CONCERNING THE RELATIVE RESPONSE TO BLOOD GAINS AND BLOOD LOSSES; AND HABITUATION TO AN EXCESS OF BLOOD PIGMENT

By MACDONALD DICK, M.D.

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

The experiments to be described were begun with the aim of determining the amount of blood, as red cells, formed each day in rabbits. It was assumed that if this amount were supplied from without the erythropoietic tissue would cease to work. Robertson (1) had noted that repeated large transfusions resulting in a superabundance of hemoglobin caused a practical disappearance of reticulated red cells from the circulation of rabbits, indicating that the marrow had become less active. When, after a time, the alien blood was suddenly destroyed as result of the formation of immune bodies by the recipient, a profound anemia developed, this fact as well as others showing that the transfused cells had functioned so effectively that the marrow had largely left off erythrocyte production. In some recipients no blood destruction was evident, and these formed no antibodies demonstrable on test. For the purpose of the present work such a state of affairs was essential. A measured small amount of compatible blood was introduced into the circulation each day, with the expectation that the marrow would lessen its erythropoietic activity in proportion as its task was taken over, the amount of strange blood necessary to induce it to cease work being presumably that which it would form each day under normal conditions. The results of the experiments have proved the primary assumption to be incorrect. No indication has been found that marrow activity lessens when small quantities of compatible blood are added to the circulation day after day. On the contrary, the marrow keeps on working and itself contributes to the increase in hemoglobin. Not only this, but when no more blood is introduced from without, and the superabundance of hemoglobin begins to diminish, the marrow becomes abnormally active, producing red

cells in such quantity as to maintain the abnormal state of affairs. An habituation of the organism to this state has come about.

There would appear to be no papers in the literature on the consequences of bringing about a superabundance of hemoglobin gradually by experimental means, though there are many describing the consequences of doing this abruptly, and many dealing specifically with the relation between marrow activity and hemoglobin concentration. Boycott and Oakley (2) have recently dealt with the latter theme comprehensively, in a paper appearing since the present work was completed. Interested primarily in the regulation of marrow activity, they undertook to stop it by supplying blood from without; but this they found themselves unable to do. The conditions were drastically altered in their experiments, and their findings differ in many respects from those now to be reported. Their data, considered in parallel with our own, provide numerous enlightening contrasts. They discuss in detail some of the problems of marrow activity and for these, as for not a few other pertinent matters, it has seemed well to refer the reader to their paper rather than to attempt to recapitulate what has been admirably summed up.

The Effects of Gradual Additions to the Blood

A rough, first test was made to find how much blood must be introduced daily into the circulation of rabbits in order to cause the erythropoietic tissue to stop working. As a criterion of stopping work the disappearance of reticulated red cells was looked for; and at the end of the series of transfusions the red marrow of some of the animals was examined for its content of these cells. A group of six normal rabbits were given transfusions of whole compatible blood, 6 days in 7, the daily amounts being $\frac{1}{2}$, 1, 1$\frac{1}{2}$, 2, 3, and 4 cc. for the respective animals.

An extensive literature shows that the number of reticulocytes in circulation varies with the erythropoietic activity; and dependence is now justifiably placed upon the count as indicative of what is occurring in the red marrow. Boycott and Oakley discuss these points at length and they have succinctly stated the general conclusion of investigators:—" . . . while changes in the proportion of reticulocytes are a good index of changes in marrow activity they are not a directly quantitative measure of it." Robertson (1) found that the marrow of rabbits maintained in plethora by the injection of large amounts of blood showed but few reticulocytes as compared with the normal content.

The rabbits, normal animals of mixed breed from stock, weighed from 1200 to 1550 gm. at the beginning of the transfusions and from 1600 to 2100 gm. at their end. They were kept in separate cages and fed hay daily, with the addition three times a week of a mixture of equal parts of oats and commercial food pellets. Water was available to them at all times.

In this series we followed the method employed by Robertson (1), aspirating the blood directly from the heart into a syringe containing 1 cc. of a 1 per cent solution of sodium citrate in normal saline. Normal compatible donors were employed in rotation, each being discarded after two or three bleedings to the amount necessary for all of the transfusions of a day. The method of Rous and Turner (3) was employed in the tests for compatibility. For 20 days before the transfusions were begun and just prior to each of these latter, reticulocyte counts and determinations of the blood hemoglobin were made. During the preliminary period the amount of hemoglobin did not vary significantly, but in several cases (Charts 1 and 2) the number of reticulocytes increased gradually.

