximately 1/3" long are thus secured for fixation and sectioning.

Stage 3. Several methods were tried before developing the following method of preparing satisfactory rib marrow sections. I wish to acknowledge the very efficient services of Miss P.L. Whitney of the Mayo Foundation Laboratories in perfecting the method of fixation, decalcification, sectioning and staining marrow.

a. Place piece of rib in Zenkers solution (without glacial acetic acid) for twenty-four hours.

b. Wash in running water for twenty-four hours.

c. Place in 5% nitric acid and change daily until block softens enough to cut. This requires from three to seven days. Rarely

a 10% solution is necessary in case of a rib with very thick hard bone cortex.

d. Wash in running water for twenty-four hours. An attempt to facilitate the process of washing out the acid by use of sodium bicarbonate solution was made, but the sections were invariably "hazy" or "muddy" and the prolonged use of plain tap water was found to produce much clearer and generally more satisfactory sections.

Place specimens in ---

e. 80% alcohol for twenty-four hours.

f. 95% alcohol for twelve hours.

g. Absolute alcohol for three hours.

h. Equal parts of absolute alcohol and cedar oil for one hour.

i. Cedar oil for one hour.

j. Chloroform (two changes) for one hour

k. Parts of chloroform and paraffin for one hour.

l. Paraffin (two changes) for two hours.

m. Imbed.

Stage 4. Sectioning.

Sections should be made uniformly thick; (in this study, sections were made seven microns in thickness. Uniformity of thickness very much facilitated comparison of different specimens.)

Stage 5. Staining.

a. Run sections through toluol and graded alcohol to water as follows: (about two minutes in each solution)

Toluol (two changes)

Absolute alcohol(two changes)

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95% alcohol

70% alcohol

50% alcohol

Water (distilled)

b. Stain for about five minutes in 5% eosin. (This period varies practice only will discover the length of time needed. The tendency is to over-stain with eosin.)

c. Wash through two waters.

d. Stain with polychrome methylene blue for two minutes (Good-pasture's)

e. 95% alcohol (two changes) for about two minutes each. (Here check up stain with microscope; if too blue return to 95% alcohol; if not blue enough, return to water and then restain for an additional period in polychrome methylene blue.)

f. Absolute alcohol about two minutes.

g. Xylol or toluol about two minutes.

Stage 6. Mount with Canada Balsam.

IV.

Study of Types of Blood Cells Paralleling Study of Marrow Cells in the Same Animal.

In a series of three animals a study was made of the type of cells in the blood to parallel the study of marrow cells in the same animal. Two dogs, D 230 and D 232, splenectomized animals, had blood smears taken both before and after splenectomy, every three to five days for a period of about two weeks after splenectomy. Similar blood smears were made from D 233, a non-splenectomized dog; which was a rib marrow control animal.

In my study of blood cells seen after splenectomy for Kala Azar (3), I noted great numbers of immature leukocytes of the "horse shoe" nucleus type in the blood stream the first week following removal of the spleen. These cells were interpreted to be intermediates between metamyelocytes or mature leukocytes and their premature release into the blood stream was considered to be a result of splenectomy. In conjunction with the present marrow study after splenectomy I was, therefore, particularly interested to note if the same reaction followed removal of the spleen in normal dogs. Careful differential study of blood cells during the first two weeks after splenectomy showed only a very few such immature cells in the blood stream. A very few such cells were found in the blood stream of non-splenectomized animals and the finding of a slightly greater number in the blood of splenectomized dogs seems to be of no significance.

In two animals, D 232 and D 233, a definite lymphocytosis was seen but this condition was only temporary and apparently had no relation to splenectomy because one was a splenectomized and one a non-splenectomized animal.

Only in one animal, D 233, a non-splenectomized dog, was

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there seen any marked number of eosinophiles. Neither of the two splenectomized dogs showed an abnormal number of eosinophiles, at least in this early period after splenectomy.

In none of the animals were nucleated red blood cells seen.

The series was too small (three animals) and the period of study after splenectomy too brief (two weeks) to constitue a comprehensive study of parallel blood and marrow cells. The findings, however, agree with what one would expect to find in the blood in conjunction with a known marrow activity.

V.

Histology of the Bone Marrow.

I agree, in all main respects, with the admirable description of marrow cell groups by Pearce and Krumbhaar(4), whose groupings were a modification of those previously made by C.H. Bunting.(5).

Marrow cell groups are usually mixed erythrogenic and leukogenic. It is very rare to see a purely erythrogenic or purely leukogenic group. But it is very common to observe groups of marrow cells where erythrogenesis or leukogenesis so largely predominates as to justify the description of the group as essentially one or the other.

After a close study of the function of femur marrow and rib marrow of the same animal I have been convinced that leukogenesis is more active in the rib. Leukogenesis occurs in both marrows but the rib marrow is the main factory of leukocytes.

The rib marrow quickly shows changes in marrow activity. It also reveals small degrees of marrow change and is therefore well adapted for marrow study. The central third of the femur marrow, on the other hand, is tardy in showing changes in marrow activity. Only after quite marked changes in the marrow function can we detect a modification of the marrow of the central third of the femur. For these reasons the rib marrow is a better index than the femur marrow.

The marrow of the dog was not observed to be an active seat of lymphocyte production. Because certain observers, notably Gulland and Goodall, (6,7,8,9,)describe very active lymphogenesis in some marrows, I made a special study of this matter in the following manner: Specimens of spleen, lymph glands, hemolymph glands and numerous rib specimens were taken from the same animal.

Then direct comparison of similarly stained specimens was made between a known lymphocyte series of cells in the spleen and glands with lymphocyte-like cells in the marrow, the micrometer being frequently used to exactly compare the relative sizes. By such a direct comparison of cells in specimens arranged under adjacent microscopes, I was convinced that a few lymphocytes do occur in the marrow and are apparently generated there. A significant degree of lymphogenesis was observed, however, in isolated marrow specimens of only two animals. It appears that lymphogenesis can occur in the marrow, and to a slight degree, may be common but a noticeably active lymphogenesis is rare.

One very interesting phase of the histology of the marrow is the method by which the rib marrow increases its capacity. This increase is accomplished by several changes which may operate separately or all together. The earliest and most common method is for marrow cells to replace the fat cells, which are abundant in a sluggish marrow. If there is a continued demand for hyperplasia and hyperactivity of the rib marrow beyond what can be met by simple replacement of fat cells by marrow cells, then an enlargement of the rib marrow cavity is produced by a thinning of the bone cortex and the bony septae which divide the rib cavity into compartments. This process consists not only of a general thinning of the bone cortex of the rib but also a "honey-combing" by small islands of marrow cells which become larger and larger, as hyperplasia increases. During the experiments it was observed that when specimens were prepared from rib resections soon after splenectomy it was necessary to use a sharp saw and cut through quite a hard thick bone cortex--but as hyperplasia more and more increased in the rib marrow the rib specimens became changed so that even macroscopically, the thinning of the bone was evident, and at times it was even possible to cut the rib sections with a knife instead of a saw.

As the rib marrow space was thus increased in size there were simultaneous changes in the character of the marrow cells therein. In a sluggish marrow most of the cells are primite cells, myeloblasts, and myelocytes, with only an occasional normoblast, megaloblast, or metamyelocyte. But as the marrow becomesactive there is a greater preponderance of these intermediate forms. Great numbers of megaloblasts and normoblasts are interpreted to mean active erythrogenesis. Numerous metamylocytes fringed by more and more mature leukocytes are likewise interpreted as a sign of active leukogenesis. When a series of eight or nine marrow specimens, taken at regular intervals during a year or more after splenectomy are studied under the microscope so as to trace the marrow changes step by step (using a specimen taken before splenectomy as a control) it is of fascinating interest to observe how wonderfully nature accomplishes hyperplasia and hyperactivity at the same time. (Microphotographs numbers 1,2, 3, 4, pages 18 and 19, illustrate this process.)

It should be admitted that we have no proof that numerous intermediate cells like normoblasts and metamyelocytes are absolute indices of increased marrow function. As pointed out by Ashby, (10), there is a possibility that there is a slowing up of the rate of development and a more tardy release of these intermediate cells from the marrow into the blood stream.

All the evidence we have, however, supports the hypothesis that numerous normoblasts and megaloblasts are indications of active erythrogenesis because meanwhile, as shown by concurrent study of the blood, the red blood cells count simultaneously increases. Also there is a very significant parallelism of a high leukocytosis of the blood stream with the presence of enormous numbers of metamyelocytes, etc., which we interpret as evidence of increased leukogenesis. This subject is dealt with more fully in the concluding discussion.

A verbal description of the histology of the marrow is not very satisfactory. I have, therefore, prepared the drawings and microphotographs of actual marrow cell groups to illustrate the histology and function of the marrow.

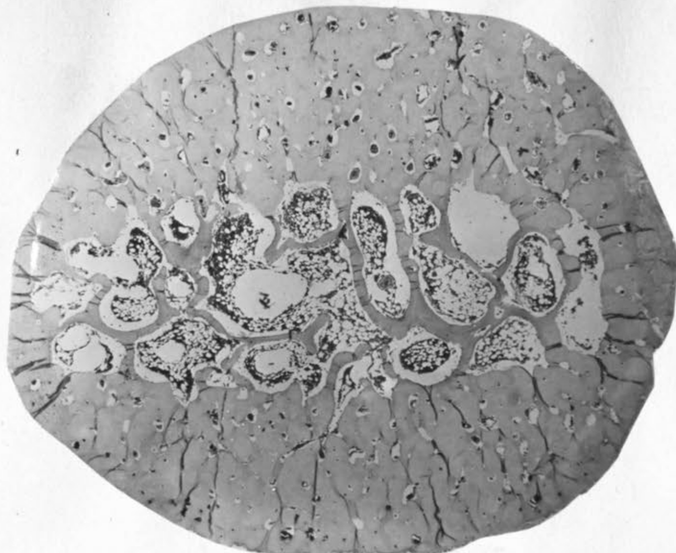


Fig. 1. 208 X18. A type of sluggish marrow. Note large amount of fat, thick bone cortex, and thick bony septae. There are numerous small islands of marrow cells honey-combing the bone cortex. Should the marrow become active, these small spaces will enlarge and become filled with active, dense groups of marrow cells, thus thinning out the bone cortex and adding greatly to the total quantity of marrow. Associated also with such an increase in activity there will be a decrease in the fat now seen to be abundant in the central islands of marrow.

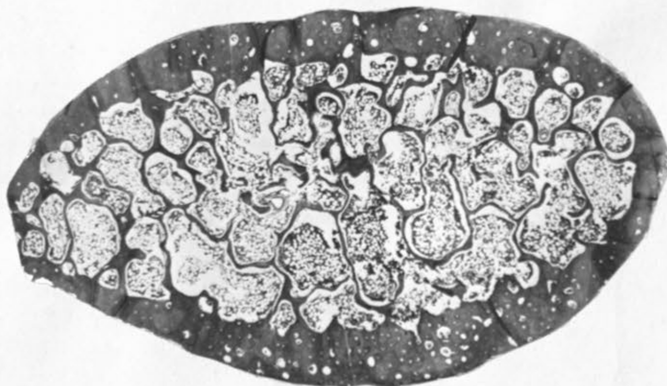


Fig. 2. 239 R_1. X18. Example of rib section showing moderately active marrow. Note large quantity of fat (white) with only a few marrow cells (black dots). The bone cortex (black), however, is very thin as also are the bony septae that separate the islands of marrow cells.

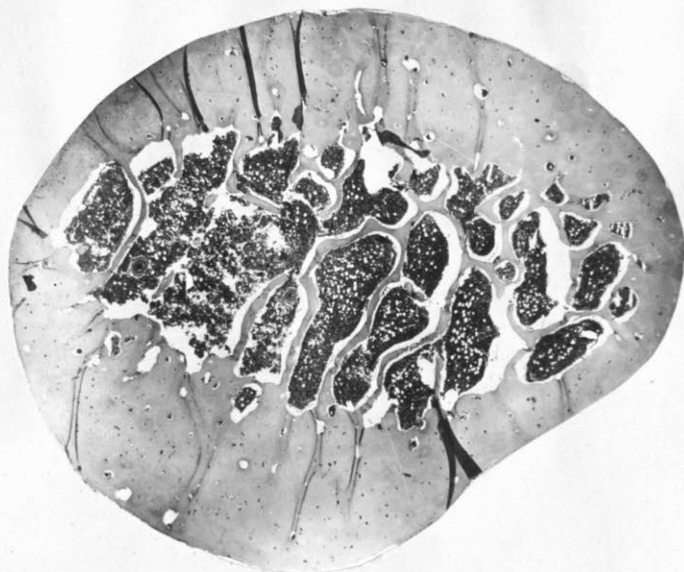


Fig. 3. 232 R5 18x. An example of very active rib marrow. Note the heavy black color of the marrow photograph (due mostly to numerous deeply staining nuceli of normoblasts and magaloblasts). There is still a sprinkling of fat cells (white dots) but marrow cells predominate. Islands of marrow cells are large and in places are seen to become confluent. There is still a thick bone cortex and this rib can yet greatly increase its capacity by a "honey-combing" of the bone and by progressively enlarging the islets of marrow cells (See Fig. 4).

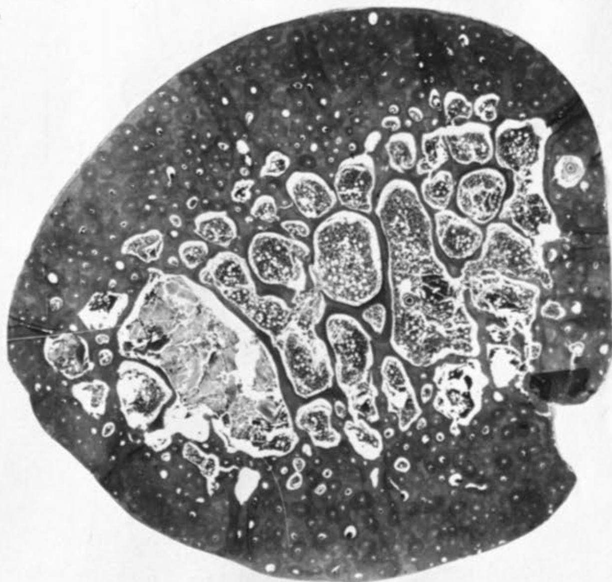


Fig. 4. 230 R_2 a 18x. Moderately active bone marrow showing how new islands of marrow cells are developing in the bone cortex which may become very thin in a very active rib marrow.

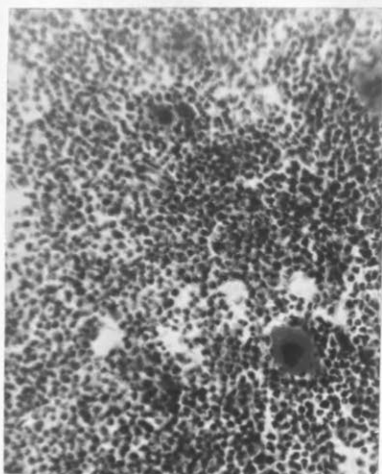


Fig. 5. 32 R_2 a X200. Dog D32. Low power picture of rib marrow two and one-half weeks after splenectomy. In places as here illustrated, the marrow cells are quite dense, but there are also large fields where the marrow consists largely of fat.

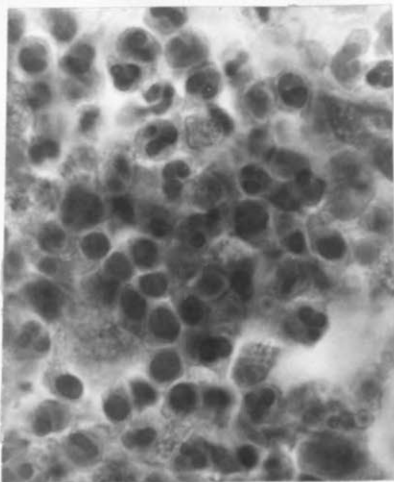


Fig. 6. 32 R_2a X1000. Dog D32. 1000 X magnification of marrow in rib 2, two and one-half weeks after splenectomy. Note active leukogenesis as shown mostly by rather advanced metamyelocytes.

