THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

IX. The Changes in the Bone Marrow after Splenectomy.*

By RICHARD M. PEARCE, M.D., and O. H. PERRY PEPPER, M.D.

(*From the John Herr Musser Department of Research Medicine of the University of Pennsylvania, Philadelphia.*)

In connection with the various investigations[1] carried out in this laboratory on the subject of the spleen in its relation to blood destruction and regeneration, the bone marrow of splenectomized dogs has been examined with a view to determining the compensatory or other changes following the removal of the spleen. As the material now consists of marrows representing periods varying from three weeks to twenty months after splenectomy, we consider our study sufficiently comprehensive to justify a detailed report.

In the literature of the subject, the references to changes in the bone marrow following splenectomy are for the most part casual and presented but incidentally in connection with the associated changes in the lymph and hemolymph glands. In Warthin's (1) collection of the literature up to 1903 the following references occur: Tizzoni and Fileti (2) (1880) and Tizzoni (3) (1882) observed in splenectomized dogs a transformation of the fatty marrow of long bones into red marrow. Mosler (4) (1882), working likewise with dogs, concluded that following splenectomy there may be compensatory action on the part of both lymph glands and bone marrow, the latter appearing to play an important part. In one animal ten months after splenectomy the bone marrow resembled that of leukemia. This change, however, was not constant. Laudenbach (5) (1893) observed in one dog (ten to twelve years of age), with severe anemia, signs of increased blood formation in the marrow 145 days after splenectomy. Ceresole (6) (1895), on the other hand, found in splenectomized rabbits no clearly defined new formation of the marrow. Warthin (1) (1903) states that after splenectomy in the sheep and goat slight lymphoid changes in the fatty marrow occur, but he gives no histological description. Of these changes he says:

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"The beginning lymphoid changes in the fatty bone marrow in the second and fifth month after splenectomy, are to be regarded as compensatory only for the increased destruction of red blood cells and not for any abrogated splenic function of red cell formation."

Other references may be found to changes in the bone marrow in the presence of diseases of the spleen in man (7) and in experimental anemias of animals with or without splenectomy, but few findings after simple removal of the normal spleen are available. Among the latter are Pugliese's (8) observation that after total splenectomy the bone marrow of the hedgehog becomes filled with giant cells. This change Foa (9) has found not to be characteristic of the rabbit. Vulpius (10) who in 1894 reviewed thoroughly the subject of the surgery and physiology of the spleen, and adds some experimental observations, supports the theory of increased activity of the bone marrow after splenectomy. Winogradow (11) found red marrow in the long bones of a dog 132 days after splenectomy, but yellow marrow was present in two after 517 and 760 days respectively, though one of the latter was slightly streaked with red. Hodenpyl (12) in the description of a case of absence of the spleen in man makes no mention of the bone marrow. Taylor (13) describes the marrow of two splenectomized dogs; that from an animal receiving albumoses by mouth and by hypodermic injection, and killed after nine months, was red; a second splenectomized animal, not receiving albumoses, showed a yellow marrow at the end of one year. Freiberg (14) states that he found red marrow in splenectomized animals, and Gibson (15) notes that in a dog killed five and a half months after being deprived of the spleen, the marrow was apparently in the process of change from yellow to red.

In some of these accounts brief mention is made of the increase of giant cells or of pigmented cells or of the numerical relations between the myelocytes and the white and red cells, but we have been unable to find an adequate account of the histology of the bone marrow after splenectomy based on modern conceptions of the cytology of this tissue. Histological descriptions exist, but they are either brief and fragmentary or are based on views current before the attainment of our present detailed knowledge of the morphology of the cells of the blood.

METHODS.

Our studies are based chiefly on the changes in the marrow of the long bones and particularly in that of the femur. As this marrow in the adult dog is normally fatty, objection may be raised against its use, and to overcome this objection, we attempted to study the marrow of the compact bones. The methods of decalcifying the tissues have, however, in our hands, failed to yield satisfactory