The blood samples for counts and hemoglobin determinations were regularly taken in the morning, before the feeding of the day. Both counts and determinations were done by the same person in all of the experiments. They were made on blood from the vein of an ear rendered hyperemic by contact with a bottle of warm water. For the hemoglobin determinations, 20 c.mm. of blood was mixed with 5 cc. of 0.1 N hydrochloric acid and allowed to stand at least 1 hour. The readings were made by means of a Duboscq colorimeter supplied with the yellow glass matching-disc of Newcomer (4). The findings are expressed in grams per 100 cc. of blood.

A white cell pipette was used for the reticulocyte counts. Blood was drawn up to the 1 division and diluted to 11 (1–10) with the staining mixture employed by Friedlander and Wiedeman (5). Staining took place for 15 minutes when a drop of the mixture was placed on a slide and counting was begun at once of the reticulocytes occurring among a thousand cells in uniform fields.

The eventual reticulocyte determinations on the red marrow were made in two ways. In one a small piece of marrow was smeared on a slide previously prepared by allowing a concentrated alcoholic solution of brilliant cresyl blue to dry upon it, thus leaving a film of the dye. Such preparations could be counterstained with Wright's stain and were useful in examining for other manifestations of bone marrow activity. The second method was to wash out a portion of the marrow by forced injections into it of normal saline through a hypodermic needle after the method of Robertson. The mixture thus obtained was spun and counts were made on the sediment diluted with staining mixture as in the case of the blood samples. The period of transfusion ranged from 30 to 70 days.

In only one of the six recipients did an evident incompatibility develop to mar the findings. In this animal, which received 3 cc. of blood each day with result that the hemoglobin percentage mounted rapidly, there occurred the characteristic phenomenon first described by Robertson; namely, a suddenly developing, pronounced anemia although the transfusions were continued. With the anemia there was associated an appearance of strong isohemagglutinins in the blood.

The circulating hemoglobin diminished from 11 gm. to 4 gm. per cent between the 7th day and the 14th day of transfusion, and then rapidly mounted again, the percentage of reticulocytes rising from 20 to 550 per thousand as repair took place. The animal will not be considered further.

No hemoglobin increase developed in the rabbit receiving $\frac{1}{2}$ cc. of compatible blood per day during more than 1 month of transfusion, and its reticulocytes varied throughout within the limits of the pre-transfusion normal. In each of the other four rabbits, those receiving daily 1, $1\frac{1}{2}$, 2, and 4 cc. of blood respectively, the hemoglobin percent-
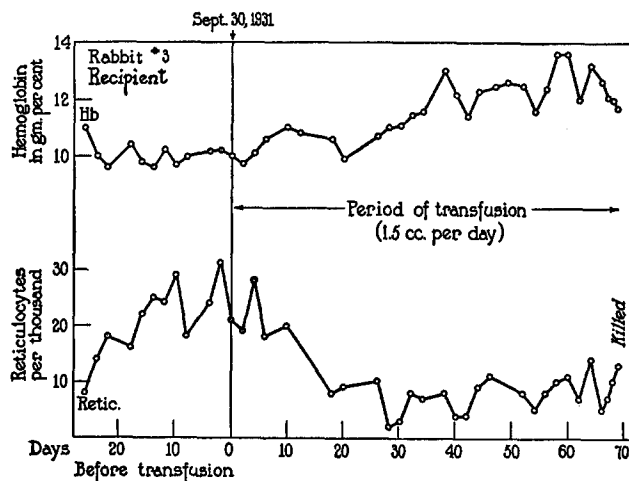

CHART 1

age increased,—gradually in the case of the rabbits receiving the smaller amounts of blood, promptly and greatly in the one injected with 4 cc. In all these instances some depression of marrow activity occurred as evidenced by a drop in the reticulocyte percentage; but it did not fall enduringly below the level at the beginning of the pre-transfusion period, and at autopsy the marrow count of reticulocytes was not significantly different from the normal, great numbers of these cells being present. Charts 1 and 2 illustrate the findings.

