# THE FACTOR DETERMINING THE SPREAD OF RED MARROW DURING ANEMIA.

By PHILIP D. McMASTER, M.D., and HERBERT HAESSLER, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATES 47 TO 49.

(Received for publication, June 20, 1921.)

It has long been known that in certain forms of anemia the red marrow extends throughout the bones, whereas in other forms equally chronic, little if any marrow spread occurs. The cause for this striking difference is not clear. Some experiments here to be detailed bear on the problem thus presented.

Several observers have noted that blood regeneration takes place less rapidly after hemorrhage than when a destruction of corpuscles has occurred within the body.[1,2] The extraordinarily rapid improvement of cases of pernicious anemia after the so called blood crises, as well as the active regeneration of erythrocytes which follows hemolysis by poison, have long excited comment. Itami and Pratt[3] found that rabbits rendered anemic by destruction of red cells within the body recover much faster than control animals deprived of blood by hemorrhage; and Robertson[3] showed that animals rendered plethoric by transfusion recovered with extraordinary rapidity from anemia due to intravascular blood destruction. An obvious explanation of these diverse facts is to be found in the conservation within the body of the products of the destroyed cells, and it has been shown that injections of laked blood will hasten regeneration after bleeding.[4] What is the part played by the stroma substances in this recovery? May one perhaps deplete the organism of them and thus lead to the production of fragile cells, similar possibly to the cells that are characteristic of hemolytic icterus? Our experiments were devised originally to answer this question. Animals were bled repeatedly and

[1] Ritz, H., *Folia hæmatol.*, 1909, viii, 186.

[2] Itami, S., and Pratt, J., *Biochem. Z.*, 1909, xviii, 302.

[3] Robertson, O. H., *J. Exp. Med.*, 1917, xxvi, 221.

[4] Itami, S., *Arch. exp. Path. u. Pharmakol.*, 1910, lxii, 104.

the hemoglobin was replaced in the hope that blood regeneration would thereby be so greatly enhanced as to exhaust the store of stroma substances.

Four series of twelve rabbits each were rendered anemic by almost daily bleedings from the heart during a period of 6 to 8 weeks. Prior to the first bleeding the animals were kept under observation for several days and repeated hemoglobin estimations were made by the Palmer method, and the resistance of the cells to hypotonic salt solution was determined. On the basis of the findings, the animals were grouped into pairs having similar weights and hemoglobin readings; and repeated bleedings from the heart were begun by an aseptic technique. One individual of each pair received a solution of concentrated hemoglobin immediately after each bleeding by subcutaneous injection into the anterior and lateral abdominal walls, care being taken to avoid the veins. The injected pigment was distributed in the tissue by gentle massage. The hemoglobin solution was prepared by the method of Sellards and Minot[5] from fresh rabbit blood obtained by cardiac aspiration, and was made up with salt solution to a percentage (140 per cent, Palmer) somewhat in excess of that found in the blood. The amount injected, 5 to 7 cc. each time, or slightly more pigment than was removed at a single bleeding, was rapidly absorbed. Usually a slight reddish stain alone remained at the end of 24 hours and there was never any noticeable tissue reaction.

To avoid introduction of the factor of malnutrition which is known to depress marrow activity (Naegeli), the bleedings were so proportioned as to cause only a mild anemia. The weight was carefully followed and usually a gain was noted during the experiment. 10 cc. of blood was taken (in large animals 15 cc.) every day until the hemoglobin reading had fallen to just below 50 per cent. At this approximate level it was maintained. Any rabbit showing a less amount of hemoglobin was not bled until regeneration had again brought the pigment above 50 per cent. As the rabbits had, to begin with, hemoglobin readings of only 75 to 100 per cent, it will be seen that the degree of anemia induced was not great. The individuals given injections of hemoglobin received it every week-day, whether bled or not. After 2 or 3 weeks of bleedings and injections, they regularly formed hemoglobin much more rapidly than the controls, as shown by the fact that far more blood had to be taken from them to keep their hemoglobin at the 50 per cent mark.

The resistance of the red cells to hypotonic salt solution was taken as the index to their fragility. It was determined frequently according to the usual technique. From time to time a color index was obtained, that is to say the ratio of hemoglobin to total cell bulk in a given amount of blood as determined by Epstein's microhematocrit method.[6] The observations on the blood were always made in the morning and the bleedings and injections followed in order.