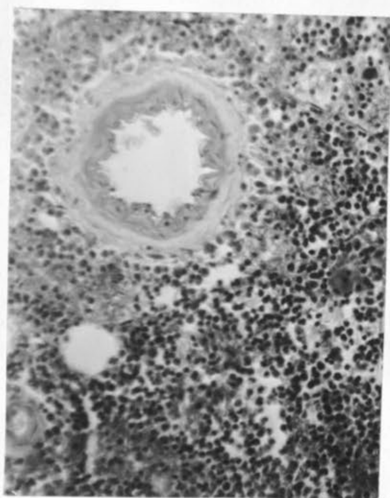


Fig. 7. 32 R_4a X200. Dog D32. Rib 4 taken nine weeks after splenectomy. Erythrogenesis Grade 2. Leukogenesis Grade $2\frac{1}{2}$. Note that only part of the marrow shows great activity, there being large spaces which are relatively inactive. Rib 5 (see Fig. 9) on the other hand shows a marrow active throughout its extent.

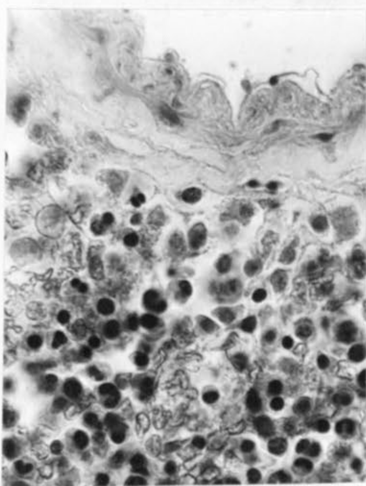


Fig. 8. 32 R_4a X600. Same as above with 600 magnification, showing mixed erythrogenesis and leukogenesis.

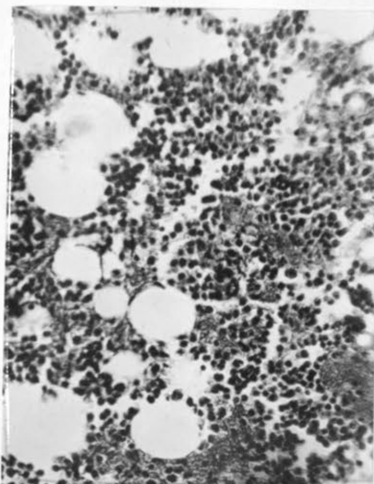


Fig. 9. 32 R_5a X200. Dog D32. Rib 5, seventeen weeks after splenectomy illustrates very active erythrogenesis. Erythrogenesis Grade $3\frac{1}{2}$. Note numerous normoblasts.

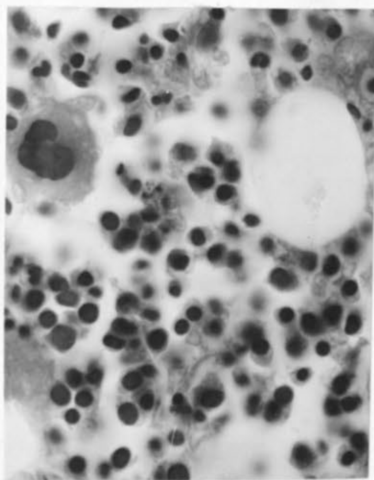


Fig. 10. R_5a X600. A rib (#5) marrow showing active erythrogenesis (almost pure erythrogenesis) from a dog splenectomized seventeen weeks before this marrow was taken. Erythrogenesis is here seen at its maximum degree of activity after splenectomy.

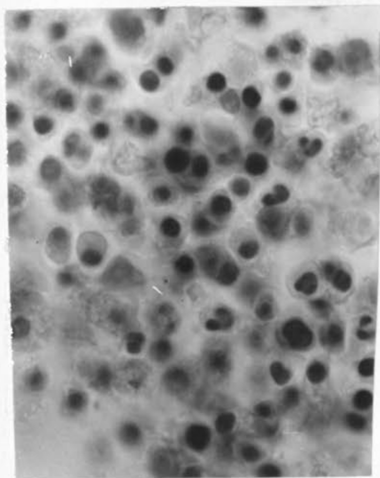


Fig. 11. 233 R_1:X1000. Mixed erythrogenesis and leukogenesis in a normal dog. This is the condition usually seen, pure erythrogenesis or pure leukogenesis being very rarely observed.

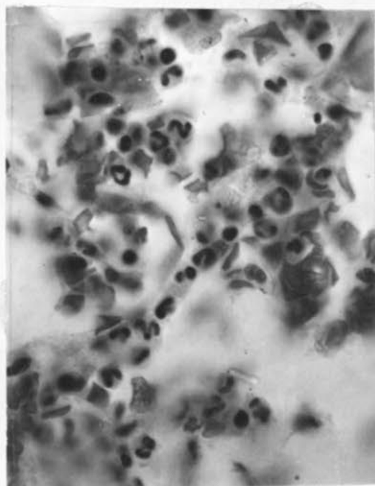


Fig. 12. 208 F a. X600. An example of very active leukogenesis in the femur. There are a few normoblasts but leukogenesis much predominates.

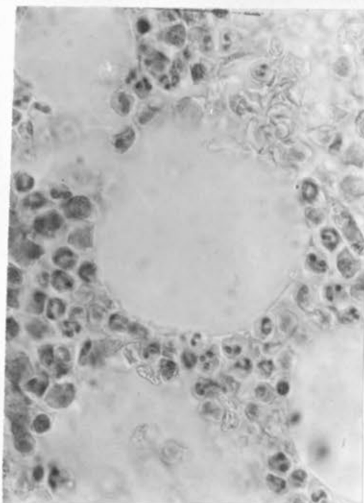


Fig. 13. 230 Rib 2a X600. Shows almost pure leukogenesis in a dog's rib taken two weeks after splenectomy. Myelocytes (showing very faintly), metamyelocytes and mature polymorphs are seen in the marrow. The primitive undifferentiated mother cells do not show well.

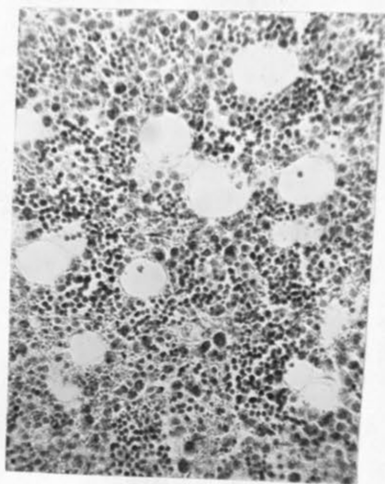


Fig. 14. Goat 82. Femur. X200. Femur marrow taken fifteen months after splenectomy. Comparison with marrow of control animal (Goat 79) shows this marrow to be the more active and marrow cells more dense.

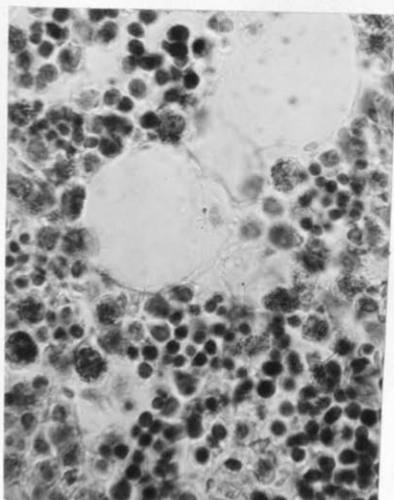


Fig. 15. Goat 82. Femur X600. Marrow of splenectomized Goat 82 (fifteen months after splenectomy). Note numerous eosinophilic myelocytes and active erythrogenic groups. Comparison of this with nonsplenectomized Goat 79 shows much more active marrow in the splenectomized animal.

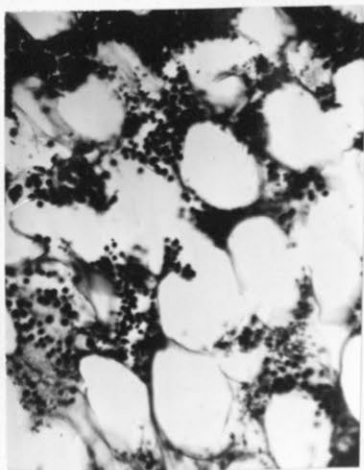


Fig. 16. Goat 79. Femur. X200. Femur marrow of a nonsplenectomized goat. Comparison with Goat 82 (splenectomized) shows this marrow to be less dense and less active.

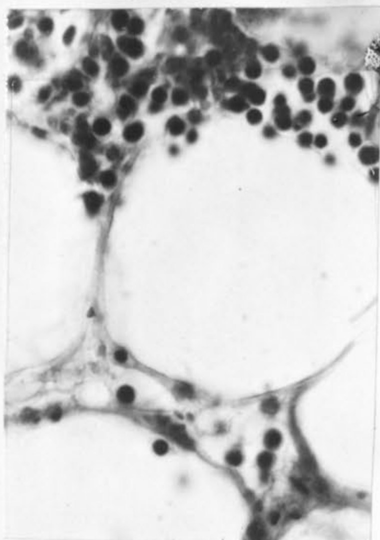


Fig. 17. Goat 79. Femur. X600. Femur marrow of nonsplenectomized Goat 79. Shows framework of marrow with large fat spaces and groups of erythrogenic marrow cells here and there in a relatively inactive marrow.

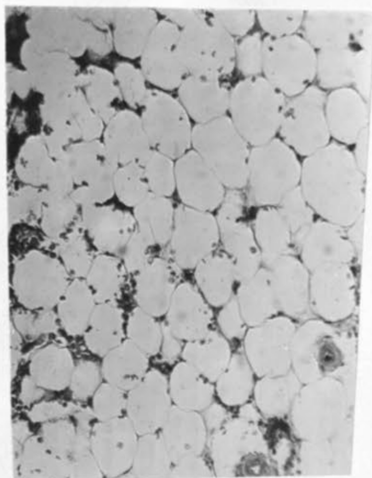


Fig. 18. 88 Femur. X100. Femur marrow of dog, showing very fatty marrow with groups of marrow cells scattered here and there; a very sluggish type of marrow.

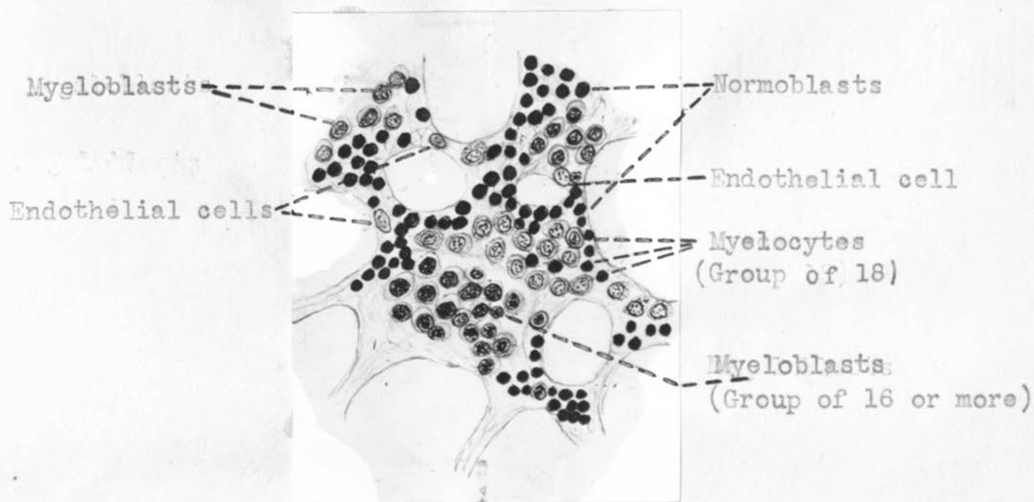


Fig. 19. Photograph of an original colored drawing of Goat's marrow. Goat 82 splenectomized fifteen months. Drawing shows a group of goat's marrow cells which is predominatingly erythrogenic. Note how small are the marrow cells of the goat in comparison with rabbits, dogs and human marrow cells.

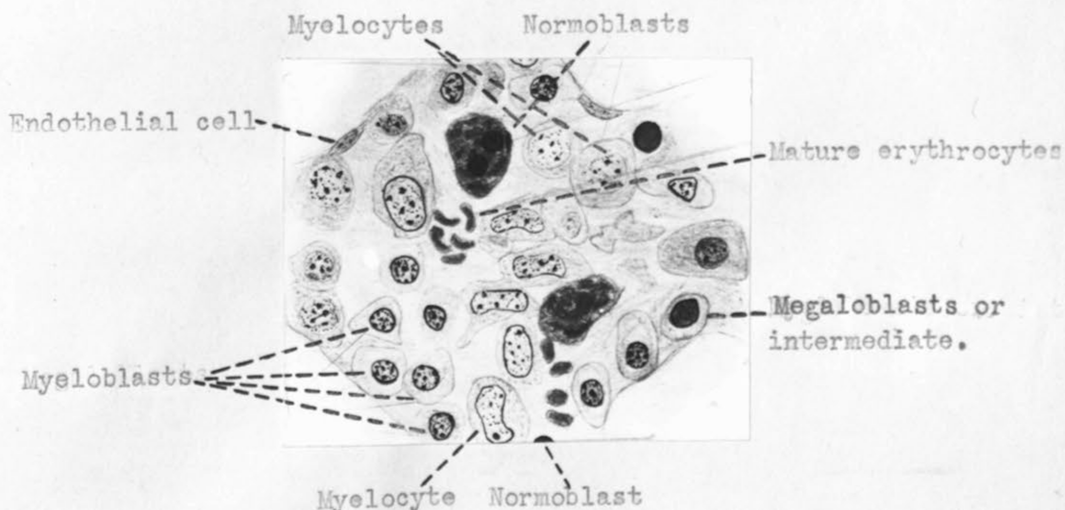


Fig. 20. Photograph of an original colored drawing of marrow cells from femur of a normal dog. 10X ocular, 1/12 objective. Scale 1 m.m.= 1 micron. Eosin and polychrome methylene blue.

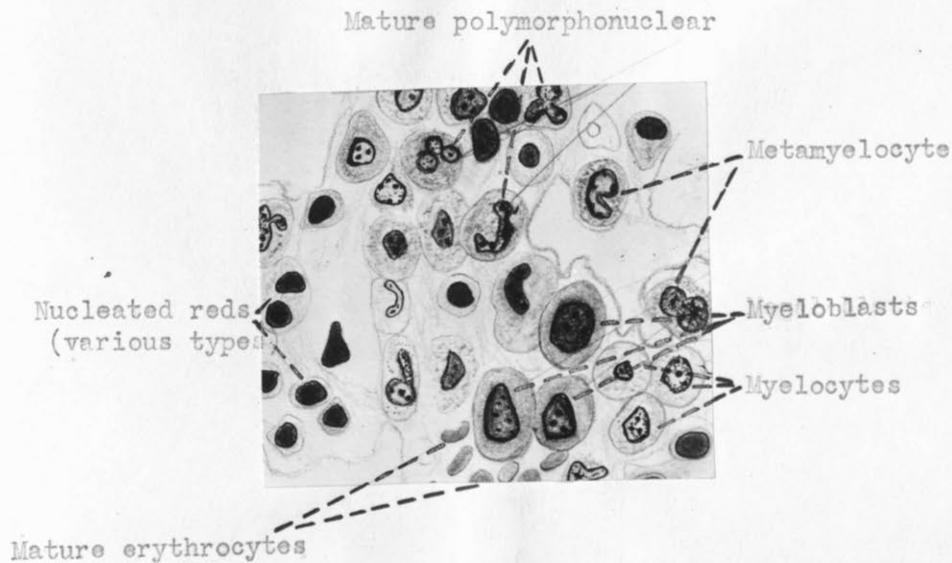


Fig. 21. Photograph of an original Drawing of group of marrow cells from normal rabbit. Rabbit 222. 10x ocular, 1/12 objective (immersion oil). Scale 1 m.m. = 1 micron. Eosin and hematoxylin stain.