As already stated, the animals serving as recipients were "normals" selected from stock. It seemed possible that their initial amounts of

hemoglobin (9 to 10 gm. per cent) might have been near the lower limit
for normality, and that the hemoglobin increase, after the smaller
transfusions at least, might not have constituted a superabundance
of the pigment but have been only a natural betterment consequent
upon unusually favorable conditions for blood production. The rab-
bits were still growing rapidly, which introduced another variable. In
the attempt to control conditions more strictly, a new group of ani-
mals was studied, adults selected as having large initial quantities of
hemoglobin in the circulating blood. Two had 12 and 13 gm. per
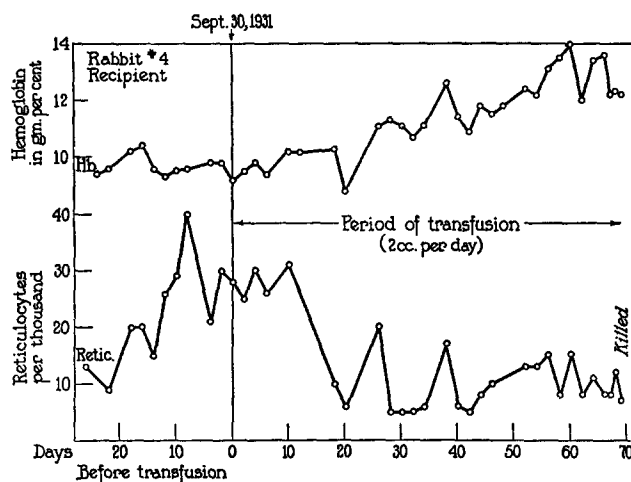cent, and these received every day for 35 days without exception $\frac{1}{2}$ cc.

CHART 2

of compatible blood; while three other animals with 11, 12, and 12.1
gm. per cent of hemoglobin respectively were given 1 cc. *per diem*.
The weights ranged from 1880 to 2280 gm. at the beginning of the
transfusions and from 2100 to 2450 gm. at their end. The same tech-
nique of transfusion was employed as in the previous experiment.

The observations on the two animals first mentioned were marred by inter-
current illness (snuffles) in one case and by the development of antibodies against
the alien blood in the other; but these complications did not develop until after a
progressive increase in hemoglobin had occurred as result of the transfusions.
Despite this increase no drop in the reticulocytes took place. In the rabbits
receiving 1 cc. of blood each day for 35 days there were more considerable increases

in hemoglobin, to as much as 14 gm. in one case; yet the reticulocyte percentage, though touching zero on a single occasion in one of the animals, underwent no enduring, significant reduction.

The results in this series confirmed the previous observations.   Even in rabbits with what appeared to be an abundance of hemoglobin, the introduction from without of 1 cc. of blood each day caused a considerable increase in the amount of the pigment per 100 cc. of blood.   Yet
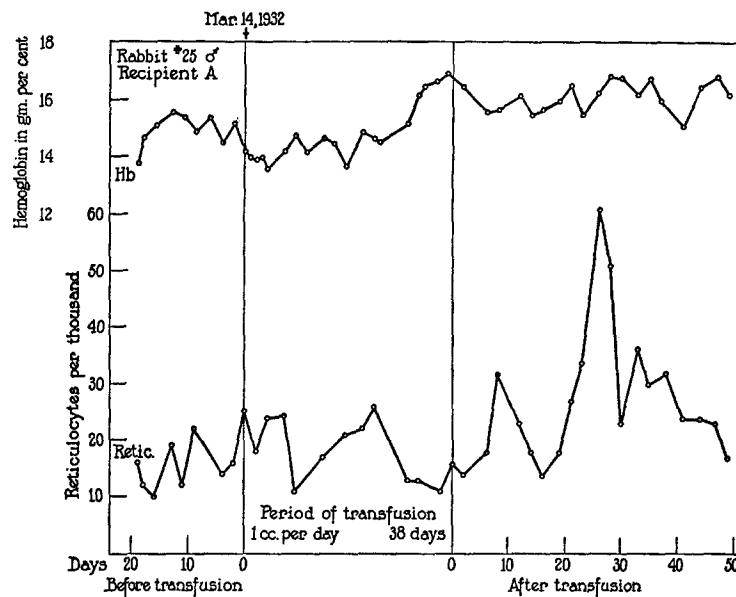

CHART 3

reticulated cells continued to circulate in good numbers, whence one might infer that there was no considerable marrow depression.   The findings will not be recorded in detail since better controlled and far more convincing evidence was obtained in the next group of animals.