---

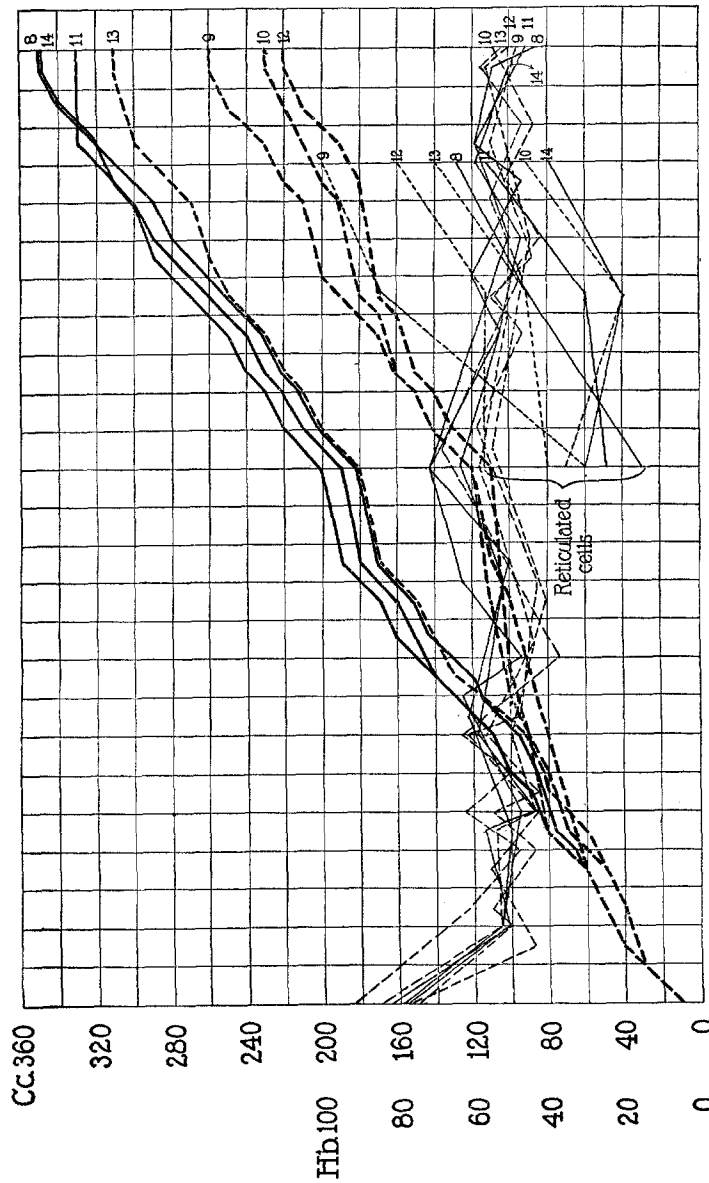
[5] Sellards, A. W., and Minot, G. R., *J. Med. Research*, 1917, xxxvii, 161.

[6] Epstein, A. A., *J. Lab. and Clin. Med.*, 1915–16, i, 610.