histological preparations. The alternative, the use of film preparations obtained successively at intervals over long periods of observation being impracticable, the study of cover-glass preparations was limited to a single observation at the time of the death of the animal. At the same time, however, in many instances marrow squeezed from the ribs has been obtained in sufficient amount to section and thus to allow a comparison with changes in the fatty marrow. We have, however, depended largely upon the study of sections of the marrow of the long bones and in particular of the femur. We are satisfied, as the result of our study of the marrow form a large number of normal dogs, that this is, after all, the most rational method of studying compensatory changes, for it is unusual even in a definitely fatty marrow not to find numerous centers of blood-forming cells. These may be limited to the periphery of the marrow or be scattered throughout, but whatever their position they afford an excellent starting-point for the study of increased cellular content as well as of changes in the character of the cells. The fatty marrow is of especial value in the study of the late changes, for in well fixed and well stained marrow there can be no doubt about the change from a purely fatty marrow to a red marrow rich in cells. This is so striking as to remove all doubt which exists when one examines the marrow of compact bone, as of the ribs or vertebræ, by either the section or cover-glass method.

We have worked exclusively with the marrow of the middle third of the femur, avoiding the marrow at either end, partly on account of its bony nature, but chiefly because of the occasional occurrence of more or less red marrow at the ends of the shaft. As only adult dogs have been used, we feel that the constant use of the middle portion of the marrow allows fairly comparable results. In removing the marrow half the circumference of the bone through the greater part of its length has been chipped away, and after separating the marrow from the bone and cutting it at either end it has been easily removed as a solid cylinder by gently rolling it on to a piece of filter paper. In carrying these tissues through the process of fixation and imbedding, the filter paper, which is firmly adherent to the marrow through the coagulation of the attached blood, allows the necessary manipulations without injury to the marrow

itself. The routine procedure has been to fix in Zenker's fluid without previous decalcification, imbed in paraffin, and stain with eosin and polychrome methylene blue. Other stains have, however, been used when necessary to bring out certain details.

RESULTS.

It may be stated at the outset that we have found no evidence of an early change in the bone marrow. Splenectomy does not cause, as do successive hemorrhages and hemolytic poisons, a rapid change of fatty marrow to red marrow. This latter change we have produced readily and rapidly in non-splenectomized control animals by the use of specific hemolytic serum and by causing hemorrhage, but we have never seen a frank change from yellow to completely red marrow in the ordinary course of events in the splenectomized animal until many months, usually six or more, had elapsed, and this despite the fact that many of the animals have had, as has been shown in our earlier work, a moderately severe anemia. This anemia has frequently been of as severe degree as that caused by severe successive hemorrhages in the normal dog, but changes in the marrow analogous to those caused by hemorrhage have not been evident in the earlier periods following splenectomy.

In this connection it may be recalled that the anemia of splenectomy in the dog follows a gradual downward course for three to six weeks, the decrease in hemoglobin being relatively more marked than the decrease in red cells, and that an equally gradual repair causes the red cell count and hemoglobin content to approach normal after three to four months or more. At the same time there is a transient initial leucocytosis due chiefly to polymorphonuclear leucocytes, and a more or less constant lymphocytosis with a late eosinophilia. Not infrequently the eosinophils disappear from the circulating blood from the third week until the end of the third month.

We have, therefore, in the course of our studies attempted to determine whether the hyperplasia in the bone marrow after splenectomy is compensatory in the sense of (1) overactivity in red cell formation chiefly, or (2) peculiarly active in the formation of the white cells of the blood, or (3) in the sense of an orderly reproduction of a new marrow with normal activities in the formation of all cells arising within it.

THE NORMAL MARROW OF THE FEMUR OF THE DOG.

In our study of the marrow of both normal and splenectomized animals we have used as a basis for orientation Bunting's (16) conception of erythrogenetic and leucogenetic centers, Muir's (17) descriptions of erythroblastic and leucoblastic reactions, and have received also much aid from Dickson's (18) study of the cytology of marrow. The arrangement described by Bunting is by no means a constant and definite one, but in the masses of marrow cells may be seen groups composed mainly of myeloblasts and surrounded at times by a nearer zone of myelocytes and an outer zone of leucocytes; in other groups with the same center, the outer zone may be made up of nucleated red cells with a still more distant zone of normocytes. We are not convinced that centers for the production exclusively of red cells or of white cells exist, for frequently an intermingling of the two types is seen in one center, but this conception of definite centers is of great assistance in the interpretation of marrow changes.