VI.

Method of Making Records of Observations on Bone Marrow.

Observations involving a close microscopic study of the marrow with oil immersion lens, were essentially a process of comparison, pathological marrow being compared with normal, hyperactive marrow being compared with a normal or sluggish marrow, very vascular marrow being compared with relatively slightly vascular marrow, a marked density of marrow cells being numerically compared with a marrow containing a few scattered marrow cells amid masses of fat cells.

In order to compare and record marrow findings, the following system of grading activity, vascularity, marrow cell density, etc., was adopted. It is a purely arbitrary standard of recording data, but if the observations are all made by one investigator who carefully maintains the same grading throughout the study, the figures thus secured, furnish a convenient and accurate basis for comparison of different marrow specimens. Such a system of records also permits the plotting of curves representing marrow activity, etc., and it thus becomes possible to present the results of the experiments in a graphic form that is definite and readily comprehended.

Grades of Activity of Bone Marrow.

(a) Erythrogenesis.

Grade I, constitutes the most sluggish erythrogenesis seen. It consists of a field where most of the cells are primitive cells, myeloblasts, myelocytes, and fat cells along with a few scattered normoblasts and megaloblasts as the only evidence of red cell production.

Grade 4, on the contrary, indicates the most active type of erythrogenesis seen in any rib marrow.

Grades 2 and 3 are intermediate grades between 1, the most sluggish, and 4, the most active.

To get a clear conception of these grades requires a great deal of practice. However, by many tests, each time recording the grade estimated for that particular specimen and each test being made at such intervals that the examination can be made independent of any memory of a previous grading, it finally proved possible to grade specimens of marrow so that the gradings of the same specimen varied no more than one-half of one grade, even though the examinations were made weeks apart.

(b) Leukogenesis.

Gradings of leukogenesis are made in exactly the same manner as erythrogenesis.

(c) Marrow vascularity.

Grades 1,2,3, and 4 for marrow vascularity have the same relative significance. Grade 1 means a marrow with only a few small capillaries moderately filled with erythrocytes. Grade 4 signifies a marrow containing numerous capillaries, and sinuses congested with erythrocytes. Grades 2 and 3 are intermediates between Grade 1, the least vascular and grade 4 the most vascular.

(d) Marrow Cell Density.

Grade 1 of marrow cell density, signifies a rib marrow with small islands of marrow cells with considerable fat; the bone cortex is quite thick as also are the bony trabeculae and septae which divide the marrow compartments in the rib. Grade 4 on the other hand, signifies large islands of

dense marrow cells with little or no fat; often these large islands of marrow cells become confluentand the bony septae become fewer and thinner. The bone cortex also undergoes a change and is thinned out and honey-combed by numerous new small islands of marrow cells.

Grades 2 and 3 constitute intermediate grades.

(e) GeneralActivity of the Marrow.

General activity of the marrow is estimated by computing the mean of four factors, namely, erythrogenesis, leukogenesis, marrow vascularity, and density of marrow cells. Thus, for example, if in a given specimen of marrow, erythrogenesis is graded grade 3, leukogenesis, grade 2, vascularity, grade 2, and density of marrow cells, grade 3, the general activity would then be computed to be grade $2\frac{1}{2}$ by averaging the four grades.

(f) Explanation of Charts on this Basis of Grading.

Nine curves were made for each of the ten splenectomized and eight non-splenectomized dogs in this rib marrow study.

The nine curves plotted for each animal experiment form three groups: Group 1 consists of five curves (namely, erythrogenesis, leukogenesis, vascularity, marrow cell density, and general activity.) This group of curves, therefore indicates the condition of the marrow. Group 2,consists of three curves, the white cell count, the red cell count, and the hemoglobin estimation. These show the parallel blood findings. Group 3 consists of one curve and notes which give the animal's weight, general health and wound condition, and finally the autopsy findings, all being made to parallel the marrow and blood observations.

The charts are ruled perpendicularly into periods of one week intervals and points of time at which blood and marrow specimens, and so forth, were made are marked thereon. The horizontal lines denote the degree of activity or the quantity of the various factors being studied. (See Charts,pages 58-72 inclusive).

VII.

A Study of the Marrow of different Animals in order to determine whether the Marrow of one apparently normal healthy Animal can properly be used as a Control for another (Splenectomized) Animal.

Hitherto it has been customary to use one animal as a control for another animal in a study of the marrow. For instance, two dogs are taken; one is splenectomized and the other not. At the end of a certain period, say six months, the two animals are killed by ether, and their marrows (the central third of the femur marrow, for instance,) are compared. If the marrow of the splenectomized animal is much more active than that of the non-splenectomized animal, then it is presumed that splenectomy causes an increase in marrow activity.

In the first three series of my experiments I undertook to thus use one animal to control another, but certain observations of the marrow led me to suspect that apparently healthy and normal animals differ markedly. Therefore, I determined to make a special study of this question, and I have, I believe, clearly demonstrated that animals of the same species and even animals of the same litter, differ very much in respect to the character and activity of their marrow.

Series "A"

A comparison of the marrow from the center of the femur of ten healthy dogs--all approximately the same age, and all adapted to laboratory living conditions with a standard laboratory diet. Both gross and microscopic examinations were made of each specimen of femur marrow. The following is a brief summary of the findings. In this series the grades refer to the general activity of the marrow:

1. Dog No. D 213

Marrow red but only on the outer surface. The large central core is almost pure fat. Microscopic examination shows only a thin layer of marrow cells on the outer surface, with a few small groups of marrow cells scattered here and there in the fat. This marrow is graded Grade 1 to $1\frac{1}{2}$.

2. Dog No. D 208.

Marrow yellow and apparently almost pure fat at center of the femur. Microscopic examination showed only a few small groups of marrow cells. Marrow Graded grade 1.

3. Dog No. D 230.

Marrow dark brandy-red. Microscopic examination shows quite a thick outer layer of dense active marrow cells with "V" like projections of marrow cells into the central fat core. This marrow is graded Grade 2 or $2\frac{1}{2}$.

4. Dog No. D 207.

Marrow at center of femur is a reddish tinge but the large fat core beneath shines through the very thin mantle of marrow cells on the surface. Marrow graded $1\frac{1}{2}$.

5. Dog No. D 212.

Marrow same as No. D. 207.

6. Dog No. D 34.

Marrow dark brandy-red. Microscopic examination shows a good thick layer of active marrow cells on the outer surface. Marrow graded Grade 2.

7. Dog No. D 232.

Marrow has a reddish tinge but the yellow color of the fat predominates. There are only a few marrow cells here and there; hardly forming a thin mantle over the surface of the marrow. Marrow graded Grade 1.

8. Dog No. D 233.

Marrow deep red containing numerous fine spicules of bone. Microscopic examination shows dense groups of active marrow cells considerably predominating over the fat content of the marrow. Marrow graded Grade 3.

9. Dog No. D 294.

Marrow dark red. Cross section shows a thick layer of marrow cells about one-half the diameter of the marrow column. Microscopic examination shows dense groups of active marrow cells all through the marrow which is a very active one, and is graded, Grade 3 to 4. This marrow is an example of the most active seen in a large number of animals.

10. Dog No. D 301.

Marrow dark brandy-red color with a few yellow fat streaks faintly showing through the outer layer of dense marrow cells. Microscopic examination shows a thick layer of marrow cells which are active and a small central core of almost pure fat. Grade 2.

The marrow of five dogs was graded Grade 1 to 1½.

The marrow of three dogs was graded Grade 2 to 2½

The marrow of two dogs was graded Grade 3 to 3½.

Thus we note that in a series of ten dogs picked at random and all of them, so far as careful examination showed, healthy normal dogs, there is a very marked difference in the marrow of individual animals. It is apparent that one animal's marrow can not be used as a control for another in laboratory experiments.

When one considers that the ordinary type of dogs used for experimental purposes is of the most varied mongrel breed, it is not surprising that their marrow also varies in character.

Series"B".

A study of twelve rabbits, (all apparently normal healthy animals, and many of them from the same litter), to determine whether the tibia marrow is similar or dissimilar in individual rabbits. In this series, erythrogenic and leukogenic activity are separately graded in each rabbit's marrow.

1. Leukogenic activity.

The marrow of seven rabbits is grade 1.

The marrow of one rabbit is grade 1 to 2.

The marrow of two rabbits is grade 2 to 3.

The marrow of one rabbit is grade 3.

2. Erythrogenic activity.

The marrow of two rabbits is grade 1.

The marrow of six rabbits is grade 2.

The marrow of four rabbits is grade 2 to 3.

These findings of marrow activity in the tibia of twelve rabbits show how varied are the marrows of individual animals even though many are from the same litter and grew up under identical encironment conditions. It is also apparent that in the case of rabbits, it is unsafe to use one animal to control another in an experimental study of the marrow.

Series "C"

A similar study of the rib marrow in a series of fourteen dogs which were judged to be healthy and normal animals.The specimens of marrow were taken from a point on the rib midway between the spine and sternum.

1. Leukogenic activity.

The rib marrow of seven dogs is Grade 1 leukogenesis.

The rib marrow of two dogs is grade $1\frac{1}{2}$ leukogenesis.

The rib marrow of three dogs is grade 2 leukogenesis

The rib marrow of two dogs is grade $2\frac{1}{2}$

2. Erythrogenic activity.

The rib marrow of nine dogs is grade 1

The rib marrow of two dogs is grade $1\frac{1}{2}$

The rib marrow of three dogs is grade 2

This study of rib marrow shows that it would be an error to use one animal's rib marrow to control another's. Therefore in series 5 of the experiments, a preliminary rib marrow specimen was taken from each animal before splenectomy to act as a control for subsequent specimens of marrow from the same animal. Also non-splenectomized animals were run through the same routine of repeated rib resections in order to show how a normal dog's marrow varies from time to time and also to show the effect of ether or cocaine on the marrow.

VIII.

Detailed Summary of Findings.

A summary of the observations regarding the influence of splenectomy upon the bone marrow is essentially an analysis of the charts upon which the data has been concentrated. Due to the great length of the protocols only two, one relating to a splenectomized animal and one to a non-splenectomized control animal, have been appended as typical illustrations of the experimental proceedure and the manner of recording data. As regards the general observations on the histology of the marrow, I refer to that section in this report and will not attempt a comprehensive summary of the subject here.

(1). Changes in the Erythrogenic function of the Marrow after Removal of the Spleen of a normal Animal.

If we correlate the findings relating to the rib marrow changes in the six splenectomized animals of series V-a (operated under ether anaesthesia) the average erythrogenic reaction may be summarized as follows (see Table)

(a) There is, on the average, a latent period of one month before an increase in erythrogenic activity is observable.

(b) Increased erythrogenesis, thus noted to begin about one month after splenectomy, attains its maximum degree of activity during the second or third month.

(c) Maximum erythrogenic activity, first attained during the second or third month, persists practically unabated for about three months i.e. until the end of the fifth or sixth month after splenectomy.

(d) Thereafter the production of red blood cells decreases gradually until a period varying from seven months to a year or more after splenectomy, it reaches a more or less constant degree of activity which, however, is still considerably above normal i.e. above the degree of erythrogenesis observed in the marrow of the same animal in a specimen removed before splenectomy.

(e) Increased erythrogenesis is observable in the marrow a very considerable period, even a month or longer, before any reduction of post-splenectomy anemia is evident. This is graphically illustrated by the respective curves on the charts, the erythrogenesis curve rising a considerable period in advance of the hemoglobin and erythrocyte curves.

(f) A further comparison of erythrogenesis with other factors, which were studied at the same time, shows certain very interesting parallelisms of the curves on the charts. On the one hand, erythrogenesis, marrow vascularity, and marrow cell density simultaneously increase. On the other hand, the hemoglobin, red cell count, and the animals'weight form a group whose curves run a remarkable parallel course first downward (during the development of the anemia) and then upward until they reach a normal or above normal level.

(2) Changes in the Leukogenic function of the Marrow after removal of the Spleen of a normal Animal.

(a) Three of the six animals whose spleens were removed under ether anaesthesia, showed a spectacular increase in marrow leukogenesis. This reaction, occuring in only half the animals operated under ether anaesthesia, was immediate in occurence but only temporary in duration lasting usually two to five weeks after splenectomy.

If in conjunction with the above observations of the rib marrow (in specimens of marrow taken usually about two weeks after splenectomy

under ether anaesthesia) we also consider the leukogenic reaction noted in the tibia marrow of twelve splenectomized rabbits whose marrow specimens were taken a long period after the ether anaesthesia was used to remove the spleen, i.e. too long after the anaesthetic for any conceivable ether influence to persist, we are inclined to believe that splenectomy itself is a factor in causing increased marrow leukogenesis. The ether used as an anaesthetic for splenectomy also seems to be at least a contributing factor in causing the leukogenic reaction. This question of the real cause of the spectacular increase in leukogenic activity after splenectomy will be considered more fully in the discussion which follows.

(b) There is an exact parallelism of the high leukogenesis curve of the marrow findings and the concurrent high leukocytosis observed in the blood stream. (see charts of splenectomized animals D 32 and D 230 whose curves very graphically illustrate this point.)

(3). Marrow changes in Rabbits' Tibia Marrow following Splenectomy.

During the early months of this study of the marrow, I used one animal's marrow to control observations of marrow changes in another's. This method of marrow study proved unsatisfactory and a new system of control, was devised (see rib marrow studies of Series V.) The findings in this rabbit marrow study were charted and retained in this report, however, to illustrate two points. First, that there are very marked differences in the marrow of apparently healthy animals so that comparisons appear peculiar and conflicting in most respects. Second, that when the twelve splenectomized rabbits are compared with the thirteen non-splenectomized rabbits marrow there is seen to be, in the former, an increased leukogenesis during the first few months after splenectomy which is not evident in the control animals.

(4) Marrow changes in Dogs Femur Marrow following Splenectomy.

The method used in series IV of taking a control specimen from the center of the femur before splenectomy and comparing this with a final specimen of marrow taken from the other femur at a certain period after splenectomy also proved unsatisfactory because in the first place the single (final) specimen of marrow was often taken after the main marrow reaction had subsided (thus missing it) and also because the fatty marrow at the center of the femur proved to be only a tardy index of marrow changes. Thus, in every respect, the rib marrow studies proved the most satisfactory and the bulk of this report is therefore confined to the data furnished by rib marrow studies Series V.

(5) Marrow changes observed in the Control Animals.

The marrow of many non-splenectomized animals was studied at intervals over periods varying from six months to fifteen months. In these studies we have an opportunity to observe how the marrow of normal animals varies from time to time. There is a little up and down fluctuation of the curves (see charts illustrating erythrogenic and leukogenic activity in control animals) which suggests that hemopoiesis is somewhat periodic but in the main the curves run as constantly level and parallel as one could expect considering that the observations extended through different seasons of the year and included many minor and unavoidable variations of environment.

IX.

Discussion.

It has been clearly established that removal of the spleen, presumably a normal spleen, of a healthy animal is followed by a hyperplasia and hyperactivity of the marrow which begins early after splenectomy and persists for a long time--more than a year at least-after removal of the spleen. But this marrow reaction may not be primarily the result of absence of the spleen. It seems more probable that the marrow activity is secondary to the anemia, which follows removal of the normal spleen, and is compensatory to that anemia.

Conclusions regarding marrow changes, especially those which occur very soon after splenectomy, were made possibly only by comparing a series of successive marrow specimens with a control specimen taken from the same animal before splenectomy. In addition to this means of direct control it was necessary to observe a similar series of marrow specimens in a non-splenectomized control animal in order to know the fluctuations of marrow activity which are possible in a normal animal. With such a system of double control of the marrow it becomes possible to interpret marrow changes after splenectomy. Thus, when it is stated that erythrogenesis in a certain marrow specimen is "above normal " it is meant that such activity was above that observed in the same animal's marrow when that animal was in a normal healthy condition before removal of the spleen. Also the term "above normal", as shown by the marrow activity of the control animals studied over a long period of time, signifies a degree of activity which exceeds the natural variations which may occur from time to time in a normal, healthy (non-splenectomized) animal.