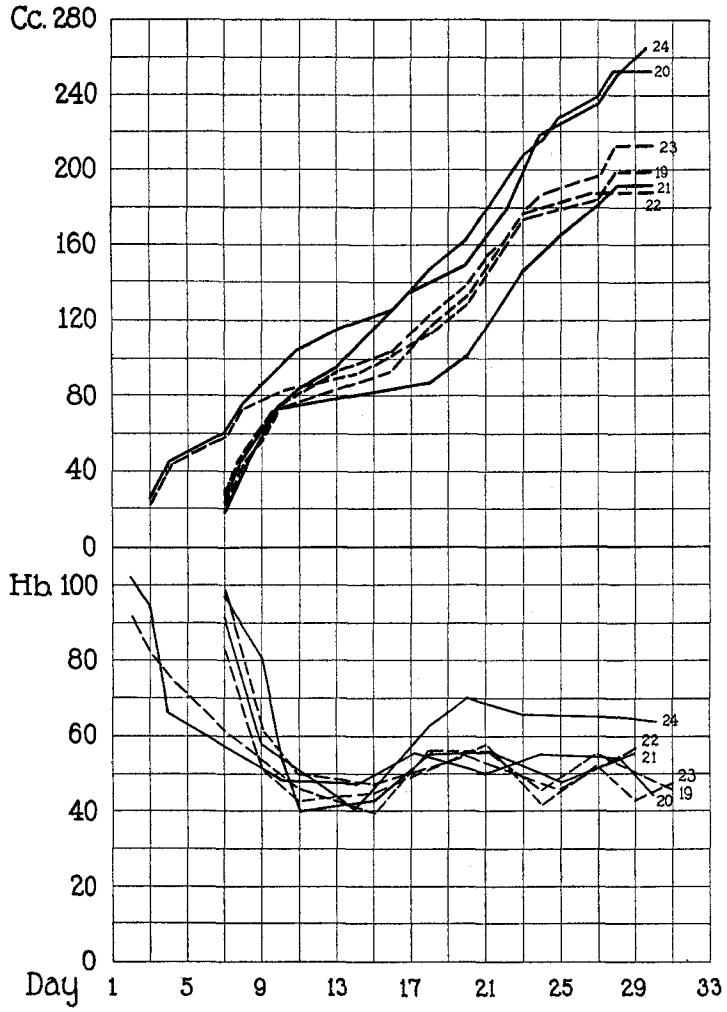
*Blood Changes.*

The most marked difference, manifest during life, between the experimental animals and the controls was the greater rapidity of blood regeneration in the former. This was expected, and save on the assumption of its occurrence our experiments would not have been undertaken, for only through its means could the opportunity for stroma depletion be rendered relatively great in the experimental animals. That the removal of corpuscular substance was far more considerable in them than in the controls is shown by relative amounts of blood removed (Text-figs. 1 to 4), taken in connection with the color index. For this latter, as already stated, the ratio was used, not of hemoglobin to cell number, since the size of the cells may vary, but of hemoglobin to corpuscle volume, that is to say, to actual bulk of hemoglobin-containing tissue. This ratio remained practically the same in the injected and control animals, whence it follows that the depletion recorded in the charts refers not merely to hemoglobin but to total corpuscle substance as well. Though the depletion was far greater in the injected animals, the resistance of their cells gave no indication of a dearth of stroma substance, showing only the same insignificant variations observable in the controls.

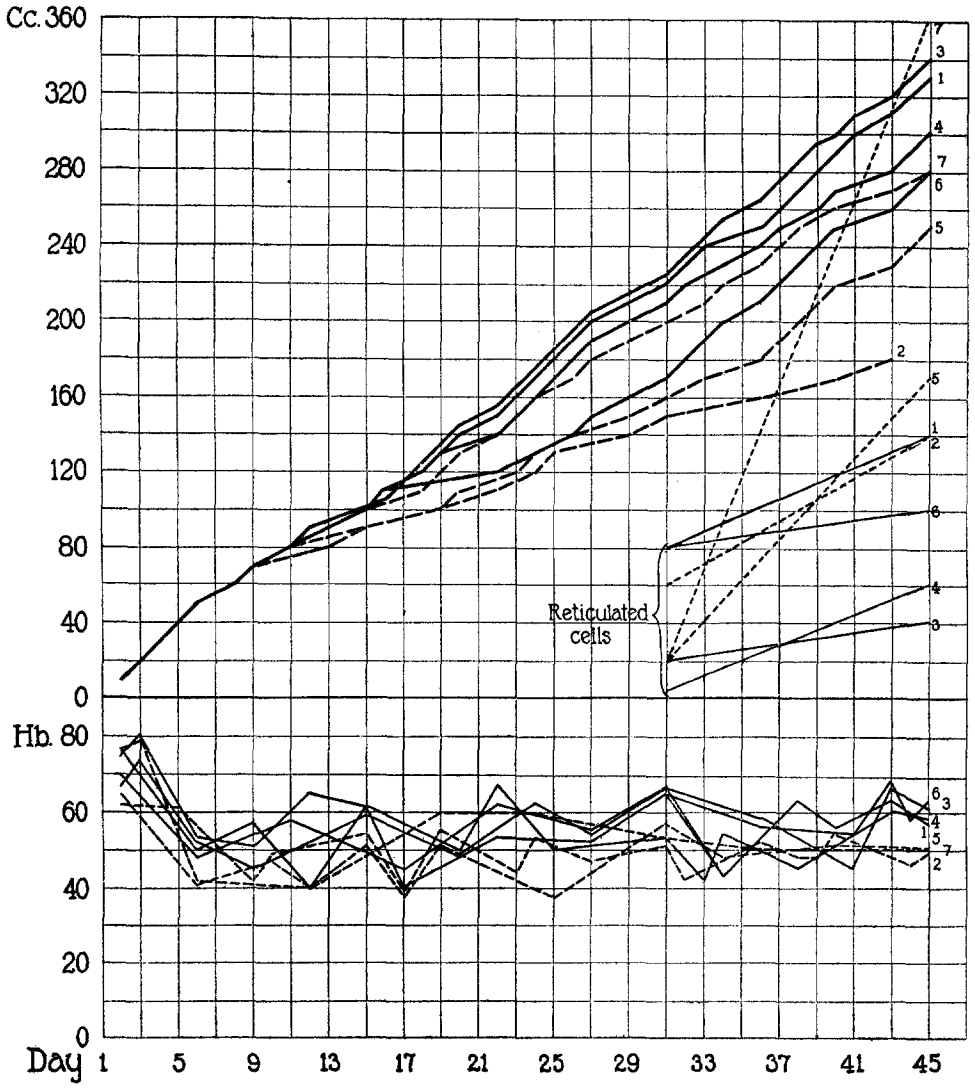
By another clinical method evidence of a stroma lack was sought for. Under the conditions of active regeneration from anemia an increased number of reticulated red cells is found in the blood. Many, indeed most, of these cells as there seen are polychromatophilic and obviously imperfect. It was thought that marrow strain might be relatively great in the injected animals, since they were manufacturing more blood than the controls and had less stroma material to do it with, and that, therefore, a larger proportion of reticulated red cells would be found circulating in them. The exact opposite proved to be the case. Though with the development of anemia the reticulated cells increased in both sets of animals, they were, after the first few weeks, most numerous in the controls and at length strikingly so, as is shown by Text-figs. 1 and 3. Only very occasional nucleated red cells were found.