The study of the marrow of the femur from many normal dogs has led to our recognition of four definite groups of cells: (1) Groups of undifferentiated cells and myelocytes. These lie between fat cells and seem to be in no way connected with blood channels. In all these centers the cells of the connective tissue reticulum are in evidence. (2) Groups of the character described above, but with a peripheral accumulation of cells in which those of the leucocyte series predominate. (3) Groups as in (1), but with a mantle of cells in which those of the erythrocytic series are most in evidence. (4) Groups as in (1), but with an indiscriminate mingling of cells of red and white series.

These groups cannot always be differentiated for not infrequently an indiscriminate mingling of cells obscures the recognition of centers. Moreover, at times may be seen groups composed purely of white cells or of red cells without myelocytic centers. We have, however, found that search for the groupings described greatly facilitates the study of complex marrow pictures and leads readily to a decision as to whether leucoblastic or erythroblastic activity predominates.

In one respect the study of normal marrow has not helped us

greatly. Megakaryocytes and polykaryocytes are so infrequent in the normal fatty marrow that we have no basis, in regard to them, for a comparison with hyperplastic marrow. The same holds true for the large endothelial cells which are phagocytic for red cells and are found so frequently in hyperplastic marrow to contain remnants of red cells and fragments of pigment.

THE MARROW OF SPLENECTOMIZED ANIMALS.

In table I the general results of our observations are presented. The terms "yellow" and "red" refer to the gross appearance, not of the surface of the marrow, but of the cross section. "Slight streaking" and "streaked" refer to an intermingling of yellow and red marrow. A marrow is described as "red" only when it is uniformly so. As will be seen by a comparison of gross and microscopic appearances, a marrow "yellow" to the naked eye may, microscopically, show evidence of beginning hyperplasia. The

TABLE I.

Hyperplasia of the Marrow of the Femur after Splenectomy.

Dog No.	Period after splenectomy.	Gross appearance.	Microscopic change.
50	24 dys.	Yellow	Slight.
23	39 dys.	Yellow	None.
21	40 dys.	Yellow	Slight.
86	42 dys.	Yellow	Slight.
79	60 dys.	Yellow	Slight.
82	63 dys.	Slight streaking	Slight.
17	84 dys.	Yellow	Slight.
10	6 mos.	Red	Complete.
39	7 mos.	Red	Complete.
32	8 mos.	Yellow	Slight.
44	9½ mos.	Yellow	None.
41	10 mos.	Yellow	None.
24	1 yr.	Red	Complete.
59	1½ yrs.	Red	Complete.
57	1½ yrs.	Streaked	Partial.
33	1¾ yrs.	Red	Almost complete.
51	1⅝ yrs.	Yellow	Slight.

early changes are indicated by the word "slight." The word "complete" indicates that only an occasional fat cell is seen microscopically. "Almost complete" means that fat cells are present in the proportion of one part to nine of marrow cells, in the surface area of sections studied. Several purely fatty marrows represent-

ing periods between five and twenty-four days after splenectomy are not included in the table.

The bone marrows representing the earlier periods of splenectomy, in that they show practically no changes, may be dismissed briefly. This is true of a series from animals killed at various intervals from five days to three months. Some of these marrows cannot be distinguished from those of the normal dog. In others, slight replacement of fatty tissue is seen. Thus, one representing the twenty-fourth day shows here and there between the fat cells single rows of blood-forming cells with now and then clumps of ten to thirty or more. These areas are neither purely erythrogenetic or leucogenetic, though in some of the groups with an older type of cells there is a predominance of polynucleated cells. The endothelial cells of the reticulum not infrequently contain large masses of old blood pigment.

Another, representing the fortieth day, presents practically the same appearance with a tendency, however, to greater erythrogenesis. On the other hand, a thirty-nine day dog shows a simple fatty marrow with no evidence of active blood formation. Three other marrows of this period, however, show already the early stages of hyperplasia. In one of these (forty-second day) showing a slight general hyperplasia, both types of cell groups can occasionally be isolated, but usually the groups are mixed. Greater numbers of eosinophil cells, both myelocytic and polymorphonuclear, are present than have been evident in earlier periods. A number of cells throughout the section correspond to Longcope's (19) small lymphocytes and a smaller number to Longcope's large lymphocytes. The small lymphocytes are not, however, in pure groups. The picture as a whole is more one of leucogenesis than of erythrogenesis. Very few giant cells are seen and only occasional phagocytes. Polymorphonuclear leucocytes are abundant.