It is remarkable that a definitely increased erythrogenesis in the marrow may precede, even by several weeks, any signs of a reduction of

A SUMMARY OF THE CHARTS OF SIX SPLENECTOMIZED DOGS

SHOWING EFFECT OF SPLENECTOMY ON ERYTHROGENESIS IN THE MARROW

	D32	D230	C981	D207	D208	D979	Average
Latent period after splenectomy before an observable increase in erythrogenesis	5 to 9 weeks	2 to 4 weeks	2 to 4 weeks	5 to 9 weeks	8 to 12 weeks	0 to 2 weeks	About 1 month
Time after splenectomy when erythrogenesis reached its maximum activity	17 weeks	8 weeks	8 weeks	13 weeks	12 weeks	2 weeks	2 to 3 months
Period during which maximum erythrogenic activity persisted (approximate)	6 weeks	20 to 25 weeks at least	8 to 12 weeks	6 to 8 weeks	12 to 20 weeks	15 to 20 weeks	3 months
Time after splenectomy when erythrogenesis has decreased to a more or less constant degree of activity (but still somewhat more active than before splenectomy)	30 weeks	Still very active when etherized at 37th week	20 weeks	33 weeks	44 weeks	Still very active at 44th week	7 months to a year or more

CHART SHOWING AVERAGE REACTION OF RIB MARROW AFTER SPLENECTOMY

the anemia. It is of course logical that signs of increased production of red cells in the marrow should precede evidence of improvement in the blood, as cause should precede effect, but the fact that a very active erythrogenesis so long precedes a reduction of the anemia indicates that whatever destructive agent becomes active after removal of the spleen, is of so potent a nature as to be compensated only by prolonged hyperactivity and hyperplasia of the marrow.

The presence of a prompt and markedly increased marrow activity after splenectomy shows that post-splenectomy anemia is due to no fault of the marrow. The presumption is, rather, that some extra medullary factor, possibly a hemolysin, is the cause. Warthin(11) has suggested that after removal of the spleen there is excessive hemolysis in the lymph nodes which often hypertrophy. The spleen being a main site of antibody production, it is possible that after splenectomy, the ordinary hemolytic factors act unchecked and unregulated(12). Whatever the cause and mechanism of post-splenectomy anemia, it is overcome by an increased activity of the marrow. It is possible that the destructive factor(or absence of a normally present "favorable factor") gradually subsides or entirely disappears a few weeks after splenectomy so that the marrow has only to overcome temporary disadvantage. The increased resistance of the erythrocytes after splenectomy is also a factor which probably aids in recovery from post-splenectomy especially as this increased resistance improves with the length of time after operation(13, 14).

In order to be sure that I correctly interpreted the changes observed in the marrow, I carefully compared marrow changes with the blood changes to see if they harmonized. Every observation supported the hypothesis that the marrow is really hyperactive when it is very vascular, densely crowded

with marrow cells (the fat content having correspondingly decreased and the marrow spaces having enlarged) and particularly when the marrow is abundant in normoblasts, megaloblasts, and metamyelocytes. The fact that exactly as we observe these changes in the marrow, we find corresponding increases in the red and white cells of the blood stream is strong evidence that new blood cells are being rapidly manufactured somewhere--and presumably in the marrow. It occurred to me that the lymph nodes might be the site of active production of blood cells in the absence of the spleen and I therefore removed specimens of axillary and mesenteric lymph glands from each animal to investigate this point. While there were, occasionally, very noticeable signs of hyperplasia in the lymph nodes and in some germ centers there were collections of normoblasts which were at least very suggestive of moderately active hemopoiesis I could not see that any considerable amount of blood cell manufacture was going on; certainly nothing to compare with the extremely marked changes seen consistently in the marrow. The parallelism of marrow changes and blood stream changes is so exact that an intimate casual relationship seems certain. It may be objected that there is no real increase in blood cells but only a decreased destruction. But such an interpretation is not supported by what we see after removal of the normal spleen. All the evidence we have (so far as we can interpret it) points rather to an increased destruction of red cells after splenectomy. Splenectomy for hemolytic jaundice, however, is a striking example of a decreased destruction of cells after operation.

While I am convinced by numerous parallel observations of marrow and blood stream changes) that the above hypothesis is correct,i.e. that increased vascularity, increased marrow cell density, and particularly the presence of numerous groups of normoblasts, megaloblasts, and metamyelocytes and evidence of increased marrow function, I must admit that such marrow changes

might be explained, theoretically, on the basis of a heightened threshold of cell output (a delayed release of cells from the marrow into the blood stream) or by a retarding of the rate of marrow cell development, or both these factors combined to produce an accumulation of cells in the marrow as suggested to me by Miss Winifred Ashby.

My investigations have been limited to a study of marrow changes which follow removal of the spleen of healthy and apparently perfectly normal animals. What occurs in the marrow after removal of a diseased spleen or the spleen of animals affected by such disease as pernicous anemia, Banti's disease, Kala Azar, leukemia, etc., may be fundamentally different. If, for instance, in pernicious anemia splenectomy is followed by a long continued hyperplasia and erthrogenic hyperactivity such as is known to follow removal of the spleen of a normal animal, then we may have the explanation why some pernicious anemia patients have shown a definite and quite lasting improvement after splenectomy as reported recently by Giffin and Szlapka(15).

It is probable that the type of reaction following splenectomy depends on the condition of the animal whose spleen is removed. When we compare the results of splenectomy for various diseases we are also led to believe that there are several factors operating to bring about the complex changes. For instance, Wolferth (16) has shown that removal of the enlarged spleen of albino rats caused a rapid and usually fatal anemia, hyperleukocytosis, marked increase in the number of reticulated cells, and in two cases there was a distinct jaundice. He therefore, considered that in diseased rats (with enlarged spleens) the splenomegaly was compensatory for a damaged condition of the other hemopoietic organs. Thus, when the spleen was removed death often resulted because the other already damaged hemopoietic tissues were unable to compensate for the loss of the spleen.

The severe, often fatal anemia and especially the distinct jaundice observed in some of his cases, indicate an increased hemolysis after removal of such spleens. On the other hand, splenectomy for hemolytic jaundice is followed by an apparently decreased hemolysis.

A very important consideration in connection with splenectomy is the anemia, sometimes severe, which follows removal of the normal spleen. If such an anemia, even though only temporary in duration, also followed removal of the spleen in patients already suffering from a high grade anemia, thus adding, temporarily to the anemia, the result might be very serious. Fortunately, splenectomy in the presence of anemia diseases, does not seem to have such an effect, i.e. it does not add to the anemia but rather reduces it. Giffin states this observation as follows:(17) "the effect of splenectomy in all forms of disease in which splenomegaly is associated with a secondary type of anemia is that the anemia itself is less severe after splenectomy than it was before------". Here again we have evidence which seems to support the contention that removal of a diseased spleen, or the spleen of a diseased animal, is followed by a different reaction from that which results from removal of a normal spleen.

Having demonstrated that a highly increased erythrogenesis persists a long time, at least a year or more, after splenectomy and moreover that this hyperactivity persists even long after the post-splenectomy anemia has been thoroughly overcome, we are concerned to know why a marked polycythemia does not develop. It is true that in some animals the erythrocytes increased one to one and a half millions per c.m.m. above the blood condition observed when the animal was in a healthy and normal condition before removal of the spleen. But such a change is within the natural variations seen in the blood stream of apparently healthy animals. The failure of a persistent hyper-erythrogenesis to develop a marked polycythemia is possibly due to a persistent action of the

destructive factor which causes post-splenectomy anemia, the destructive and productive factors thus finally becoming balanced. If, on the other hand, we suppose that this destructive factor or lack of a normally present favorable factor is only temporary as it appears to be, then we are obliged to find another explanation. It may be that there is a regulatory mechanism of the hemopoietic system which, normally, prevents the accumulation of more than a certain density of red cells in the blood stream--as for instance, the sugar content of the blood is kept constant, under normal conditions, in spite of the amount of carbohydrate intake.

The question of the influence of ether on the marrow is a difficult one. I planned an attack on this problem by studing a series of four animals (two splenectomized and two non-splenectomized with repeated rib resections for marrow study) using cocaine anaesthesia throughout the experiments. These furnished a control for the larger series operated under ether anaesthesia. As regards erythrogenesis ether does not appear to be a significant factor. The splenectomized animals all showed increased erythrogenesis whether ether or cocaine was used. The non-splenectomized animals, equally subjected to ether and cocaine, failed to show any considerable increase of erythrogenesis. Thus, splenectomy, or its resultant anemia, is clearly the precursor (and apparent cause) of increased erythrogenesis. But when we study the relation of the anaesthetic to leukogenesis individual variations are so marked and a leukogenic reaction so inconstant that we doubt if a series of four animals operated under cocaine anaesthesia is a large enough series to cover ordinary variations (only half the dogs splenectomized under ether anaesthesia showed a marked leukogenic reaction). So far as my data goes, it appears that ether is a very important factor in stimulating leukogenesis. But the fact that none of the control (non-splenectomized) animals showed a considerable change in leukogenic activity

7-20 2M

although they were subjected to the same degree and length of ether anaesthesia as the splenectomized animals, is evidence that at least a brief ether anaesthesia is alone insufficient to affect the leukogenic function of the marrow. Mann(18) observed that a leukocytosis follows ether anaesthesia if maintained long enough, without removal of any organ (as the spleen for instance). He noted a leukocytosis usually appeared after three or four hours of ether anaesthesia. His study was limited to the blood changes however. The concurrent marrow function can only be inferred from the changes seen in the blood stream.

It should be pointed out that in my series of experiments no marrow observations were made earlier than two weeks after splenectomy and the use of an anesthetic.It is possible that this is too long after the time of stimulus to note the reaction. Further investigation is necessary to arrive at definite and clear cut conclusions regarding the effect of an anaesthetic on leukogenesis. Fortunately it is clear that the erythrogenic reaction is independent of the anaesthetic.

It has already been suggested that the marrow reaction which follows splenectomy may be really secondary to the anemia which is known to follow the removal of that organ. It is possible that hemorrhage or repeated bleedings can cause the same marrow changes as removal of the spleen. Drinker(18) Drinker, and Kreutzman, in their study of the influence of hemorrhage and infusion noted in their post-hemorrhagic cases, a hyperplasia of the marrow with extension of erythorcyte bearing tissue into the shafts of the longs bones and many islands showing erythrogenic activity.

Thus hyperplasia and hyperactivity of the marrow after splenectomy is probably a physiologic response to a demand for more blood cells and may have no direct relation to the absence of the spleen. Moreover the long persistence of this marrow reaction, a persistence of hyperfunction which far exceeds the duration of post-splenectomy anemia, may be likewise an expression of the pathologic law of regeneration in excess.

General Summary.

The principal findings regarding marrow changes after splenectomy may be summarized as follows:

Removal of the spleen of healthy animals is followed by a definite hyperplasia and hyperactivity of the marrow. Moreover these changes occur immediately or soon after splenectomy.

The most constant, the most permanent, and apparently the most important sequel of splenectomy is an increased activity of the erythrogenic function of the marrow. In some animals this occurs immediately after removal of the spleen. In others there may be a latent period of a few weeks. Maximum activity is attained usually about the end of the third month, and persists through the fourth, fifth, and even into the sixth month. Thereafter erythrogenesis subsides until a period varying from seven months to a year or more after splenectomy where it reaches a more or less constant level of activity which, however, is still considerably above normal.

It should be emphasized that such is the average reaction. There are marked individual differences which may be of great significance.

This increase of erythrogenesis is probably secondary and compensatory to the more or less severe anemia which follows splenectomy. Such prompt and vigorous erythrogenic activity shows, that post-splenectomy anemia is due to no essential fault of the marrow. On the contrary the anemia appears to be due to some destructive factor which becoming active after removal of the normal spleen is of so potent a nature as to be overcome only by prolonged and excessive compensatory activity of the marrow.

Leukogenic changes in the marrow after splenectomy are

inconstant and temporary. In some animals, splenectomized under ether anaesthesia, there is an immediate spectacular leukogenic activity, which quickly subsides.

Ether, when used as an anaesthetic to remove the spleen, appears to be at least a contributing factor in causing this leukogenic reaction in the marrow. The erythrogenic reaction is, however, independent of the anaesthetic used in splenectomy.

In conclusion I wish to emphasize that the findings regarding bone marrow changes after the removal of the normal spleen were obtained by a new method of marrow investigation which obtained accurate experimental control. This method also furnished a connected record of marrow conditions at regular intervals for a period of more than one year after removal of the spleen. It is hoped that such a procedure will prove applicable to further study of the marrow, especially of the changes which may occur after splenectomy for such diseases as pernicious anemia, Banti's disease, the leukemias, Kala Azar, and so forth, - diseases in which the hematopoeitic system appears to be mainly involved.

XI.
Protocols

The charts are really a condensed form of the protocols and the routine of the experiments has been fully discussed. Therefore, only two protocols will be summarized in order to give illustrations of typical procedures and records in Dogs D 32 (a splenectomized animal) and Dog D 239(a control animal). V means marrow vascularity, A, general activity, L, leukogenesis, E, erythrogenesis, and D marrow cell density; figures refer to grade.

D32. Adult mongrel terrior; wt. 9.2 kg; tan and white color; in good condition. 7-11-19, Hb. 80%; R.B.C. 8000000; W.B.C. 13050, animal in healthy condition. 7-14-19, splenectomy and removal of piece of rib right side; all operations done with aseptic technic, specimens of rib and spleen preserved in Zenker's fluid and sent to laboratory. Spleen weighed 22 gms. 7-28-19, W.B.C. 31900; R.B.C. 3736000, and Hb. 65%. 8-1-19, rib specimen number two removed, time, 12 minutes, ether anaesthesia. Dog in good condition. 4 pieces of rib sent to laboratory in Zenker's solution (without acetic acid). 8-12-19, Hb. 35%; R.B.C. 3296000; W.B.C. 14700. Dog appears to be in good condition except he is thin. Blood is thin and watery-wounds healed without infection. 8-13-19, rib marrow specimen number three removed from left side of chest, 8th rib, from point midway between spine and sternum. Pleura not injured; 3 pieces of rib sent to laboratory in Zenker's solution. 9-19-19, wounds healed clean; Animal is very thin; wt. 5.5 kg.; Hb. 40%; W.B.C. 20000; R.B.C. 4290000. Rib marrow specimen number one (control taken before splenectomy) slide "A" shows moderate leukogenic and erythrogenic function of about equal activity. Possibly leukogenesis predominates slightly. Note marked contrast between this specimen and those of rib two and rib three. 9-10-19, rib marrow specimen number four

removed. Pleura not injured. Time, 12 minutes (only very brief ether anaesthesia). 10-3-19 review of first four rib marrow specimens. Close comparison made several times both under low and high power of microscope. Rib one contains large islands of marrow cells and many erythrocytes. A few normoblasts indicate sluggish erythrogenesis. Leukogenesis is more active. Rib two, leukogenesis greatly predominates. Fairly numerous normoblasts suggest some erythrogenic activity. In rib two, marrow is definitely more active than that in rib one (control). Leukogenesis is very active. In rib three marrow (slide "A" is excellent for detail study--good clear stain), large islands of active marrow cells. Leukogenesis definitely predominates over erythrogenesis. In general rib three marrow is about the same as rib two. Rib four, slide "A", there are large islands of marrow cells showing active hemapoiesis. Leukogenesis still predominates over erythrogenesis. In this rib erythrogenesis is, however, definitely increased over rib two and three. Slide "B" (of rib four) shows the same condition. Erythrogenesis has increased but leukogenesis still predominates as in ribs two and three. 10-4-19, a second review of marrow specimens of ribs one to four with special bifocal microscope was made in Dr. MacCarty's laboratory (Notes and drawings omitted because findings essentially corroborated those of former studies). 11-4-19, wt. 7.8 kg., condition good. R.B.C. 5070000, W.B.C. 15800, HB. 50%. 11-5-19, rib marrow specimen number five removed. Pleura not injured, i.e not opened into the pleural cavity. 1-6-20, review of ribs one to five (close microscopic comparison). Rib 1 has small central cavity quite well filled with marrow cells. Bone cortex is thick with small islands of marrow cells "honey-combing" it. V-1, A-1, L-1, E-1, D-2. Rib one shows mostly myelocytes with only a few normoblasts, metamyelocytes etc. to indicate even a sluggish activity in producing blood cells. Rib two, general description same as rib one. Formula is V-2, A-3 to 4, L-3 to 4, E-1, D-2. Note tremendous leuko-