For the purposes of this experiment, adult rabbits were selected, both as recipients and as donors, that had notably great amounts of circulating hemoglobin,—far beyond the average "normal" quantity.   A single donor was provided for each recipient in order to narrow the chances that the introduced blood might have a hidden incompatibility;

and frequent tests were made to be sure that one had not developed. In the experiments of Robertson (1) as also in Rous' (6) study of induced auto-antibodies, incompatibility leading to destruction within the organism of transfused blood was regularly accompanied by an agglutination *in vitro* of the donor's corpuscles by the recipient's serum; while in those instances in which isoagglutinins were lacking no such destruction was evident. To make sure of obtaining wholly com-
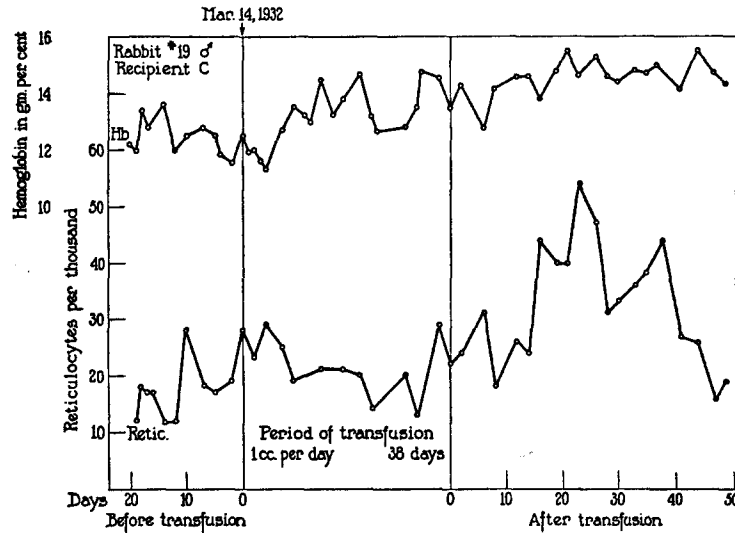

CHART 4

patible donors, especially searching agglutination tests were now made. As a check upon the general findings one recipient was purposely given incompatible instead of compatible blood.

The donors were vigorous animals which it was supposed would easily bear the loss of 1 cc. of blood *per diem*, as indeed proved to be the case. The condition of their blood was studied with the same care as was that of the recipients. Frequent hematocrit determinations of the red cell bulk were made in both groups. The observations were kept up for weeks after the transfusions had been discontinued.

Five compatible pairs of animals and one incompatible pair were employed. For the selective agglutination tests, the serum of the recipients was mixed with the washed cells of the donors, 9 parts of serum to 1 of a 50 per cent suspension of

the cells in salt solution. After 2 hours at room temperature, the mixtures were examined in the gross and microscopically. There was, in the case of the incompatible pair, well marked gross and microscopic agglutination of the donor's cells. As an accessory check upon the development of incompatibility during the transfusions, specimens of the blood of the recipients were examined for auto-agglutination (7). None transcending the normal was found save in the case of the animal receiving incompatible blood.

The initial weights of the rabbits were from 1800 to 2400 gm., and they were weighed each week, the figures showing a steady, gradual gain, as great in the donors as in the recipients, the final range being from 2300 to 3200 gm. All were kept in individual cages and on the same diet as the preceding series, save that cabbage was given three times a week.

The hemoglobin percentage, the number of reticulocytes, and the red cell bulk were ascertained at frequent intervals, usually every other day, for a period of at least 20 days preceding the first transfusion; and the observations were kept up during the 37 days of transfusion and for 6, in some cases 8, weeks thereafter. The blood specimens were taken in the morning, prior to feeding, the transfusions being done between 11:30 and 12 noon.