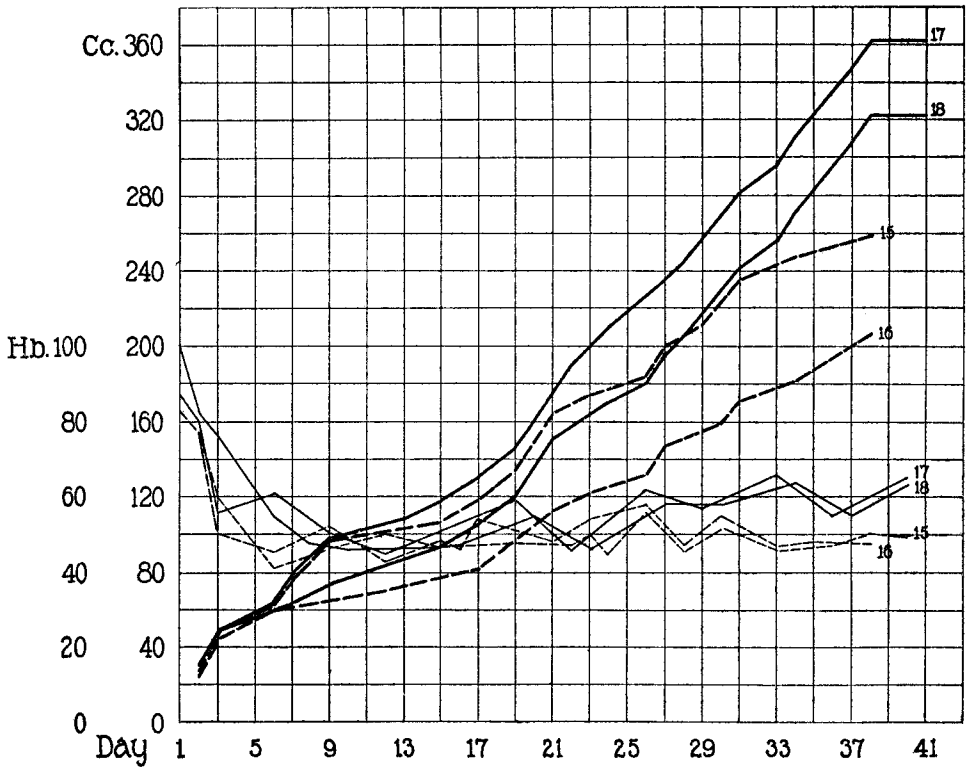

TEXT-FIG. 1. Illustrating the effect on hemoglobin regeneration of subcutaneous injections of the pigment. There were seven rabbits, three of which (Nos. 8, 11, and 14) received injections while the others served as controls. All were bled to the extent necessary to keep their hemoglobin at approximately 50 per cent (Palmer). In the chart, one set of faint lines shows the individual hemoglobin percentages from the time the bleedings were begun. The heavy lines indicate the mounting totals of blood removed from each rabbit. A second set of faint lines shows the late variations in the number of reticulated red cells in circulation. In order to simplify the chart many of the daily determinations which merely fall in with its general trend have been omitted. It will be seen that much more blood was taken from the injected animals to maintain their anemia at the level set than was necessary in the controls. The difference was not at first noticeable but became marked as time passed. Late in the experiment reticulated red cells were relatively less numerous in the injected animals despite the fact that these were forming more blood than the controls.




TEXT-FIG. 2. The same general explanation applies here as in Text-fig. 1, and nearly identical findings are illustrated. Rabbits 20, 21, and 24 received the pigment injections. Of these only No. 21 failed to regenerate hemoglobin better than the three controls. The percentage of reticulated cells is not charted.




TEXT-FIG. 3. This figure shows the same facts as those preceding. Despite the large quantities of blood removed from the injected animals (Nos. 1, 3, 4, and 6) they were not rendered quite as anemic as the controls.




TEXT-FIG. 4. In the injected animals (Nos. 17 and 18) of this series, the bleedings were eventually pushed to the point of danger, owing to the repeated sudden reductions in blood volume, yet the percentage of circulating hemoglobin was not prevented from rising above that of the controls.

*Morphological Changes.*

The reason for the differences above noted was strikingly evident at autopsy. The erythropoietic tissue of the animals injected with hemoglobin had undergone extensive hyperplasia, whereas that of the controls had increased little if at all.