genic activity as contrasted to rib one. Rib three, bone cortex still thick but marrow cavity is almost solidly packed with marrow cells. V-2, A-2 to 3, L-2, E-1 to 2, D-2 to 3. Rib four, a marked contrast to rib one; marrow core is almost a solid mass of active marrow cells; bone septae few and thin; the marrow islands are almost all confluent making one solid core of marrow cells. V-3, A-3, L-2, to 3, E-2, D-2 to 3; note the definite increase in erythrogenesis which now is equal to leukogenesis. 1-16-20, rib five, examination of this shows an active dense mass of marrow cells; V-3, A-3, L-2, E-3 to 4, D-3 (three other slides of same marrow specimen all give same formula.) 1-28-20, wt. 8.3 kg; condition thin and rather listless, wounds healed without infection. Hb. 50%; R.B.C.3,610,000; W.B.C. 24300. Rib marrow specimen number six removed from a point midway between spine and sternum. 3-5-20, examination of rib six marrow: this marrow appears less active and there is quite a little fibrosis; animal very thin and listless(this animal showed a most profound post-splenectomy anemia). Direct comparison of rib five and rib six shows a decrease in activity of the marrow(query:-is marrow becoming exhausted in efforts to compensate the severe anemia?); V-3, A-2 to 3, L-2, E-2 to 3, D-2 to 3. 4-21-20, wt. 9.9 kg., condition good, Hb.58%, R.B.C.3800000, W.B.C. 28000. 4-22-20, rib specimen number seven removed. 5-19-20, examination of rib seven shows large, active and dense marrow cell islands. Bone cortex is still quite thick but it has been much "honeycombed" by small islands of marrow cells; V-3, A-3, L-3, E-3, D-3. Note active leukogenesis again in this marrow specimen. 7-13-20, wt. 9.4 kg., condition good, though anemic. Hb. 68%; R.B.C. 4790000(an improvement); W.B.C. 13950. 7-15-20, animal etherized and an immediate autopsy made; specimens of rib marrow (from numerous places all midway between spine and sternum), hemolymph nodes, mesenteric and axillary lymph nodes and liver sent to laboratory in Zenker's

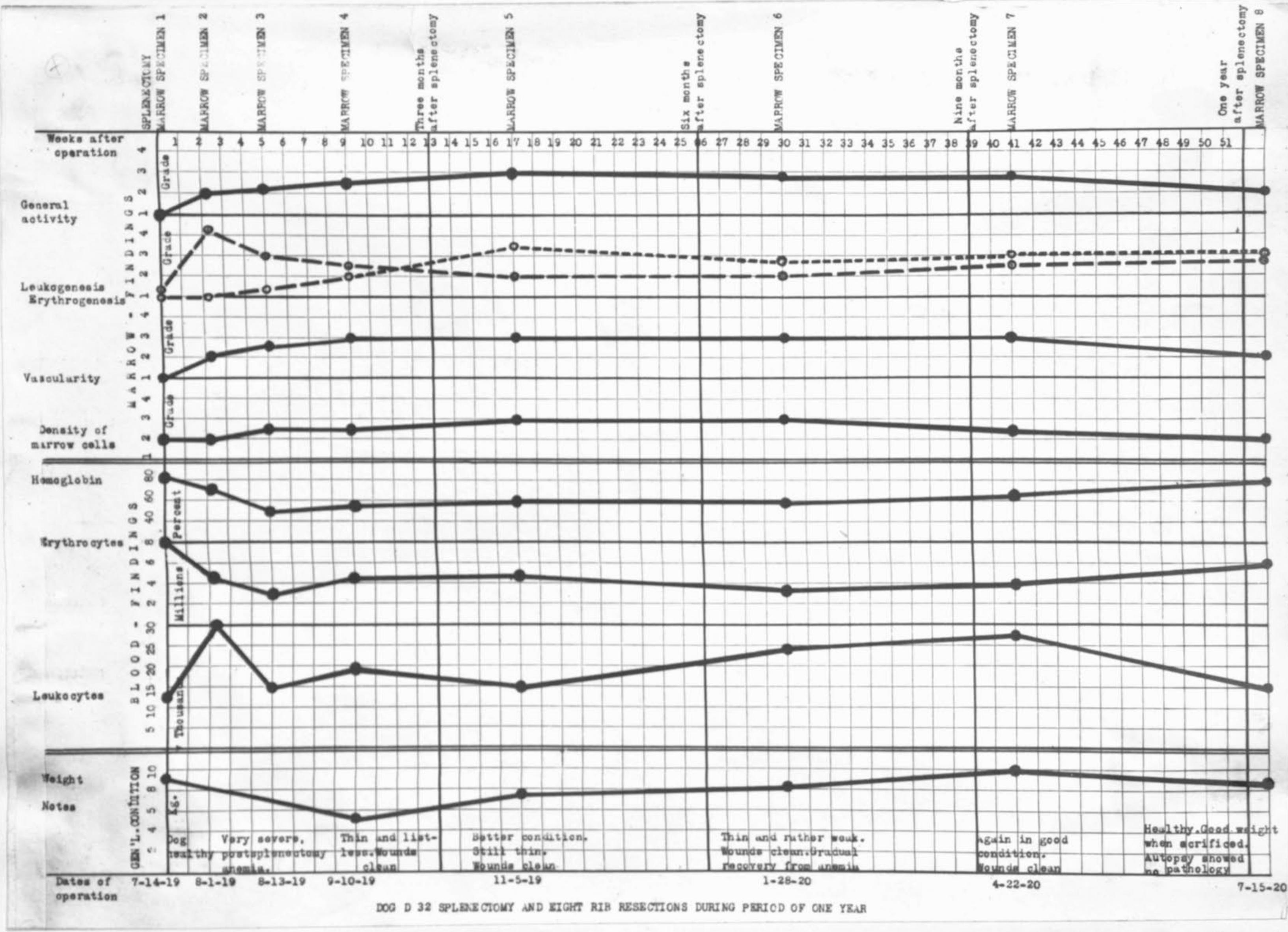
solution. Also took specimen of femur marrow (central). This is very fatty (droplets of oil). There seems to be a slight hypertrophy of hemolymph nodes, the largest being a 4 m.m. in diameter. Thorax free of adhesions and no sign of any infection; pleura shiny and smooth everywhere in spite of the numerous rib resections; no pathology discovered at autopsy; animal appeared to be healthy and entirely recovered from severe anemia. 11-19-20, rib eight (final) examination V-2, A-3, L-2 to 3, E-2 to 3, D-2. Final femur specimen shows on microscopic examination, a mixture of much fat with some fairly active marrow cell groups. Erythrogenesis predominates over leukogenesis.

Dog D239. Mongrel, black and white color, in good condition, wt. 24kg. 8-25-19, Hb. 92%; W.B.C. 20250; R.B.C. 7804000. 8-26-19, under ether anaesthesia incision through right rectus. Spleen pulled out of abdomen and traction and manipulation made to a degree estimated to equal the trauma of splenectomy. Organ then returned to abdominal cavity and wound closed. Also removed specimen of rib 1½ inches long from point midway between spine and sternum. Pleura not perforated. Chisseled open central third of right femur and removed a piece of femur marrow. 10-1-19, rib two specimen removed on left side of chest. Pleura not perforated. Time, 15 minutes. Dog thin but healthy. Former wounds all healed clean. 11-3-19, Examination of femur marrow shows much fat with small islands of marrow cells scattered here and there, mostly at outer edge of marrow. Rib one, marrow specimen (control) shows large islands of marrow cells which comprise about one half of the marrow (the rest being fat, etc.) Fairly active erythrogenesis predominates. Only slight leukogenesis. Rib two is not so active, erythrogenesis predominates, only sluggish leukogenesis, less active than rib one. 11-7-19, wt. 20.5 kg., condition thin but active and apparently healthy. R.B.C. 5640000; W.B.C. 11650; Hb. 70%. Dog put on special milk and meat diet because thin. 11-7-19, rib number three specimen removed from left side midway between spine and sternum. 12-9-19, wt. 25.9 kg., condition good, wounds clean, animal fat; Hb. 62%; R.B.C. 6170000; W.B.C. 20000. 12-10-19, rib resection number four. Dog in good condition. 1-21-20, rib marrow specimen number one shows large islands of marrow cells(not very dense) and considerable fat. Bone cortex fairly thick, V-2, A-2, L-2, E-1 to 2, D-2. Rib two same as general description of one except a little less active and less vascular. V-1, A-1, L-1, E-½, D-1 to 2. Rib number three shows much fat in marrow islands and cells are not dense(D1). In a few small areas the marrow cells are fairly dense (D-2 to 3) and quite active. V-2, A-2, L-2 to 3, E-2, D-1 to 2. Rib four is

same as rib three except somewhat less active. V-2, A-2, L-2, E-2, D-2. 2-3-20, wt. 28kg., condition fat and healthy;wounds cleanly healed; Hb. 100%; R.B.C. 9220000; W.B.C. 20616(three counts). 2-4-20, rib number five removed from left side. Pleura not perforated. 3-18-20, rib five examination: marked erythrogenesis (many normoblasts and megaloblasts),a vascular active marrow, considerable fat in with marrow cells; bone cortex "honeycombed" with new marrow cell islands. V-2 to 3, A-2 to 3, L-1, E-2 to 3, D-2, (several slides examined and average was L-2, E-2). 3-30-20, wt. 27 kg., animal fat and frisky, wounds clean, Hb. 96%, R.B.C. 7680000, W.B.C. 18300. 3-31-20, rib number six removed from right side under ether anaesthesia, pleura not perforated. 5-20-20, rib number six marrow examination:- large islands of active marrow cells; bone cortex much "honeycombed" by large sized islands of marrow cells, small amount of fat. Slide "A" shows V-2, A-2, L-2, E-2, to 3, D-3. Slide "B" shows V-2 to 3, A-3, L-1, to 2, E-3, D-3. Slide "C" shows V-2, A-2, to 3, L-2, E-2 to 3, D-3. 6-3-20, wt. 27.2 kg., condition excellent, wounds clean, Hb. 100%; R.B.C. 7700000; W.B.C. 19650; rib resection number seven. 8-14-20, animal etherized and immediate autopsy done; condition fat and healthy at time etherized; wt. 24.5 kg., no pathology found; no hemolymph node hypertrophy; lymph glands are large and dark chocolate color with firm consistency; spleen (large) weighed 75 gms. Specimens of liver, spleen, rib marrow (numerous pieces from both sides of chest midway between spine and sternum), lymph glands (axillary and mesenteric), femur marrow (central third) sent to laboratory in Zenker's fluid. Femur marrow dark brandy red color at center of shaft (cross section showed core was fatty and only outer layer was red). This outer red layer of marrow cells was about 1 m.m. thick. Rib number seven marrow examination: about same as rib six, possibly a little less active, considerable fat, marrow cell density 2, activity 2. V-2, A-2, L-1 to 2, E-2, D-2. Final rib marrow(number eight) examination:- fine large islands of marrow cells with considerable fat, marrow cell density 2, activity about same as marrow of rib

seven. V-2, A-2, L-1 to 2, E-2, D-2.

Final femur marrow examination:- a good outer layer of marrow cells about 1 m.m. thick. There are many myelocytes and a few intermediate forms. Activity is mainly erythrogenic (E-2). Direct comparison with control femur marrow taken at beginning of experiment shows the final specimen to be somewhat more active.



DOG D 32 SPLENECTOMY AND EIGHT RIB RESECTIONS DURING PERIOD OF ONE YEAR

For explanation see Part VI, pages 29-31.

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SPLENECTOMY
MARROW SPECIMEN 1
MARROW SPECIMEN 2
MARROW SPECIMEN 3
MARROW SPECIMEN 4
Three months after splenectomy
MARROW SPECIMEN 5
MARROW SPECIMEN 6
Six months after splenectomy
MARROW SPECIMEN 7

Weeks after operation
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 4

MARROW - FINDINGS
General activity
Leukogenesis
Erythrogenesis
Vascularity
Density of marrow cells
Grade

BLOOD - FINDINGS
Hemoglobin
Percent
Erythrocytes
Millions
Leukocytes
Thousands

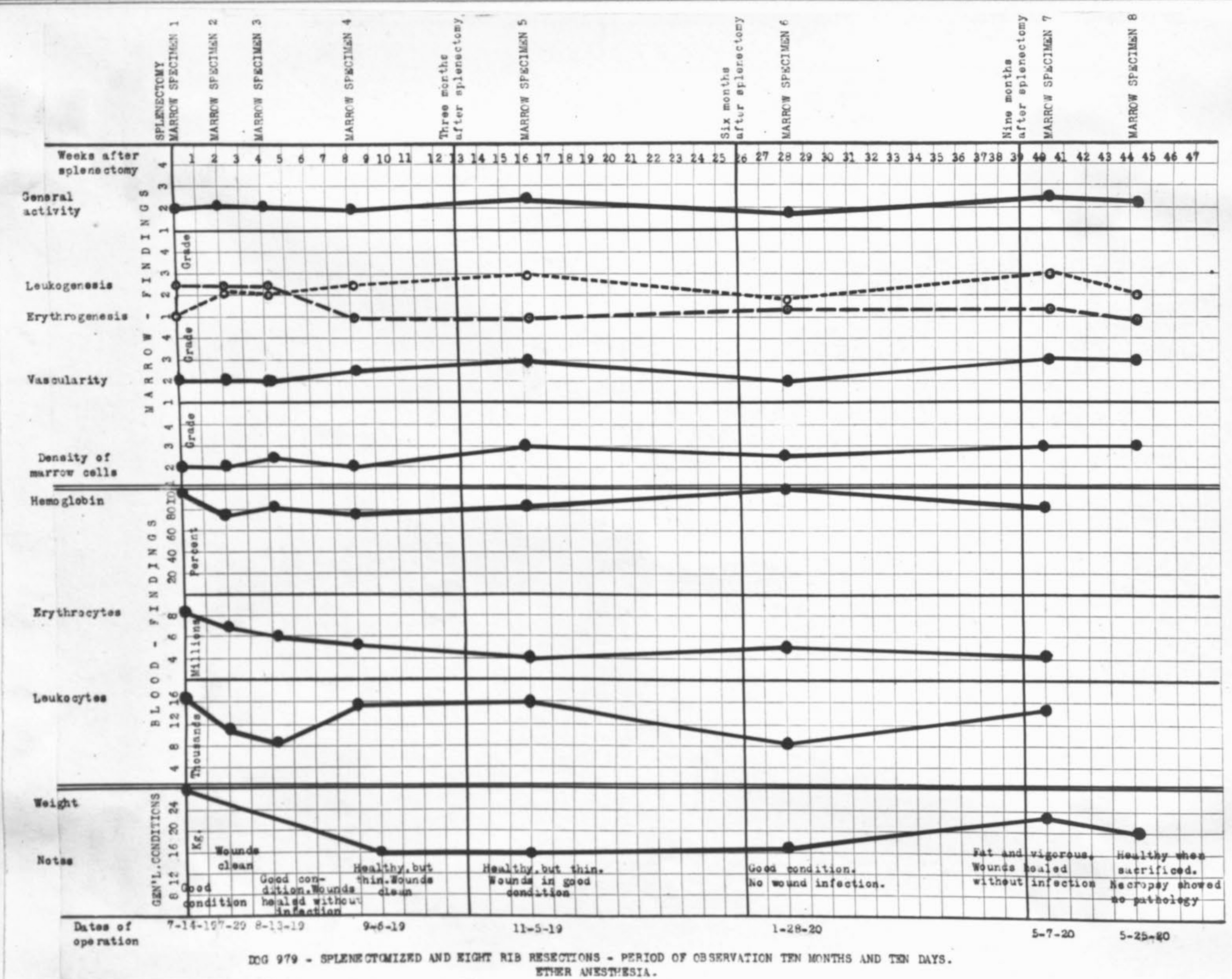
GEN'L. CONDITION
Weight
Kg.
Notes

Fine, healthy, dog
Good condition (thin) Wounds clean
Thin but apparently healthy. Wounds healed well.
Excellent condition. Wounds healed without infection
Dog in good condition. Wounds clean.
Dog healthy when sacrificed 5-6-20. Wounds clean. Necropsy showed no pathology.