No anticoagulant was employed in this series to keep the injected blood fluid. The donor was placed in a covered box from which its head projected and the recipient in another by its side on the laboratory table. The shaved and oiled ear of the donor was heated until an active circulation had developed. A small cut was made in one of the marginal veins of the ear, and as the blood gushed forth it was steadily drawn up into a tuberculin syringe. When 1 cc. had been obtained in this manner, all further bleeding was prevented by an assistant and the injection into the ear vein of the recipient was quickly made. In this way no time was lost, the interval from the nicking of the donor's ear vein to the completion of the transfusion averaging not more than 60 seconds. Such care was taken for hemostasis that usually the donor lost no more than the desired 1 cc. of blood, at most not more than a drop. It has been found possible to extend the method to the transfusion of larger amounts; but when these exceed 4 cc. the risk of clotting becomes great. The first 2 or 3 cc. are much more rapidly obtainable than larger amounts, owing to contraction of the vessels of the bleeding ear. This caused trouble in some later experiments. For the determinations of red cell bulk the Van Allen hematocrit was used, with normal saline as the diluting fluid. Care was taken so to prepare this latter that changes in cell bulk due to osmotic imbalance were excluded. The findings during the transfusion and after periods showed that the red cell bulk varied directly with the hemoglobin quantity, as one might have expected under the conditions. Hence they are only occasionally charted.

In the analysis of the charts the experiment can be divided into three stages. There was the control period after the animals had been selected from stock, during which they lived under the same conditions as obtained subsequently when they

functioned as donor and recipient. Then followed a period of 37 days, on each of which the recipient received from his paired donor 1 cc. of whole blood. And there was a post-transfusion period of observation ranging from 40 to 50 days (see Chart 3, Rabbit 25). The preliminary hemoglobin amount of the recipients ranged from 12 gm. per cent to about 15 gm., averaging 13 gm. That of the donors had the same range but was slightly less, averaging about 12.4 gm. when the transfusions were started. They were not begun until the fact was plain that the amount of blood pigment was practically constant from day to day.

Charts 3 and 4 are typical of the findings in the group of animals receiving 1 cc. of compatible blood per day, an amount approximating 1/100 of their own initial quantity. There occurred in every instance (see Chart 5 giving averages) a progressive increase in hemoglobin percentage, gradual, as one would expect under
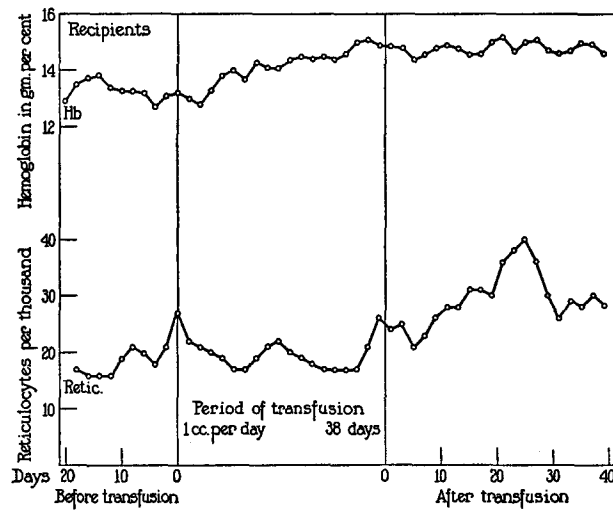

CHART 5. Composite chart of animals receiving small amounts of compatible blood daily.

the circumstances, but continuous, the average increase being about 2 gm. during the 38 transfusion days, the consequence being that sometimes the ultimate circulating amount far exceeded that encountered in any normal rabbit by the author. For example the rise in Rabbit 25 (Chart 3) was from an average of 15 gm. to one of nearly 17 gm. The reticulocyte percentage remained essentially unchanged throughout the transfusion period, a fact shown for the group as a whole by Chart 5.

Analysis of the Findings in the Recipients of Blood

The fact that the hemoglobin percentages rose markedly in the recipients does not necessarily mean that the pigment was present in

superabundance. The rises might conceivably have been the result
of a diminished blood volume. But there are observations on record,
notably the extensive ones of Boycott and his coworkers, which prove
that after transfusion the blood volume returns to normal or is only
slightly increased. A lessening in volume seems never to have been
observed.