The animals were killed with chloroform. Pieces of the liver, kidneys, and spleen were saved to determine whether there had been extramedullary blood formation. It was never notable. All of the large, long bones—femur, tibia, humerus, and ulna—were stripped of muscle and split open along one side to expose the entire length of the marrow cavity. It was early recognized that only by averages might a conclusion be arrived at as regards marrow spread, since the red marrow of normal rabbits varies greatly in extent. Thus, for example, in the femur it may occupy only the upper third of the shaft or practically the whole of it, though in the latter case there is ordinarily a considerable admixture of fatty tissue toward the distal end. In the tibia, a little red marrow is normally present at the proximal end and somewhat less is found in the humerus and ulna. All of the marrows removed from the anemic animals were fixed in Zenker's fluid and sections made,—in some series of the entire marrow length and in others of cross-sections of the proximal, middle, and distal portions.

It cannot be said that in the control animals there was any definite increase of hematopoietic tissue over the normal. Always it was rather pale, and it extended, mixed with more or less fat, throughout the upper one- to two-thirds of the femur, but was never present in any great quantity in the humerus, tibia, and ulna. A far different condition was encountered in the injected animals. Here the entire shafts of femur and humerus were filled with deep red, typical "currant jelly" marrow, and the tibia and ulna contained a similar tissue throughout their length which in the controls held almost nothing but fat. Figs. 1 to 4 illustrate the findings. We would again emphasize the fact that our statement is based not on individual instances, for these vary considerably, but on the assembled findings in 48 rabbits.

The histological pictures as a whole showed a contrast as great as the gross. In the injected rabbits the extension of the red marrow at the expense of the yellow was, of course, well seen, but far more important was a qualitative change in the first mentioned tissue, namely an enormous increase in the erythrocyte-forming elements as

distinct from the leucogenic. Under the microscope large aggregations of the pycnotic nuclei of immature red cells, the mark of erythrogenic islands of great size, at once attracted attention. All the tissue was extremely vascular. A characteristic finding is shown in Fig. 3 and should be contrasted with that of Fig. 4 obtained from the same level in the femur of the control rabbit.

## DISCUSSION.

The experiments yielded no evidence that rabbits can be depleted of stroma sufficiently to affect the resistance of the red cells to hypotonic salt solution. Rather do they indicate that the elements which go to form stroma can be provided by the body on occasion in quantities enormously in excess of those demanded by any ordinary emergency. This view is supported not only by the blood findings but by the great spread of the erythropoietic tissue in the animals receiving hemoglobin.

All of the rabbits remained healthy and well nourished, and the anemia in all was but moderate. These facts seem to us to rule out the possibility that the injected pigment served merely as a food in the ordinary sense. The diet was mixed and contained much green vegetable, so that an abundance of iron must have been available to the controls. It is possible, of course, that iron compounds readily utilized by the marrow may have been lacking in them and present in the experimental animals by virtue of the injected hemoglobin. But certainly the injections did not bring about any noteworthy short cut in the elaboration of hemoglobin-containing cells. Their effect on the blood did not become clearly evident until during the 3rd week of anemia, as the charts show. Long before this time, after but a few bleedings, corpuscles poor in hemoglobin were circulating in large numbers just as in the controls. Had any considerable amount of the material provided by the injections made its way to the corpuscular tissue by a metabolic route more direct than the normal, its effect should have been evident early in a rapid replacement of the circulating hemoglobin and perhaps in a relatively high color index. A high color index has indeed been recorded during recovery from anemia abruptly produced by a hemolytic poison or

by sudden intercurrent blood destruction in animals long plethoric from transfusion.[3] But in these instances, the index was determined on the basis of cell number, whereas in the present work the total bulk of the cells in a given amount of whole blood has been used instead. The distinction is worthy of stress since the average size of the corpuscles varies much under different conditions.

Late in the experiments when the injected animals were repairing their blood loss far more rapidly than the controls, reticulated cells were less numerous in their circulation—a fact which at first seems an exception to the generally accepted rule that in anemic states the number of reticulated cells bears a rough relationship to the rapidity with which regeneration is going on. But the exception is only an apparent one. The demands for new blood were nearly identical in all of the rabbits, but they were exerted upon very different amounts of marrow; and in those possessing most of this tissue, that is to say the injected animals, repair would inevitably take place most quickly, granting that the blood-forming elements worked at the same rate in all. Had this been the only factor concerned, the number of circulating reticulated cells should have been approximately the same in both sets of animals. But in the injected rabbits there was an additional advantage. Blood repair in them went on so fast that the 24 hours between bleedings was sufficient for recovery to a slightly higher hemoglobin percentage than in the controls. Thus the anemia from day to day was not really quite so severe as in the latter—a fact reflected in some of the charts,—and the demands on the bone marrow were less drastic and elicited the setting-free of fewer reticulated cells.

The fact is well attested that anemia induced by letting blood from the healthy body does not suffice to cause any marked marrow hyperplasia. Some other factor is necessary. This factor, as shown by our experiments, is the presence in the body of hemoglobin in excess of the amount that the existing hematopoietic tissue can utilize. Under the conditions of a moderate anemia of fixed grade, such as we have dealt with, the marrow is supplied from the body with enough hemoglobin to manufacture cells of a certain kind at a certain rate, cells which may be considered as the inevitable product of the interacting conditions. When more hemoglobin is supplied, as in our

injected animals, and the degree of anemia is not altered, the physiologic choice arises of more heavily pigmented cells to be formed by the same tissue or merely a greater quantity of cells of the same sort. The second alternative is the one chiefly taken; and as the output of the marrow increases this tissue itself spreads.