Dates of operation
8-20-19 9-2-19 9-17-19 10-15-19 12-10-19 2-4-20 5-6-20

DOG 230 SPLENECTOMY AND SEVEN RIB RESECTIONS; ETHER ANESTHESIA
PERIOD OF OBSERVATION THIRTY-SEVEN WEEKS.

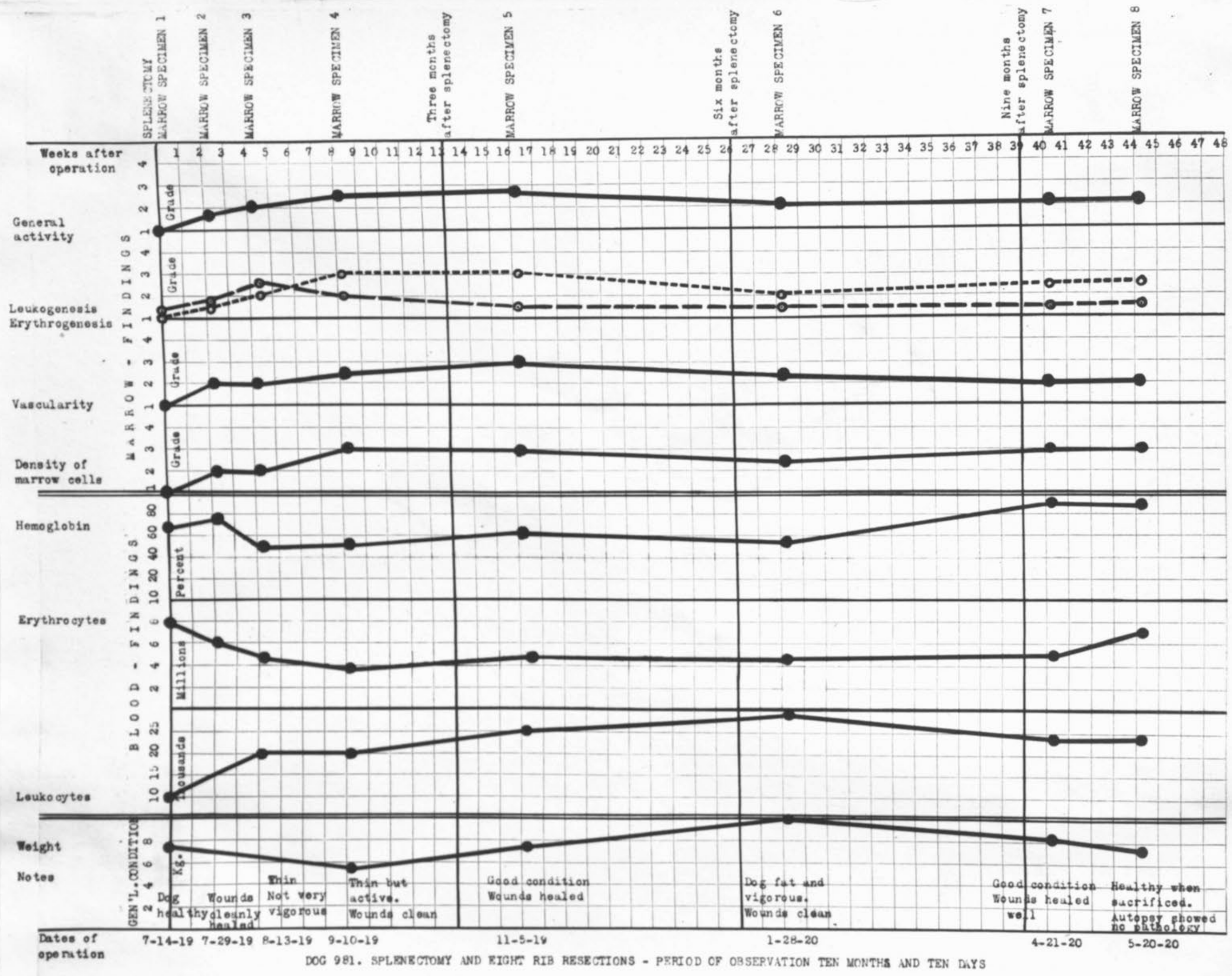
For explanation see Part VI, pages 29-31.



DOG 979 - SPLENECTOMIZED AND EIGHT RIB RESECTIONS - PERIOD OF OBSERVATION TEN MONTHS AND TEN DAYS. ETHER ANESTHESIA.

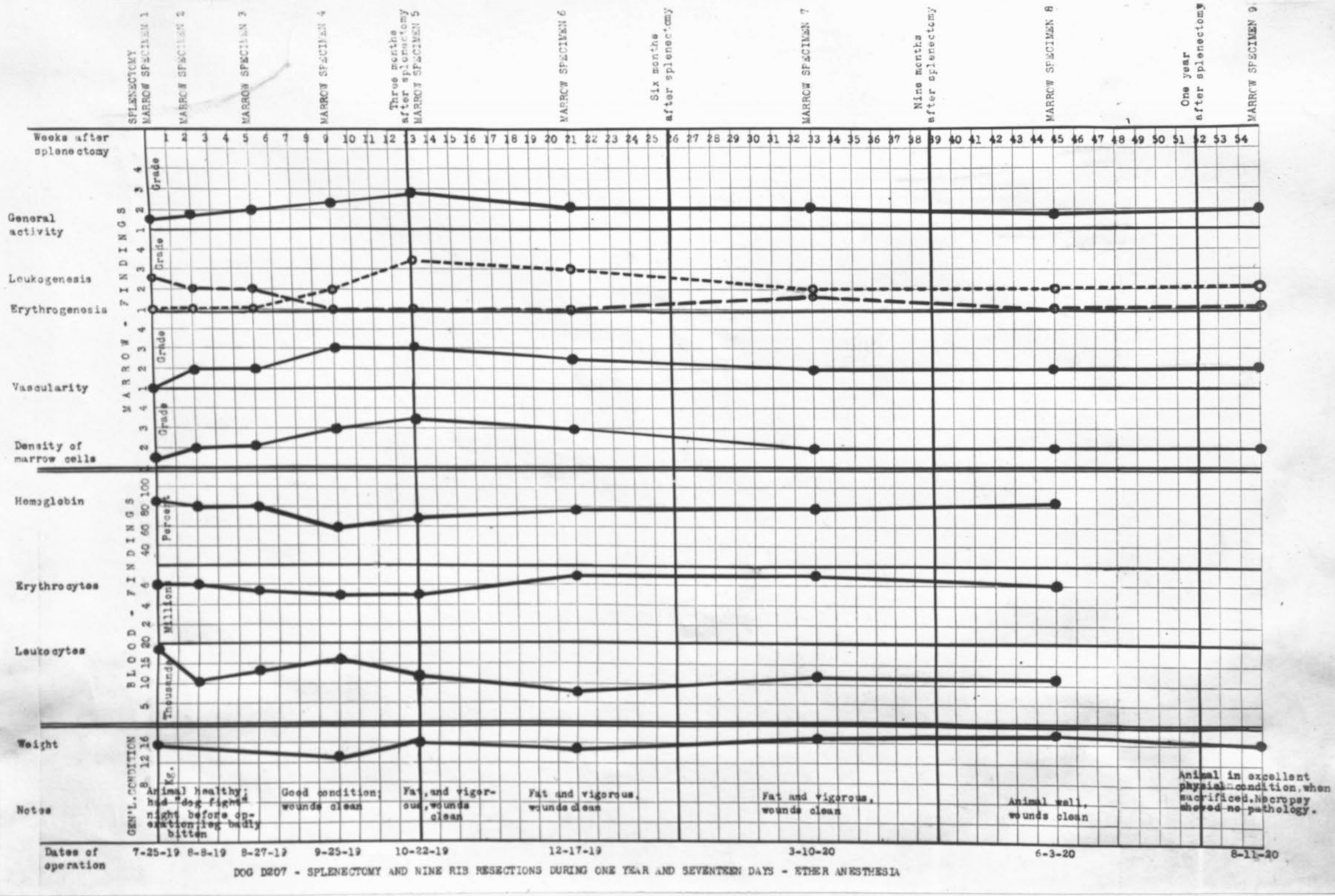
For explanation see Part VI, pages 29-31.

7-20 2M



DOG 981. SPLENECTOMY AND EIGHT RIB RESECTIONS - PERIOD OF OBSERVATION TEN MONTHS AND TEN DAYS

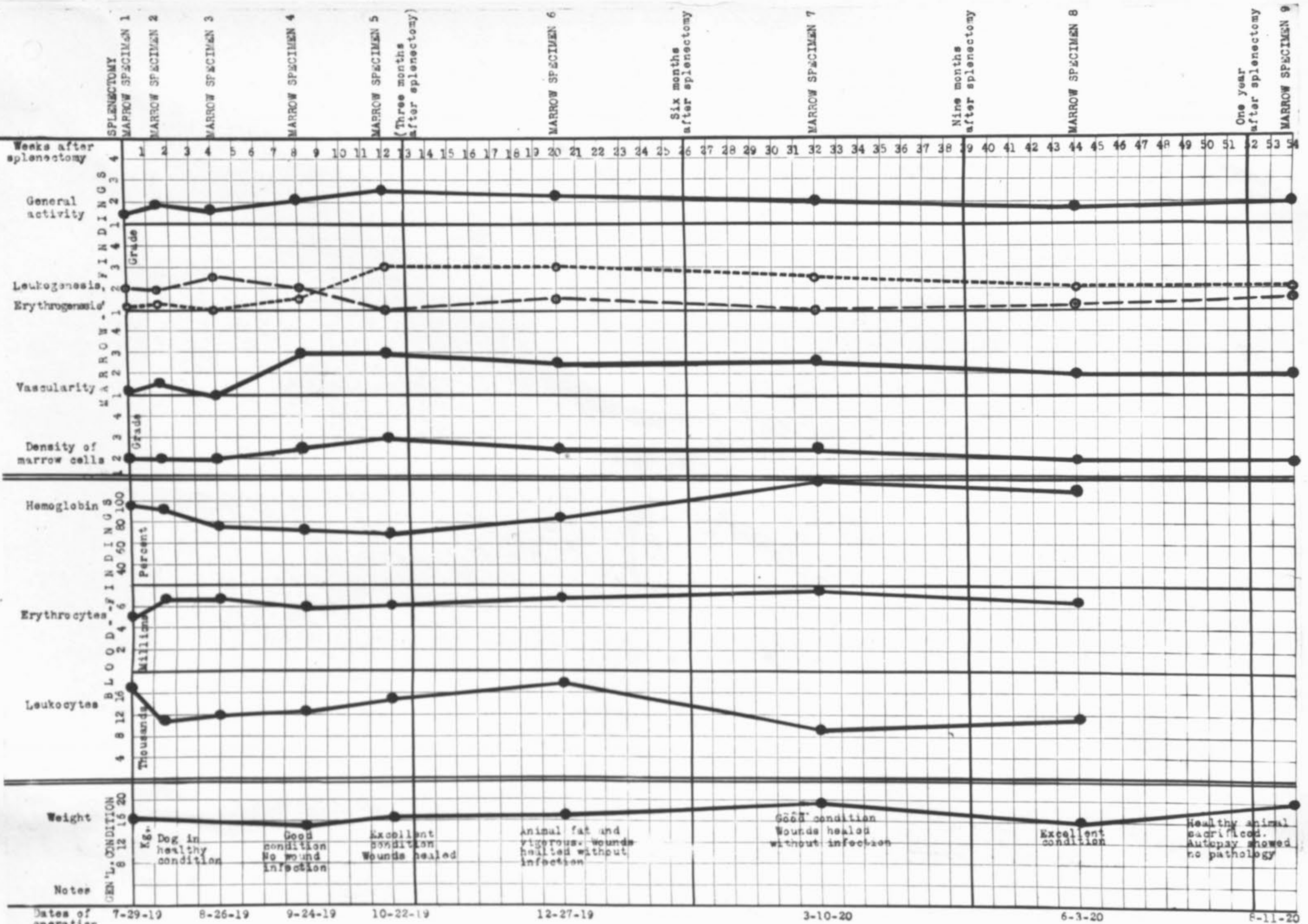
For explanation See Part VI, pages 29-31.



DOG D207 - SPLENECTOMY AND NINE RIB RESECTIONS DURING ONE YEAR AND SEVENTEEN DAYS - ETHER ANESTHESIA

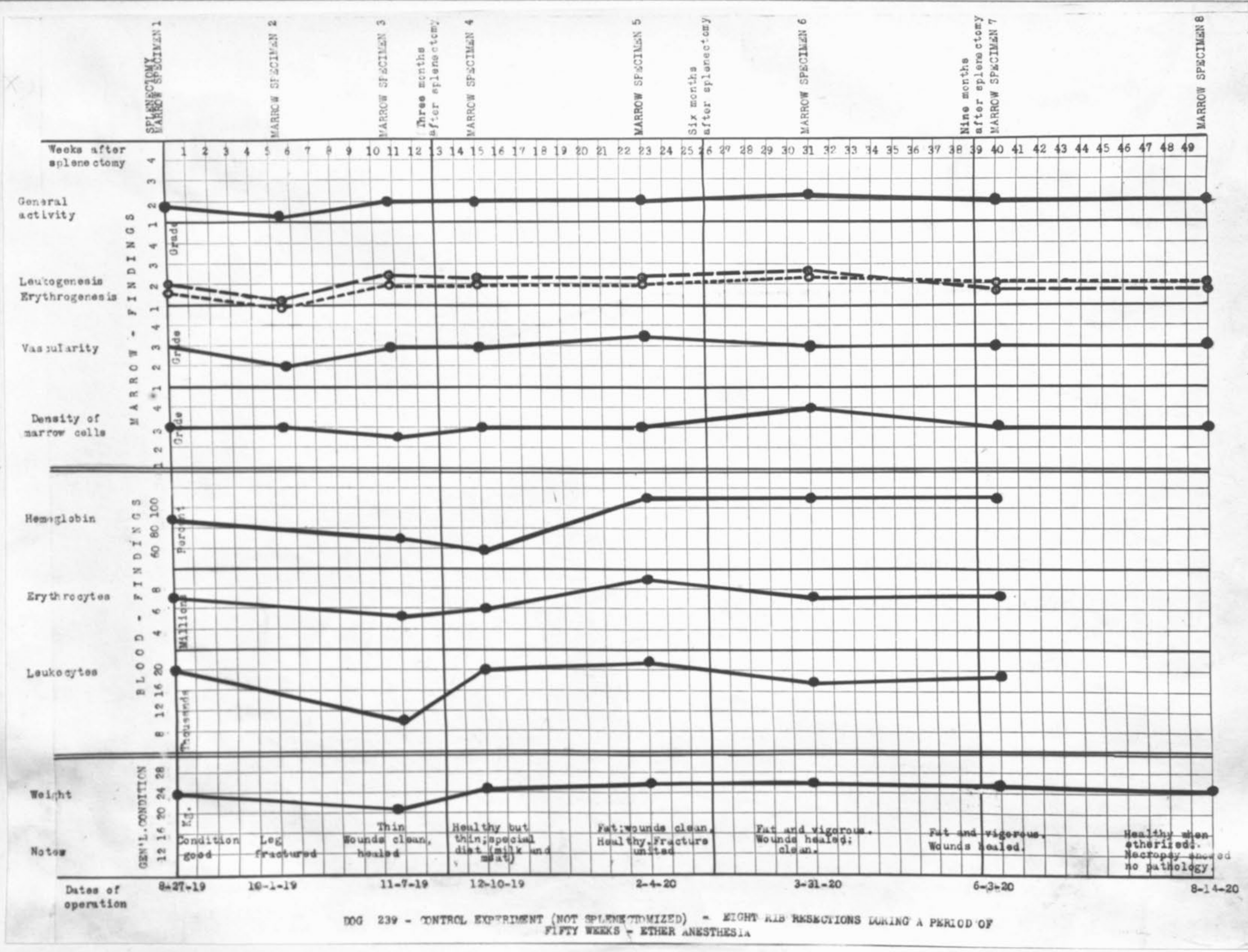
For explanation see Part VI, pages 29-31.