In another marrow of the sixty-third day, a moderate peripheral hyperplasia of mixed type is present. Marked congestion is evident between the fat cells, and in places near the periphery there is hyperplasia; in some places the erythrocytes appear to be outside the vessel, forming distinct hemorrhages. A few phagocytes are present, but giant cells are rare. Polymorphonuclears are frequent and of

mature development. At the periphery erythrogenesis seems to predominate over leucogenesis. Eosinophils and lymphoid cells are not conspicuous.

A marrow of the sixtieth day shows less hyperplasia, but leucocytic reaction is more evident, though erythrogenesis is active. Scattered throughout the section are many small lymphocytes, but nowhere are these seen in solid clumps. Numerous deposits of pigment are seen.

Again on the eighty-fourth day an essentially fatty marrow shows a narrow cellular strip at the periphery in which erythrogenesis is active. Here and there leucogenesis predominates, but in the main the process is erythrogenetic. A few nucleated red cells of the megaloblastic type are found, but the more mature normoblasts are more abundant. In some centers radiating lines of four or five normoblasts are seen. Few giant cells are present.

The changes of the fourth and fifth months after splenectomy are not represented in this study. Well marked hyperplasia is, however, present in bone marrow representing periods of 6, 7, 12, 17, 18, and 20 months after splenectomy. On the other hand, two marrows representing respectively nine and a half and ten months show no departure from the normal fatty marrow, and in a third (eight months) only slight hyperplasia is evident. In the latter are areas composed almost entirely of cells of the myelocyte or premyelocyte type with some evidence of the formation of both red cells and polymorphonuclear leucocytes. The picture suggests a proliferation of the primitive cells of the marrow without, however, a very active function on their part. With evidence of well marked hyperplasia at six and seven months and after a year and a year and a half, it is impossible to explain its failure in these three animals representing the 8th, 9th, and 10th months respectively.

The best opportunity of studying the late changes is presented by material from six animals, representing the period from six to twenty months, in all of which the fatty marrow of the femur was transformed entirely or in large part into red marrow. The histological picture of each of these will be given in detail.

Dog 10.—Splenectomized May 20, 1913. Before operation the red cells numbered 6,910,000 and the hemoglobin was 105. The severest anemia was about

July 21; red cells 4,240,000, hemoglobin 62. On Sept. 11 the figures were 5,220,000 and 92. Later the animal became pregnant and anemia recurred, the picture on Nov. 18 being red cells 4,410,000, hemoglobin 78 per cent. On Nov. 24 the animal was chloroformed. At autopsy the medulla of both femurs presented a deep red marrow.

Histological Examination.—A uniformly cellular tissue is seen with occasionally a fat space here and there at the periphery. For the most part this marrow is as definitely cellular as is, for example, a lymph node or the spleen, and indeed it has the appearance of the pulp of the latter organ in the new born puppy. In this cellular mass, which at first appears to present a hopeless confusion of cells, it is not difficult to resolve the cells into more or less distinct proliferating centers. The arrangement is by no means a definite one, but in the patchwork of cells one sees groups which correspond to Bunting's description. In speaking of these centers we shall refer to them as erythrogenetic or leucogenetic, according to whether red cells or polymorphonuclear leucocytes predominate in the mass of cells surrounding the center in question. We have made no attempt to distinguish in these centers, which may include 6 to 10 or 20 to 30 cells, between the finely granular neutrophil myelocyte and the non-granular basophil cell from which it is supposed to arise. In these centers mitotic figures may occasionally be seen but only after prolonged search. It is also in these centers that old blood pigment, which is quite abundant in this marrow, is deposited; its deposition in the loose vascular tissue elsewhere has not been observed. The erythrocytic centers appear to be more active than the leucogenetic. This impression is based on the fact that about a mass of myelocytes composed of twelve to fifteen cells may be seen twenty-five to thirty nucleated red cells and a small number of normocytes, while about the leucogenetic centers comparatively few leucocytes are seen. The red cells in question vary in size and show changes from the megaloblast to the normocyte. It is not to be supposed that about erythrocytic centers no leucocytes occur. A few are always present; for example, among the twenty-five to thirty cells mentioned above, eight polymorphonuclear leucocytes could be clearly distinguished. Sometimes on one side of a center nucleated erythrocytes may be grouped, and on the other leucocytes with little intermingling. This suggests simultaneous formation of the two cells in one cell center. When this occurs the number of red cells is always greater than the number of leucocytes, in the proportion of 30 to 8. All through the section are lymphoid cells, usually single and of the small variety. Giant cells are frequent and a few show inclusions of polymorphonuclear leucocytes. Cells containing such inclusions have a broad homogeneous gray staining protoplasm suggesting necrosis. There is considerable pigment, but not many phagocytic endothelial cells are seen. Normoblasts are seen free in the capillaries. Smears from the marrow of the ribs show active erythrogenesis, and, on the whole, much the same cellular picture as the marrow just described. In the rib marrow a considerable number of eosinophils, chiefly polymorphonuclears, are also seen. In connection with the activity in the formation of red cells shown by the marrow, it is significant that the blood count six days before death was 4,100,000, and the hemoglobin 78 per cent. On Sept. 11, four months after splenectomy, the figures were 5,240,000 and 92. In other words, despite the hyperplasia of the bone marrow