When the clinical and marrow findings in human anemias of different origin are compared, it is seen at once that there are two large classes which correspond in essentials with our rabbits. Included in the one class are pernicious anemia, malarial anemias, anemia from purpura, and that of various hemolytic origins, in all of which the blood is destroyed within the body and more or less conservation and utilization of its constituents may occur with result that the "turn over" of corpuscular material by the combined processes of destruction and restitution becomes abnormally great. In these cases as in our injected rabbits the marrow spreads. And then there are the anemias from hemorrhage, or from iron deprivation, in which as in our control rabbits there exists a lack and the marrow fails to spread, or spreads slightly and slowly if conditions render the elaboration of hemoglobin relatively easy. Anemias due to marrow depression lie, of course, outside both categories.

Repeated reference has been made thus far to a repair by blood quantity *versus* one by blood quality (altered color index). While the former means of repair preponderated in the conditions with which we have had to deal there are undoubtedly states in which the second is of much importance. In pernicious anemia, as Capps[7] has shown, the color index based on hemoglobin *versus* the volume of the massed corpuscles is often higher than normal, showing that the excess of pigment available for blood formation has had the effect of altering the blood tissue in a qualitative direction. But when this is the case the quantitative output tends to be even more markedly affected, as shown by the enormous marrow spread. That the marrow in cases of pernicious anemia can, on occasion, function as its spread would seem to warrant, is proven by the noteworthy rapidity with which blood repair occurs during remissions in the disease.

[7] Capps, J. A., *J. Med. Research*, 1903–04, x, 367.

SUMMARY.

Rabbits in which a chronic anemia of moderate grade is induced by repeated bleedings repair the hemoglobin loss much more rapidly when given subcutaneous injections of hemoglobin than when this is not the case. But the effect of the injections is not manifest for several weeks, during which many pale corpuscles are put out by the marrow; whence it follows that the introduced pigment does not find its way in quantity direct to the new-formed cells but must follow a more or less roundabout metabolic route, perhaps the same one as that of ordinary iron compounds destined for the blood.

The rapid replacement of the circulating hemoglobin in the injected animals occurs chiefly through an increased production of corpuscular substance having the same color index as that found in uninjected, anemic controls. By color index in this connection is meant the relation of hemoglobin to the volume of the massed corpuscles.

Late in the period of bleedings and hemoglobin injections the demand for stroma for the new-formed blood is far greater than in control animals that have been merely bled, yet the circulating corpuscles show no lessening in resistance to salt solution, such as might perhaps be expected were there a stroma lack. The hematopoietic tissue of the injected animals undergoes an extensive increase—a fact which speaks strongly for the view that the elements out of which stroma is formed are still abundant.

The factor which determines the spread of red marrow during anemia is shown by our experiments to be the presence in the body of hemoglobin, or perhaps of its precursors, in excess of the amount which can be utilized by the marrow already existing. Numerous illustrations in support of the point can be adduced from human pathology. Two will suffice. The widespread "currant jelly" marrow of pernicious anemia is found in an organism rendered anemic but supplied with hemoglobin in excess; while the pale, restricted marrow of cases suffering from chronic anemia due to repeated hemorrhages is associated with depletion of the constituents necessary for pigment production.

## EXPLANATION OF PLATES.

### PLATE 47.

FIG. 1. Humeri of Rabbits 1, 4, 6, and 18 at left—Nos. 5, 2, 7, and 15 at right (see Text-figs. 3 and 4). Those of the injected animals are on the left. The marrow in them is of dark red "currant jelly" character whereas that of the controls is for the most part fatty. Fresh specimens.

### PLATE 48.

FIG. 2. Femora of the same rabbits grouped in the same way and showing the same differences.

### PLATE 49.

FIGS. 3 and 4. These figures are taken from the same level in the distal portion of the femur marrow of Rabbits 11 and 13, an injected animal and its control which were bled almost equal amounts (Text-fig. 1). The replacement of fat tissue with hematopoietic elements is seen to be almost complete in Fig. 3, from the injected animal. The abundance and large size of the erythropoietic islands, as indicated by aggregations of pycnotic nuclei, are especially noteworthy.