7-20 2M



DOG D208 - SPLENECTOMY AND NINE RIB RESECTIONS DURING PERIOD OF ONE YEAR AND FOURTEEN DAYS

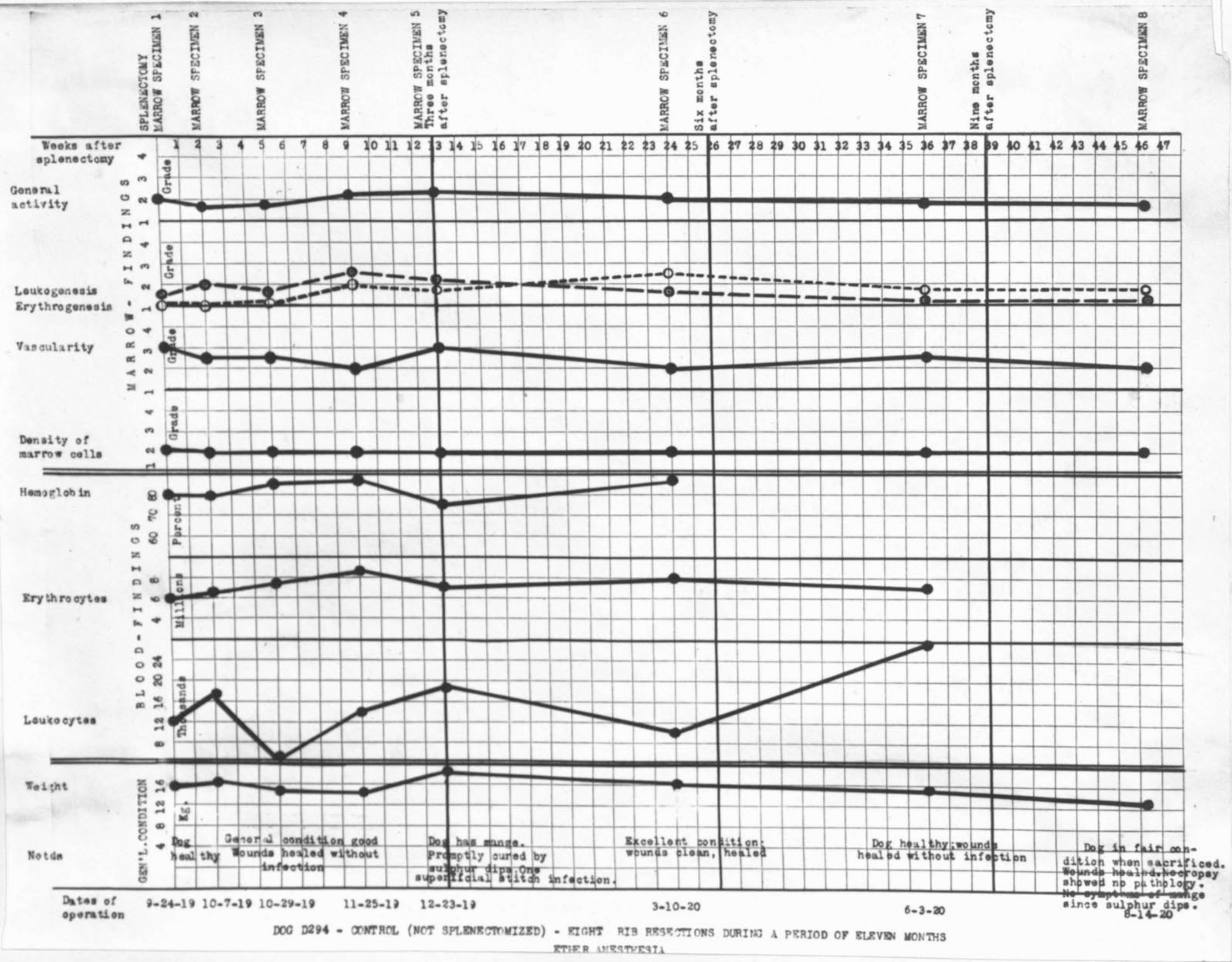
For explanation see Part VI, pages 29-31.



DOG 239 - CONTROL EXPERIMENT (NOT SPLENECTOMIZED) - EIGHT RIB RESECTIONS DURING A PERIOD OF FIFTY WEEKS - ETHER ANESTHESIA

For explanation see Part VI, pages 29-31.

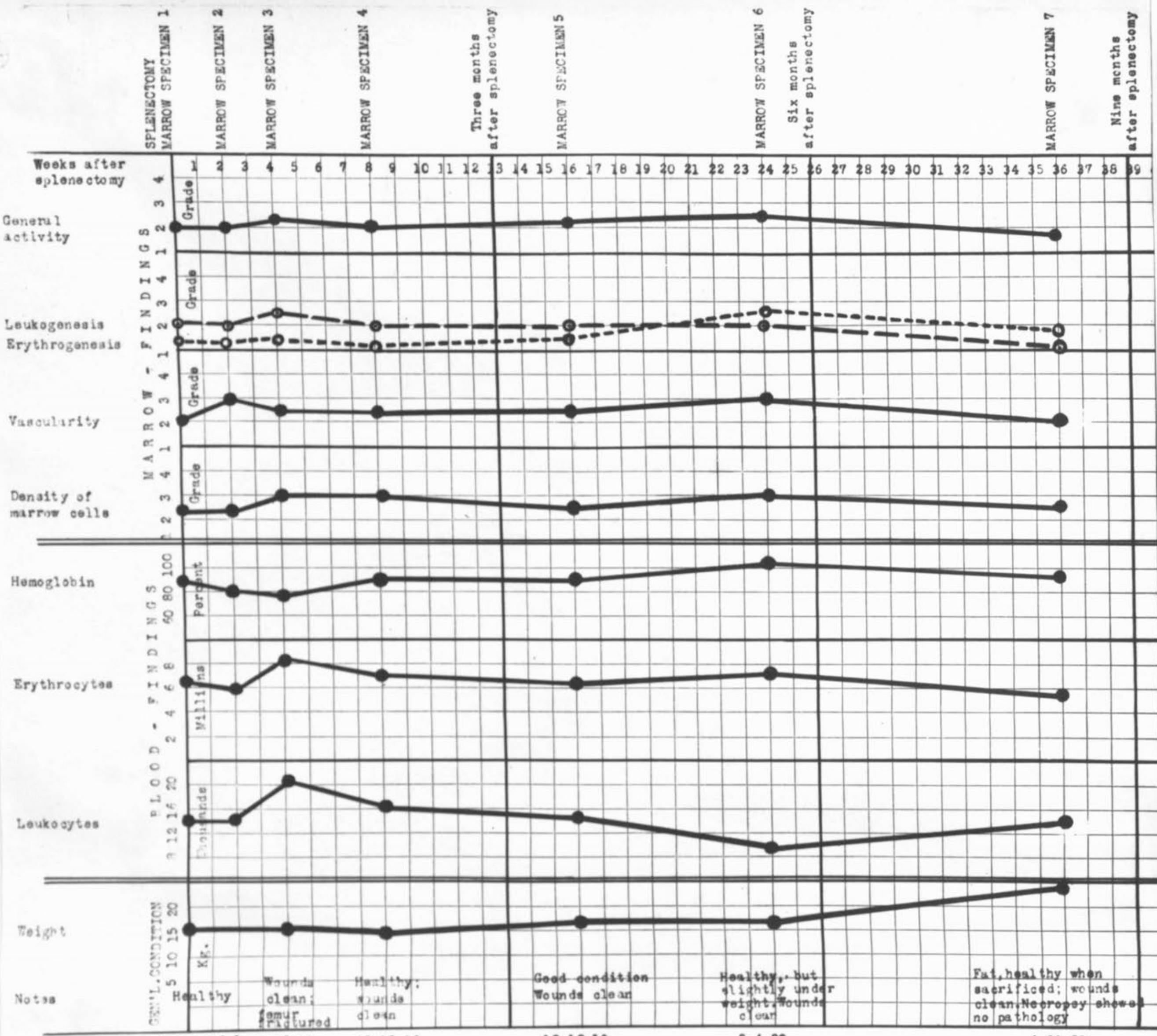
7-20 2M



DOG D294 - CONTROL (NOT SPLENECTOMIZED) - EIGHT RIB RESECTIONS DURING A PERIOD OF ELEVEN MONTHS
ETHER ANESTHESIA

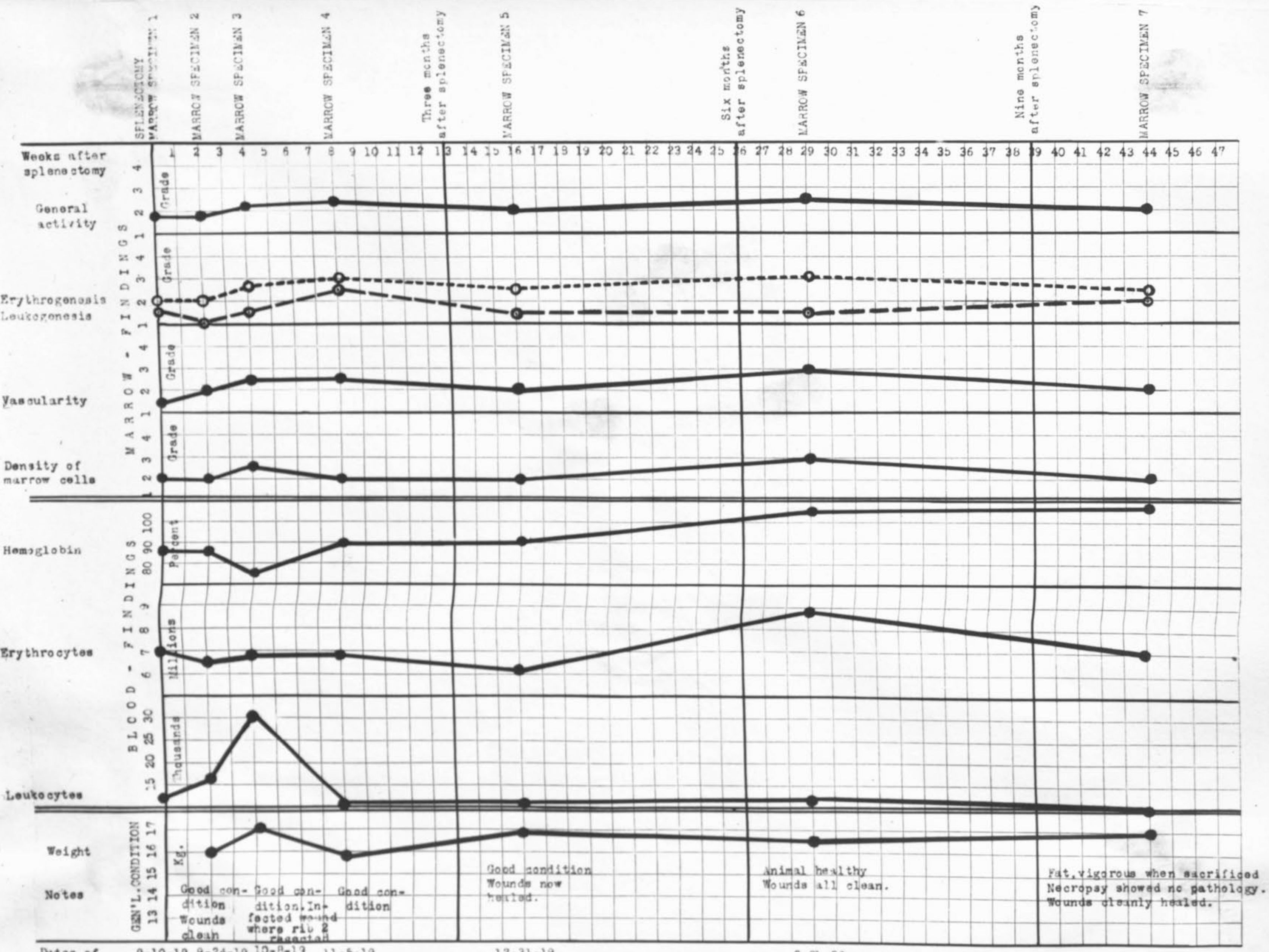
For explanation see Part VI, pages 29-31.

7-20 2M

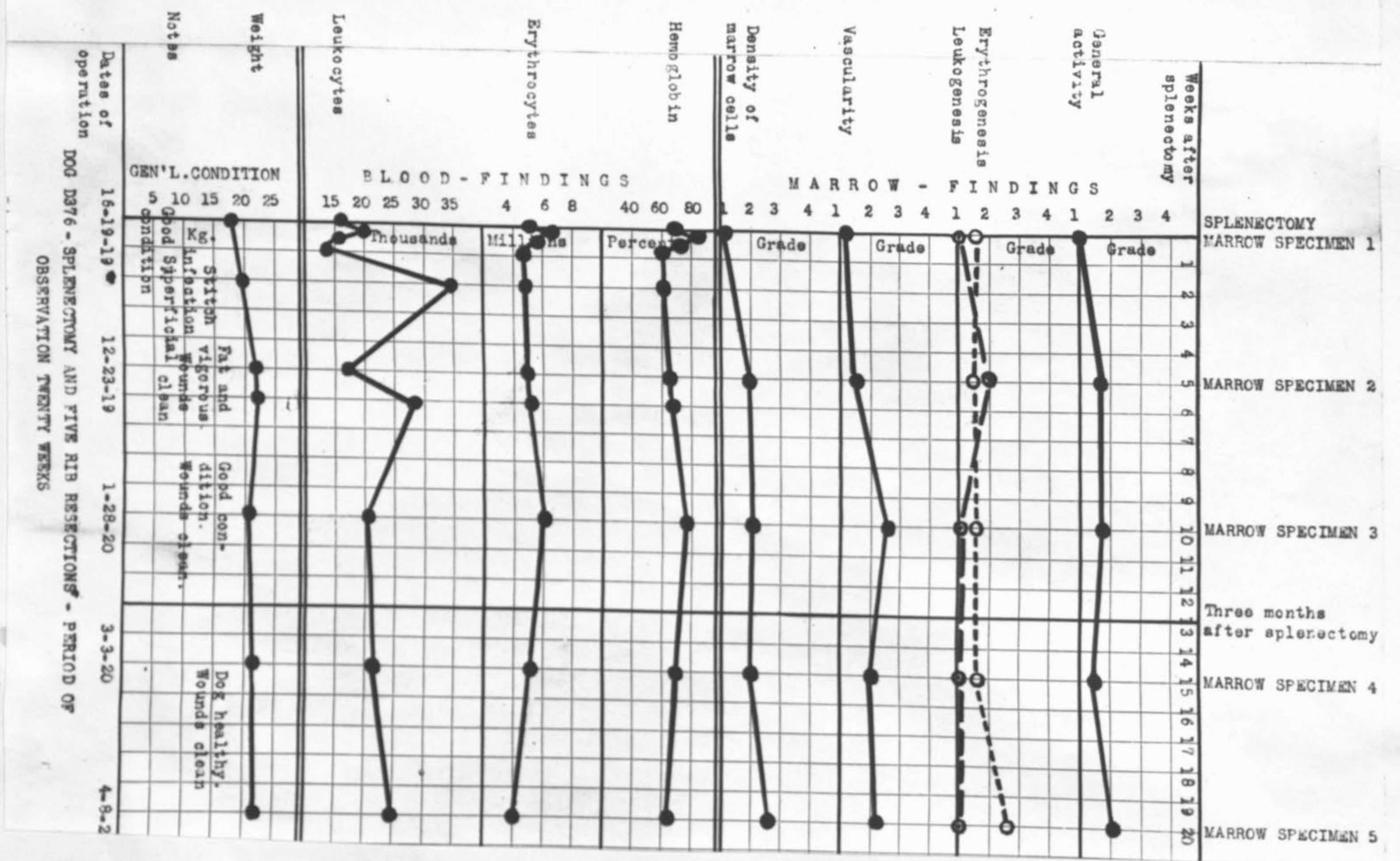


DOG D233 - CONTROL EXPERIMENT (NOT SPLENECTOMIZED) - SEVEN RIB RESECTIONS DURING NINE MONTHS
CONTROL SPECIMEN OF FEMUR MARROW ALSO TAKEN AT BEGINNING OF EXPERIMENT

For explanation see Part VI, pages 29-31.