the animal exhibited a late anemia, two and a half months after recovery from the initial anemia, following splenectomy. This may have been due to the drain occasioned by the intervening pregnancy,—an unfortunate complication from the point of view of the study of the blood. The fact remains, however, that the marrow is actively forming normal red cells. The anemia was, therefore, not due to insufficient erythrogenesis in the marrow.

Dog 39.—On Apr. 7, 1913, before splenectomy, the red cells numbered 6,528,000, and hemoglobin was 110 per cent. The lowest point of anemia was reached on June 3, the red cells numbering at that time 3,650,000; the hemoglobin was 62 per cent. By July 7 the blood picture had improved (red cells 5,080,000, hemoglobin 88 per cent.), but on Sept. 11, a late recrudescence of the anemia gave red cells 4,040,000 and hemoglobin 68 per cent. The animal was killed on Nov. 15. The bone marrow of the femur was of a definite red color. The anemia did not affect the general nutrition of the animal. On Apr. 7 the weight was 12,800 gm.; on Nov. 15 it was 13,950, and the adipose tissue was abundant.

This animal, representing practically the same period after splenectomy and the same changes in the blood, presents very much the same picture in the marrow. Of minor importance is the fact that the marrow is not so cellular, the proportion of cells to fat being in the ratio of about 3 to 2; also the myelocytic centers are not so pronounced, but in other respects the marrow is the same. Many giant cells are present but lymphoid cells are rare. The formation of red cells and leucocytes is perhaps not so rapid; that is, the numbers about any one center are not so great but, on the other hand, the activity of the marrow in connection with the former is sufficient to exclude the possibility of the bone marrow being responsible for the later development of anemia.

Dog 24.—This animal was splenectomized on Feb. 10, 1912, and was used for the injection of hemolytic immune serum on Mar. 20 and again on Apr. 7. On June 28 it had recovered from the anemia then produced (red cells 5,650,000, hemoglobin 89 per cent.), and on July 15 it was treated with sodium oleate. On Sept. 26 the red cell count was 5,780,000, and hemoglobin 90 per cent. On Feb. 19 the blood picture had improved (red cells 6,048,000, hemoglobin 110 per cent.), and at this time hemolytic serum was again injected. The animal was chloroformed on Mar. 4, 1913. The lapse of time since splenectomy was, therefore, thirteen months. At autopsy a red marrow was found.

Owing to the use of various hemolytic poisons, the bone marrow of this animal may have been influenced by other factors than the absence of the spleen. The histological picture, however, is so in accord with the marrow of simple splenectomy that, with this explanation, it is included in the series.

Histological Examination.—The marrow is uniformly solid with no fat spaces visible in any of the sections examined. It does not, however, appear to be as cellular as the marrow of dogs 10 and 39. This difference is caused by a greater congestion and distention of the blood vessels, a slight increase in the reticulum, and a lessened tendency of the myelocytic tissue to be grouped in large centers. Erythroblastic centers are very prominent and very active; leucogenetic centers, on the other hand, are made out with difficulty. Lymphoid elements are rare. Many cells of the myelocytic type are seen with coarse

basic granules and short threads in the nucleus, and with little or no protoplasm. In close relation to these are sometimes seen degenerated mitoses, but whether all these masses can be so interpreted is not clear. These degenerative changes are doubtless the result of the last injection of hemolytic serum.