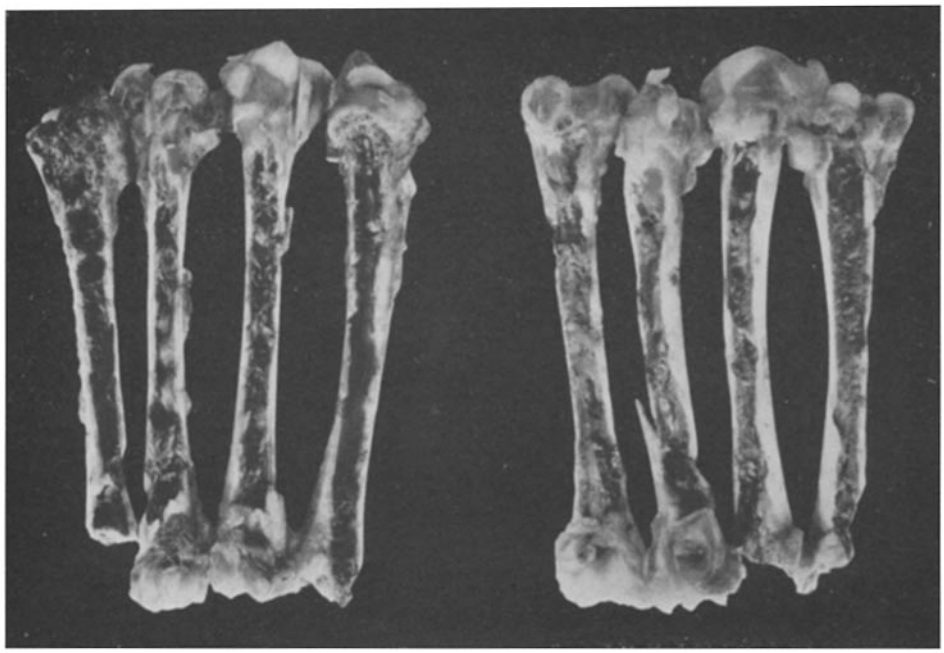


FIG. 1.

(McMaster and Haessler: Spread of red marrow during anemia.

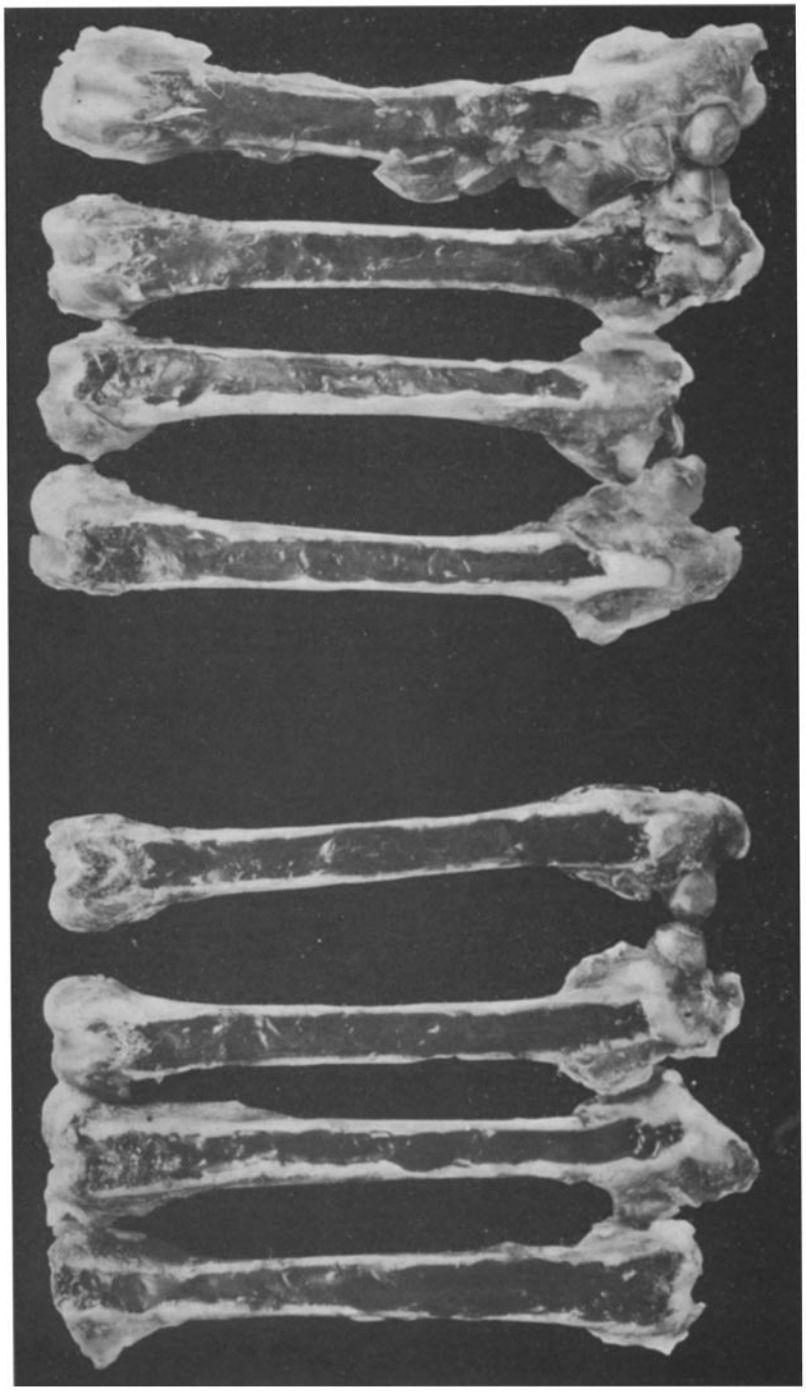


FIG. 2.