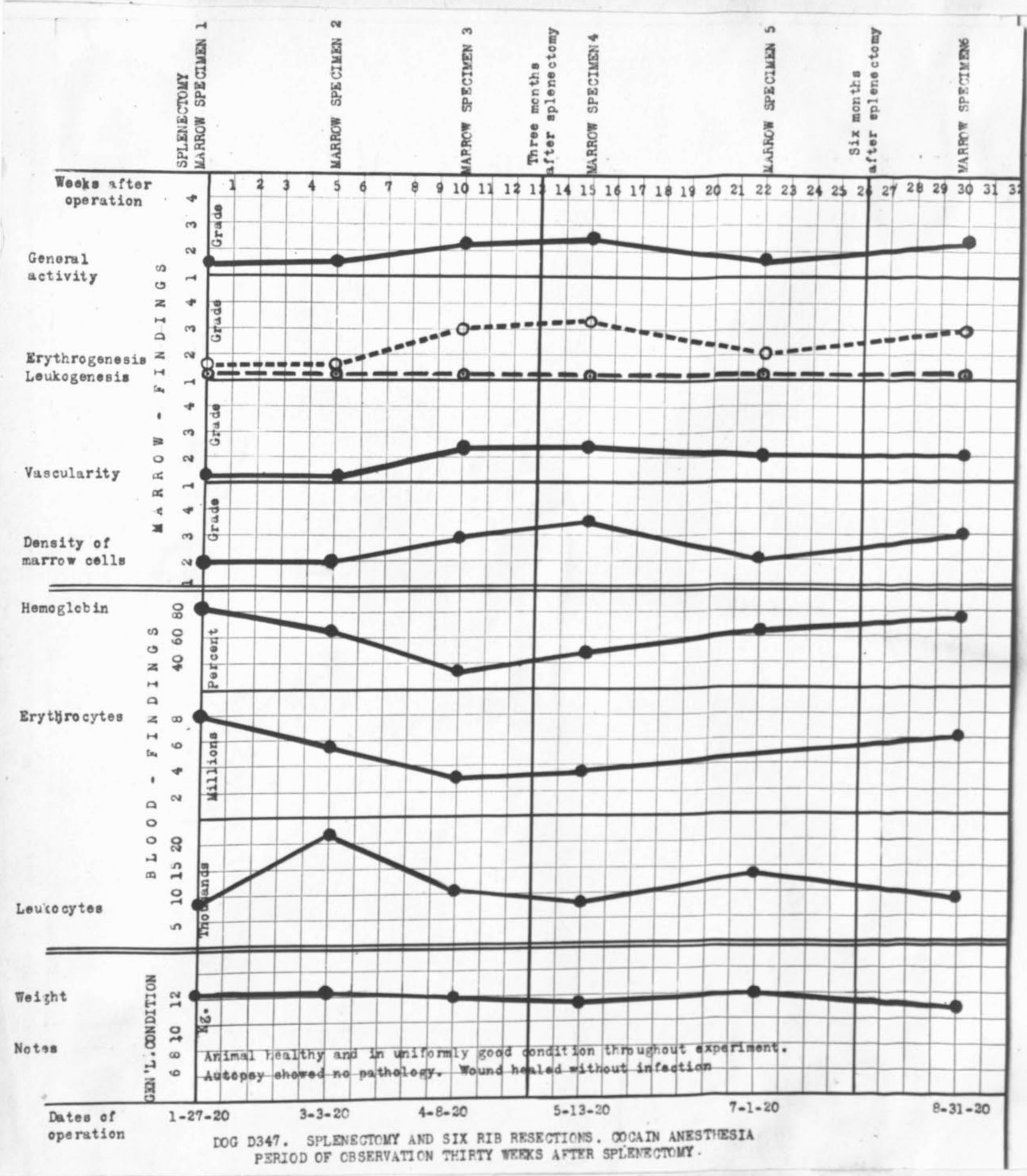


For explanation see Part VI, pages 29-31.



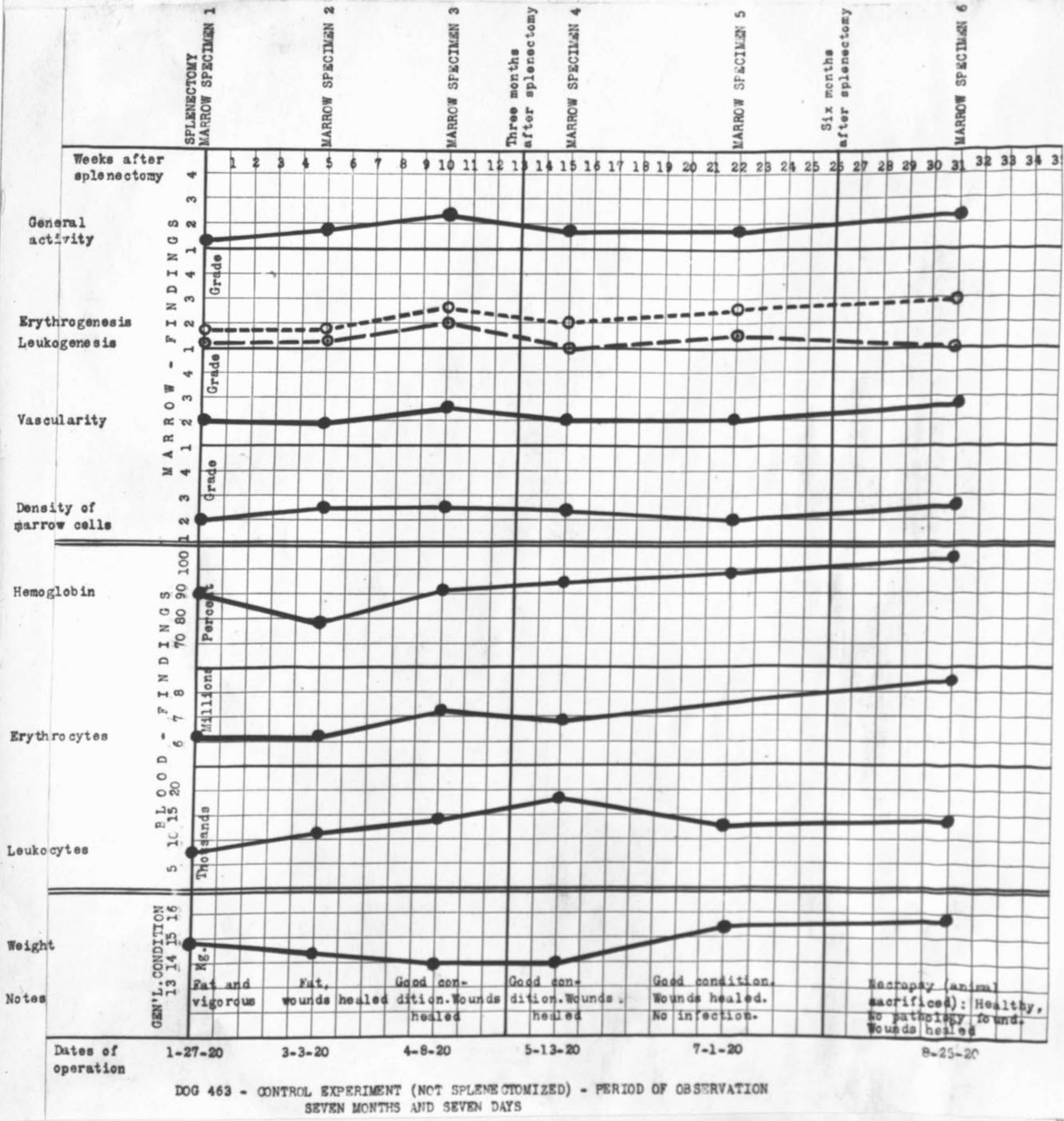
For explanation see Part VI, pages 29-31.

7-20 2M



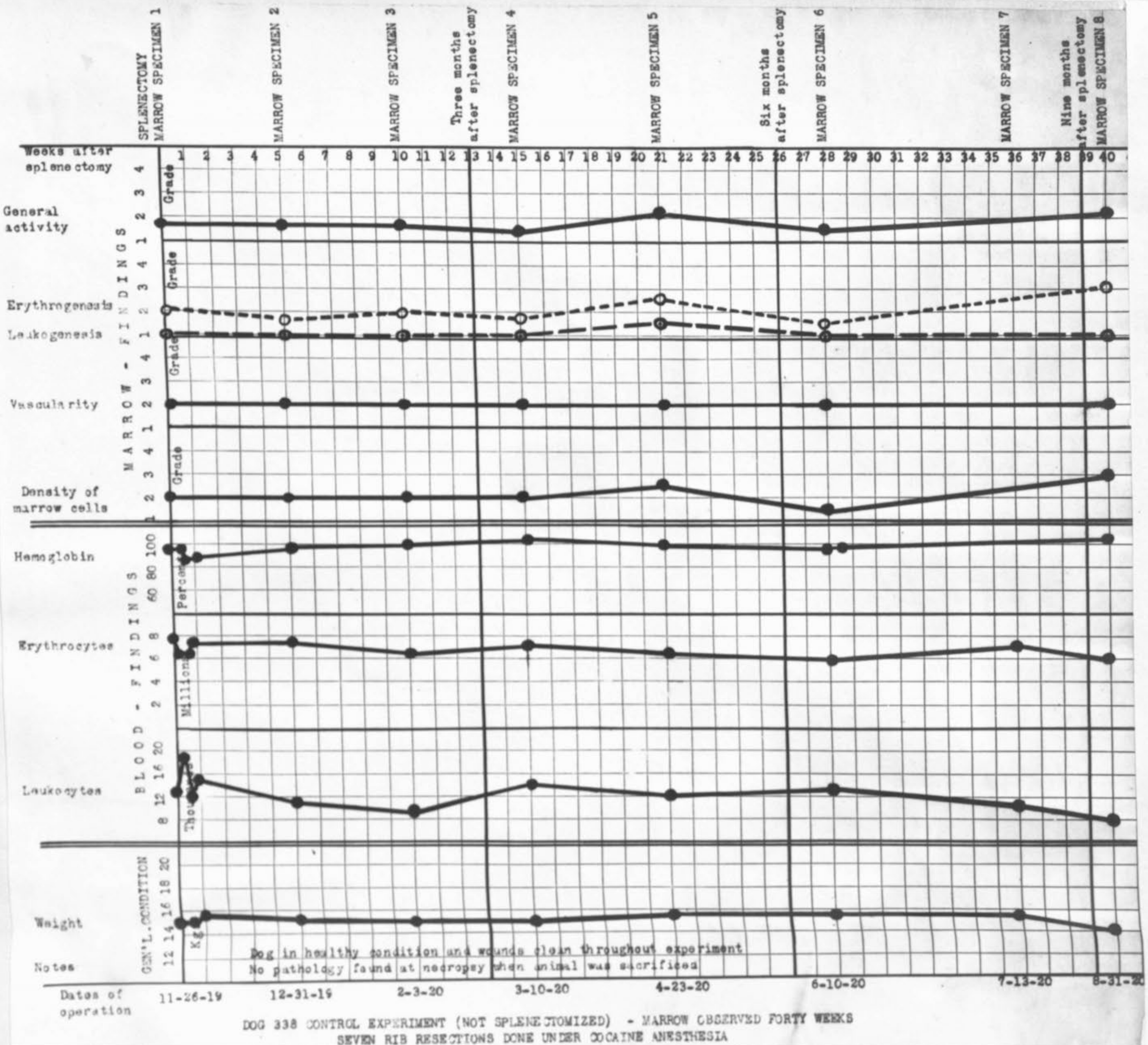
DOG D347. SPLENECTOMY AND SIX RIB RESECTIONS. COCAIN ANESTHESIA
PERIOD OF OBSERVATION THIRTY WEEKS AFTER SPLENECTOMY.

For explanation see Part VI, pages 29-31.



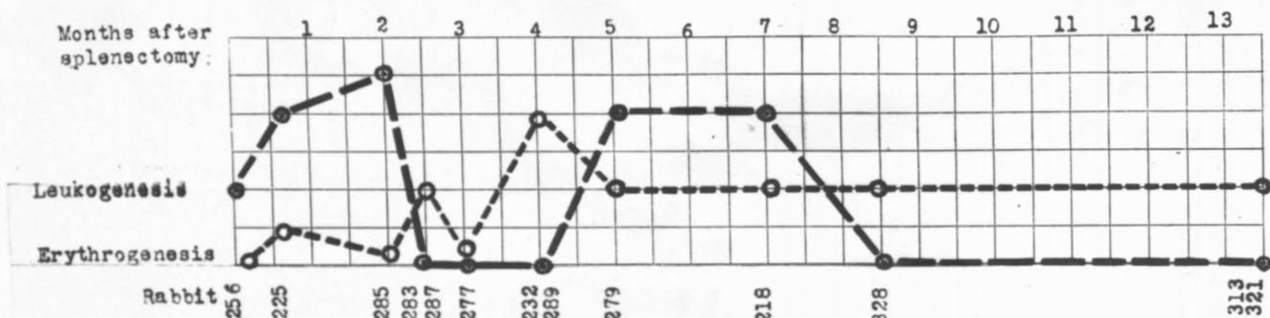
DOG 463 - CONTROL EXPERIMENT (NOT SPLENECTOMIZED) - PERIOD OF OBSERVATION SEVEN MONTHS AND SEVEN DAYS

For explanation see Part VI, pages 29-31.

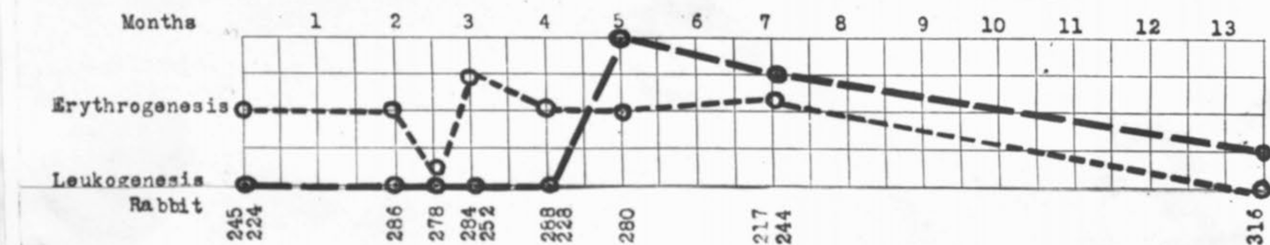


For explanation see Part VI, pages 29-31.

TIBIA MARROW CHANGES IN THIRTEEN RABBITS AFTER REMOVAL OF SPLEEN
(Composite charts)



TIBIA MARROW FINDINGS IN TWELVE NORMAL CONTROL RABBITS



For explanation see Part VI, pages 29-31.

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* These additional references have been selected as those which furnished the most valuable information regarding the relation of the spleen to the marrow.