A second possibility has been mentioned already; namely, that the
increase in hemoglobin was within the bounds of the normal, represent-
ing merely a blood betterment consequent upon favorable conditions.
The experiment just described was designed to exclude this possibility,
through the selection as recipients of rabbits having the highest initial
amounts of hemoglobin encountered in normal animals. It is, of
course, impossible to state precisely the upper limit of the normal, but
it is sufficiently demarcated for present purposes by the data of Pearce
and Casey (8), who themselves worked with Rockefeller Institute stock.
The figures on blood hemoglobin that they obtained by the examina-
tion of 174 animals procured from stock range from 28 to 90 per cent
(Newcomer, 4.7 to 15.2 gm. with the instrument Pearce and Casey
employed); but pathological instances were not ruled out, a fact suf-
ficiently obvious from the figures. The distribution frequency of the
curve representing the individual findings, as also the data of a subse-
quent paper, yield a maximum and minimum range for the generality
of animals of from 52 to 74 per cent (8.8 to 12.5 gm. per cent). The
first group of "normal" animals of the present work had initial hemo-
globin values of 9 to 11 gm. per cent and those of the third group from
12 to 15 gm. with an average of 13 gm. It is evident that prior to
transfusion the first group of animals utilized as recipients had hemo-
globin values somewhat above the lower level of the normal while
those of the third group, which had been selected for high hemoglobin
values, were at or near the normal maximum. In these latter animals
a further considerable increase occurred during the transfusion period,
one which in some instances definitely transcended the individual maxi-
mum encountered by Pearce and Casey. Such an increase can justly
be regarded as constituting superabundance.

The mounting curves expressive of the hemoglobin percentages in
the individual charts yield no indication of a "ceiling," such as could
be taken to represent the upper limit of the normal, beyond which

obstacles to the increase in pigment might conceivably be encountered. In Rabbit 25, Chart 3, the curve followed the same slant in reaching 17 gm. per cent as in Nos. 1 and 2 in which it attained to only 13.8 gm. per cent and 14 gm. per cent respectively.

In what way did the increase in hemoglobin come about? There are several possibilities. One might suppose that the daily addition of blood from without was greater than that provided by the marrow and that this tissue ceased to work, a fact masked by the persistence in circulation of the reticulocytes already present or injected with the strange blood. But reticulocytes do not persist as such in the circulation. Boycott and Oakley (2) have reviewed the numerous papers which go to show that those of the rabbit mature into ordinary red cells in from 1 to 2 days after they leave the marrow. Those introduced with transfused blood disappear rapidly. Otherwise the count of such cells could not drop to zero as it frequently does for a brief period in rabbits receiving massive transfusions (1, 2). The daily introduction of 1 cc. of blood into the recipients of the present experiments would not suffice of itself to provide enough reticulocytes to maintain the count even if those introduced persisted as such throughout the transfusion period.

It is possible to calculate the total number of reticulocytes transfused in those instances in which their number was followed in the donors (Charts 3 and 4). For example in Recipient C, the reticuloycte count after 30 days when the hemoglobin had increased from 12.4 gm. per cent to 14.6 gm. per cent was still 3 per cent, about what it had been at the beginning. During this period the animal had received 30 cc. of strange blood from Donor C, carrying an average of 2.5 per cent of reticulocytes. This was introduced into a blood bulk of 113 cc. (since the rabbit has about 4.7 per cent of its weight in blood (9)). Assuming that all of the introduced reticulocytes persisted as such throughout the transfusion period, the gradual accumulation of them would account at most for only one-fourth the number in circulation at the end of the transfusion period. And the necessary assumption is not justified.

One is forced to conclude that the continued presence of reticulocytes in normal number throughout the transfusion period resulted from a persisting activity on the part of the erythropoietic tissue.

Granting that the marrow continued to work, to what was its work due—to lack of sensitiveness to the gradual increase in circulating blood pigment, as represented by the strange red cells, or to stimulation

resulting from the intercurrent destruction of these cells? Care had been taken to provide compatible cells, yet many of them must have been destroyed in the natural course of events, together with the cells of the host; and they might even have been destroyed practically at once after introduction, a fact concealed through the activity of the erythropoietic tissue to form new ones.

The total increase in circulating hemoglobin was far from being as great as it should have been had none of the introduced blood been destroyed and the blood volume remained constant. The average final weight of the five rabbits of Chart
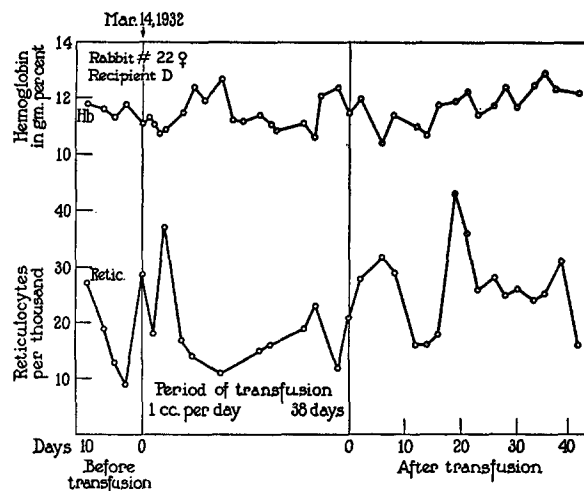

CHART 6. Effect of incompatible blood in small amounts.