Dog 59.—On July 24, 1913, this animal was splenectomized and used for the study of the progressive anemia following this procedure. On Dec. 7, 1912, the highest point of recovery was reached (red cells 5,250,000, hemoglobin 105 per cent.). Continued observation showed a slight decline to 5,200,000 red cells and 86 per cent. of hemoglobin on May 21, 1913, on which date the animal was used in an experiment with sodium oleate. From the moderate anemia caused at this time, the animal quickly recovered, the blood examination on June 9 showing 5,050,000 red cells and 86 per cent. hemoglobin, the condition slightly improving as to hemoglobin content until Nov. 18, 1913, when red cells were 5,100,000 and hemoglobin 101 per cent. The animal was chloroformed on Nov. 24. At autopsy the bone marrow of the femur was soft, succulent, and dark red in color. In connection with the general condition of this animal it is of interest to note that in the last seven months its weight increased from 10,450 to 12,580 gm., and that adipose tissue was very abundant. The administration of sodium oleate introduces a possible disturbing factor, but as this was given six months before death, it is not considered, in view of our other results, a serious matter.

Histological Examination.—The marrow shows some fat cells, the proportion of marrow cells to fat being about 10:1. Nothing new is presented. Leucogenesis and erythrogenesis proceed at about equal rate, the latter being a little more active. Mitotic figures are seen not infrequently, but the type of cell in which they occur is not always evident. Myeloblasts seem to be more abundant than usual. Giant cells are fairly abundant, but lymphoid cells are rare.

Dog 57.—On June 23, 1912, the blood of this dog contained 5,350,000 red cells per cubic millimeter, and 98 per cent. hemoglobin. On July 2 the spleen was removed. The resulting anemia reached its lowest point (red cells 2,970,000, hemoglobin 50 per cent.) on Aug. 5. On Oct. 24, two days after the blood count showed 5,240,000 red cells and 90 per cent. hemoglobin, the animal received sodium oleate intravenously; a very slight anemia (fall in hemoglobin to 62 per cent., but no change in red cells) resulted. In Jan., 1913, the red cells were 5,206,000, hemoglobin 110 per cent., and with slight variations this higher level was maintained, accompanied by an increase in body-weight, until Dec. 12, 1913, when the animal was chloroformed. At autopsy the animal was found to have a large amount of adipose tissue; the bone marrow of the femur was definitely reddish in color with faint yellowish streaks. As the sodium oleate given four months after splenectomy and fourteen months before death produced only a slight transient change, we consider that the bone marrow represents the effect of splenectomy only.

Histological Examination.—The relation of the fat to cells is about 1 : 1; otherwise nothing new is seen. The marrow is very active, leucogenesis and erythrogenesis being equally prominent. Phagocytic cells and masses of old blood pigment are quite numerous, as are also giant cells. More abundant than

in other marrows are eosinophils of the myelocytic type. Lymphoid cells are not conspicuous.

Dog 33.—This animal was splenectomized on May 14, 1912, the blood examination on the previous day showing 4,950,000 red cells and 85 per cent. hemoglobin. The anemia following splenectomy reached its lowest point on June 28 (red cells 3,550,000, hemoglobin 52 per cent.). On Sept. 20 the red cells had risen to 5,490,000 and hemoglobin to 95 per cent. In Nov., 1913, the animal passed successfully through pregnancy. In Jan., 1914, as the animal had developed mange, it was chloroformed. The blood examination on the preceding day was red cells 4,480,000, hemoglobin 70 per cent. At autopsy the bone marrow of the femur was deep red in color. It should be stated that one and two months before splenectomy the animal had received injections of hemolytic serum. From our studies of the effect of hemolytic serum in the normal dog, we do not believe that these injections, nearly two years before death, are in any way responsible for the hyperplasia of the marrow.

Histological Examination.—This marrow differs in no way from those of dogs 57 and 59 described above.

Dog 51.—The spleen was removed on May 31, 1912, and on June 26 hemolytic serum was administered. From the anemia thus produced the animal made a slow recovery, but after 200 days the blood examination showed 6,200,000 red cells and 110 per cent. hemoglobin, as compared with 6,210,000 red cells and 100 per cent. hemoglobin before splenectomy. On Mar. 26, 1914, when the animal was chloroformed, its weight was 9,750 gm. as compared with 8,270 gm. at the time of splenectomy, and 8,120 when hemolytic serum was administered. The notes made at the autopsy refer to the large amount of adipose tissue, the normal appearance of the lymph nodes, the absence of supernumerary spleens, and the presence in the long bones of a distinctly yellow fatty marrow.