(McMaster and Haessler: Spread of red marrow during anemia.)

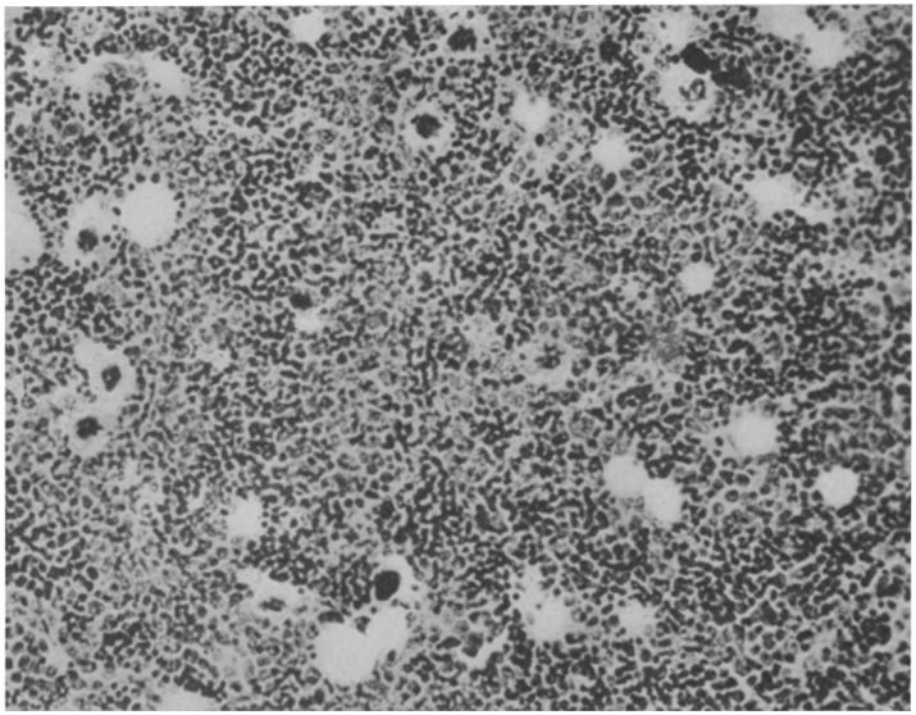


FIG. 3.

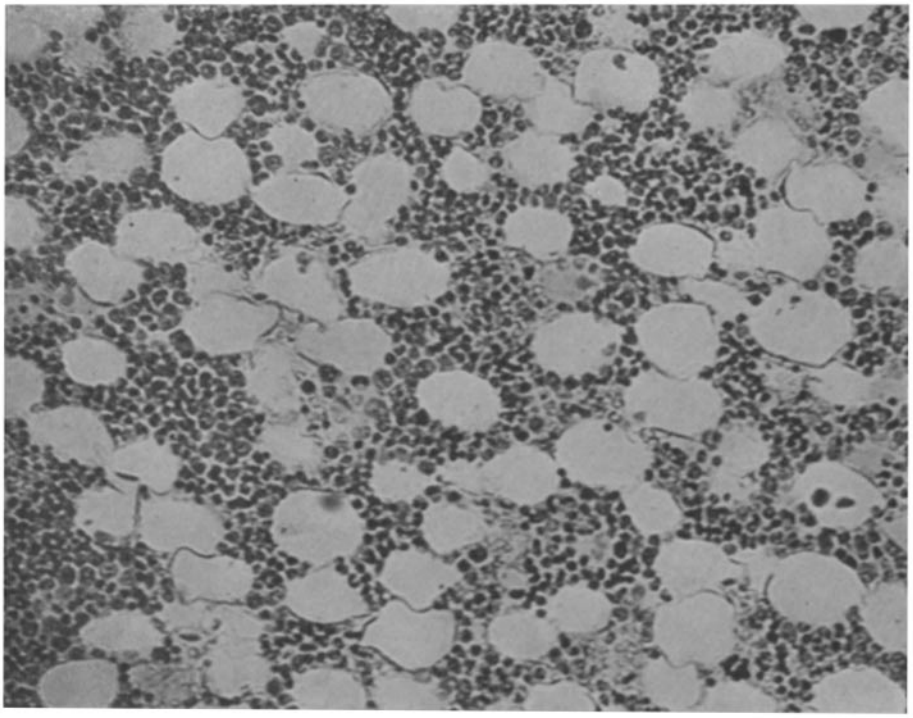


FIG. 4.

(McMaster and Haessler: Spread of red marrow during anemia.)