5 was 2580 gm. Assuming that the proportion of the blood volume to the weight remained stationary, and that it amounted to 47 cc. per kilo, the total increase in hemoglobin at its height represented an accretion of only about 18 cc. of blood, whereas 37 cc. had been injected. Boycott and Oakley found that large transfusions never raised the hemoglobin to the figure it should have reached on calculation, a finding which may have been due in some small part to an increase in the total blood volume, as may that now under discussion. The daily wastage from normal blood destruction, as shown by the bilirubin output, varies directly with the quantity of red cells in circulation (10).

Boycott and Oakley have reviewed the contradictory literature dealing with the question of whether the products of blood destruction act to stimulate the marrow. The fact has many times been proved that when the body needs blood the introduction of materials that can be used in its formation, laked blood for ex-

ample, results in an increased activity on the part of the erythropoietic tissue. Indeed McMaster and Haessler (11) have shown that increases in the amount of this tissue to meet the emergency of anemia from hemorrhage are directly conditioned by the availability to it of the materials for blood formation. But increased reparative activity consequent upon the availability of such materials is not necessarily the same as direct marrow stimulation. Boycott and Oakley themselves found no such stimulation as result of the products of blood destruction save in a special instance, that of citrated, laked blood injected subcutaneously, laked blood as such failing of effect. Robertson did not obtain stimulation by transfusing incompatible blood to rabbits. As a check upon the possibility that in the present experiments the cells transfused were destroyed soon after introduction and utilized in the formation of new blood, agglutination tests of the third series of animals were made from time to time throughout the transfusion period to find whether the recipients had reacted against the donors' blood so that it had become incompatible. As already stated, it has been the experience of those working with rabbits that when such evidence of incompatibility fails to appear, transfused blood is well tolerated. In the present instances agglutination was not encountered. As a further check a rabbit was transfused with frankly incompatible blood to learn whether its destruction would lead to a mounting hemoglobin percentage. That in this instance the strange blood was promptly destroyed can be inferred from the fact that the hemoglobin underwent none of the increase seen in the animals receiving compatible blood (Chart 6); yet the reticulocytes remained at the pretransfusion level. Throughout the transfusion period there was a lack of significant intercurrent variations in the number of these cells, such as might have been expected had the marrow been subjected to restimulation.

From all this it seems plain that the marrow activity during the period while the hemoglobin was increasing, must have been due, not to stimulation by the products of blood destruction, but to persistence at a normal task.

The Effects of Repeated Small Blood Losses

The lack of sensitiveness of the marrow to induced increases in hemoglobin contrasts strikingly with its response to slight blood losses. It has been said that in the best controlled experiment, that of the third series of animals, observations were made on the donors at the same time as on the recipients. The loss sustained by these donors did not exceed by as much as a drop per day the amount of blood gained by the recipients. Yet in all six instances (Charts 7, 8, and 9) the percentage of reticulocytes markedly increased within a few days after