Histological Examination.—The marrow shows a very slight hyperplasia with large numbers of leucocytes and deposits of blood pigment.

DISCUSSION.

In view of the slight changes seen in the bone marrow during the early periods after splenectomy, it appears that neither during the period of anemia and consequent repair nor in the period of hyperplasia of bone marrow are nucleated or other irregular forms of red cells found frequently in the peripheral blood. Careful differential counts of three dogs at regularly spaced intervals for 138 days failed to reveal in two the presence of nucleated red cells, and in one they were found only five times, the largest number seen in one count being three. In a large number of other animals in which differential counts were made at irregular intervals, changes in the red cells have been found very rarely; in one dog five weeks after splenectomy five normoblasts and two megaloblasts were

found (in 100 cells) with evidence of poikilocytosis and polychromatophilia, and a week after one normoblast and one megaloblast. These findings correspond to the first days of beginning repair, the red cells and hemoglobin having a few days before reached the lowest level observed during the experiment; hemoglobin 50 per cent., red cells 2,970,000. In another, two months after splenectomy, again at the stage of beginning repair (hemoglobin 92, red blood cells 3,650,000) five nucleated red cells were found, and a polychromatophilia was evident. In no instance did these findings persist for any length of time. If they have any significance it is that they indicate the period of beginning repair.

It is difficult to bring the changes in the bone marrow into relation with the changes in the peripheral blood. If the hyperplasia of the bone marrow is compensatory to increased blood destruction, or decreased blood formation, one would expect definite hyperplasia to be present in 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 after six months to a year or a year and a half when the blood picture is normal. It is true that in two of the animals (dogs 10 and 39) a late recrudescence of anemia occurred and the marrows of these animals were obtained during this period, but this was not the case in other animals of the series and is not characteristic of the late periods after splenectomy. It is therefore impossible, on account of the late development of hyperplasia in the marrow, to explain its occurrence as compensatory to the anemia following splenectomy.

Likewise we cannot accept Warthin's theory based upon his study of sheep and goats. In these animals Warthin found hyperplasia of the marrow to occur several months after splenectomy and to be associated with evidence of increased destruction of red blood cells in the lymph and hemolymph nodes. This destruction, greater than that in the primitive spleen, is responsible, he believes, for the anemia following splenectomy, and this is in turn compensated by increased activity in the bone marrow. We have found little to support this theory in our studies of the dog. The lymph nodes, as well as the endothelial cells of the liver, as we have shown elsewhere (20), are indeed more active after splenectomy than in the

normal animal in the phagocytosis and destruction of red cells, and this is very evident when large numbers of red cells are injured, as by the administration of a hemolytic poison; but in the ordinary course of events, after splenectomy, the lymph nodes present no evidence of excessive blood destruction. An occasional cell containing one or two red cells may be seen and small amounts of old blood pigment are occasionally demonstrable, but of excessive hemolysis there is no evidence. Microchemical tests for iron in the lymph nodes of fifteen splenectomized dogs showed a considerable amount of iron in three, slight amounts in five, and none in six. The animals examined represented periods of eleven days to twenty-two months after splenectomy. In the lymph nodes of eight normal animals similarly examined, moderate amounts of iron were found once, slight amounts three times, and in four, none. It is evident, therefore, that in the dog the iron content of the lymph nodes after splenectomy differs little from normal. The liver likewise shows no increased deposition of iron. Of fourteen livers from splenectomized dogs, four showed slight depositions of iron in the Kupffer cells, while ten showed none. At the same time the livers of six normal dogs were similarly examined; in three slight deposits of iron were found, and in three none. For this reason, and because the anemia is not persistent and progressive we cannot support the theory that the hyperplasia of the marrow is compensatory to abnormal blood destruction in the lymph nodes.

Another possible explanation is that the bone marrow, in the absence of the spleen, is concerned in the storing and utilization of iron. There is no doubt that, in the intact animal, iron set free in the dissolution of red cells is stored in the spleen. After splenectomy a readjustment in the storage of iron takes place, and there is some evidence that for a short time after the removal of the spleen iron is lost to the body. It is possibly this disturbance of iron utilization that is responsible for the early transient anemia. Our investigations[2] show, however, that this disturbance of iron utilization is transient and that after a few weeks the elimination of iron in the splenectomized animal differs in no way from the process in the normal animal. This suggests naturally that the storage of

[2] To be presented in detail in a future communication.

iron in the absence of the spleen is taken over by other tissues. As microchemical tests for iron showed no definite increase of iron in the lymph nodes and liver it seemed probable that the bone marrow might be the chief depot of iron storage. Such a view was supported by the fact that all hyperplastic bone marrows contain large amounts of altered blood pigment, sometimes free, but occurring, for the most part, in large phagocytic cells. The activity of these phagocytic cells presumably in transforming the iron of old blood pigment in order that it may be reutilized by the red cells, might, it was plausible to suppose, stimulate the other functions of the bone marrow, that is, the erythrogenetic and leucogenetic functions and cause eventually a replacement of the fatty marrow by a very cellular red marrow.

In order to prove this hypothesis it was necessary to obtain some idea of the iron content of these marrows. Direct chemical analysis was out of the question on account of the small amount of material available and the variations in blood and bone content of different marrows. We therefore made a comparative study based on the use of the microchemical reaction for iron. This demonstrated at once that all red marrows in our series have a large content of iron, and that fatty marrows contain very little or no iron. On the other hand, when the marrows of non-splenectomized dogs rendered hyperplastic by anemia or infection were examined it was found that these also had a large iron content. Thus in a group of 17 non-splenectomized dogs iron was present in the marrow in large amounts in 4, in moderate amounts in 2, in small amounts in 4, and in 7 none was found. On the other hand, in 27 splenectomized dogs, iron was present in large amounts in 10, in moderate amounts in 3, in small amounts in 4, and absent in 10.

In both groups the amount of iron was in direct proportion to the degree of hyperplasia. These observations point therefore to the conclusion that a red marrow is always rich in iron, but it is impossible to say whether the cellular hyperplasia or the iron deposition is primary. Under the circumstances, it is also impossible to conclude that the late hyperplasia of marrow following splenectomy is an attempt to conserve iron. Moreover, the irregularity of our results as shown by the failure of hyperplasia in four animals,

representing respectively the 8th, 9th, 10th, and 22d months after splenectomy, prevents in the present state of our knowledge an adequate explanation of the cause of the transformation from yellow to red marrow.

The divergent results in this study are characteristic of all phases of experimental work on the spleen and doubtless are to be explained by the fact that removing the spleen takes away only one organ of a system composed of liver, spleen, lymph nodes, and bone marrow, and that the interrelations which exist in this system may or may not under varying circumstances bring into play compensations of the greatest importance in determining the degree of blood destruction or regeneration and therefore the degree of change in the bone marrow.

A search of the literature of splenectomy in man, although it reveals evidence of the occurrence of red marrow in various forms of splenic anemia (7), offers little of importance concerning the changes which occur in the bone marrow after removal of the normal spleen. Several references (21) are made to the occurrence of pain in the long bones after splenectomy, and by some this has been assumed to be evidence of hyperplasia within the rigid bony canal. The only note of the direct examination of the bone marrow after splenectomy is that of Riegner (22), who found active proliferation of the marrow of the femur in a man whose leg was amputated for gangrene four weeks after splenectomy for trauma. It is therefore impossible, on account of this paucity of data concerning the changes in man, to bring them into relation with our experimental results.

CONCLUSIONS.

Splenectomy in the dog causes, as a rule, a transformation of the fatty marrow of the long bones to a richly cellular red marrow.

During the early periods, one to three months, the change in the marrow is slight and either focal or peripheral; after six to twenty months the replacement of fat by marrow cells is complete or nearly so. Exceptions were, however, seen in four animals representing the 8th, 9th, 10th, and 22d months, respectively. The evidence at hand does not support the theory that this hyperplasia is compen-

satory either to the anemia caused by splenectomy or to an increased hemolysis in the lymph nodes. It is possible that it may be a concomitant of the activity of the bone marrow in taking over, in the absence of the spleen, the function of storing and elaborating the iron of old blood pigment for future utilization by new red cells, but our studies do not fully support this view.

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