the bleedings had been begun, and though the blood losses caused but a transient fall, when any, in the hemoglobin percentage, and were soon followed by recovery to a higher percentage than before, the reticulocyte count persisted above the previous level so long as blood was withdrawn. When no more was taken, the count soon fell, and reached the pretransfusion level. It is plain that not only did the erythropoietic tissue become abnormally active under the stimulus of the bleedings, but it never adjusted itself to the daily loss, being
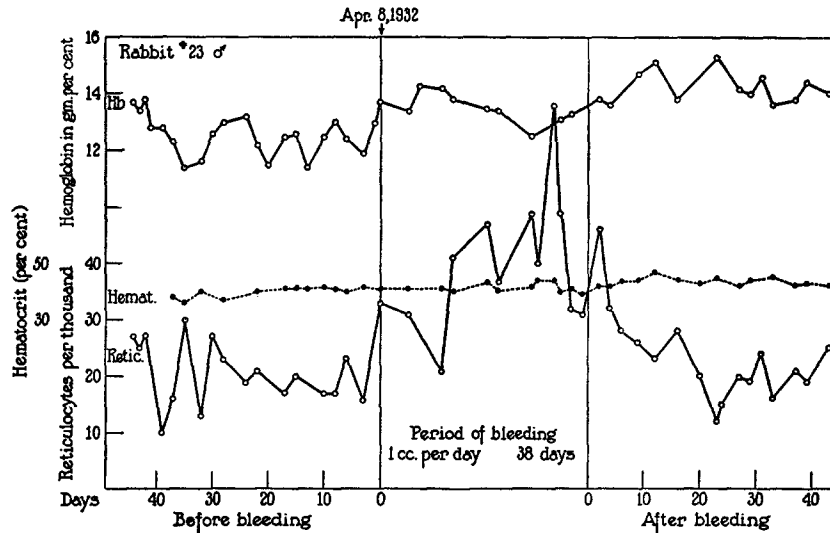

CHART 7

quite as sensitive thereto at the end of the period of hemorrhage as at the beginning.

The hemoglobin percentage of the donor rabbits,—which throughout remained in excellent condition,—continued to rise progressively after the bleedings had been stopped, surpassing the previous normal, but not attaining the level reached in some of the recipient rabbits. This rise may conceivably have been due to overcompensation, such as is frequently observed after hemorrhage, though the effect of this does not ordinarily persist for so long a period.

The observation that daily small blood losses in healthy animals may not only be repaired, but may be attended by an increase in the amount

of circulating hemoglobin, assumes special significance when the consequences are studied of removing the same total amount of blood on fewer occasions. The experiment was carried out on four rabbits selected and studied with the donors of the third experiment but deprived of 7 cc. of blood at a single bleeding once a week. In all four the reticulocyte percentage rose far more markedly than in the animals bled 1 cc. per day, yet the manufacture of blood was insufficient to compensate for the weekly loss and an anemia developed (Charts 10
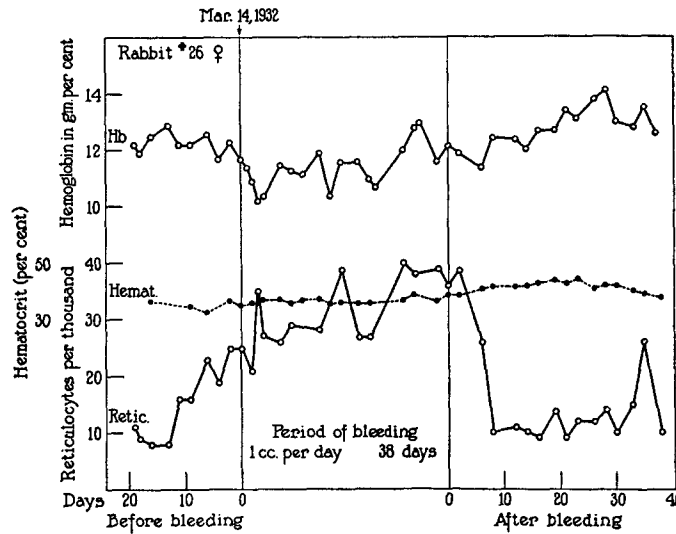

CHART 8

and 11). The chart of the average findings (Chart 11) typifies the individual instances. There was a far greater increase in the number of reticulated cells than when the blood was withdrawn as 1 cc. per day. The blood removed was in one case injected into another, compatible rabbit. The hemoglobin of this animal was increased from 13.0 gm. to 14.2 gm. and a pronounced depression of marrow activity took place as evidenced by the reticulocyte change.

Habituation to Overmuch Hemoglobin

The fact that the erythropoietic tissue is insensitive to gradual additions to the amount of circulating blood pigment, while responding

practically at once to withdrawals of the same magnitude, was evident
in the present experiments not only during the period of transfusion,
or of bleeding, but in the subsequent weeks, during which the blood
studies were continued. For some days after the transfusions of 1 cc.
of blood had been stopped the induced superabundance of hemoglobin
persisted without change. Then the pigment percentage began to fall;
and soon afterwards the erythropoietic tissue became unusually active,
—as shown by a sharp rise in the reticulocytes,—and the high per-
centage was regained. This happened in the rabbits of all three series,
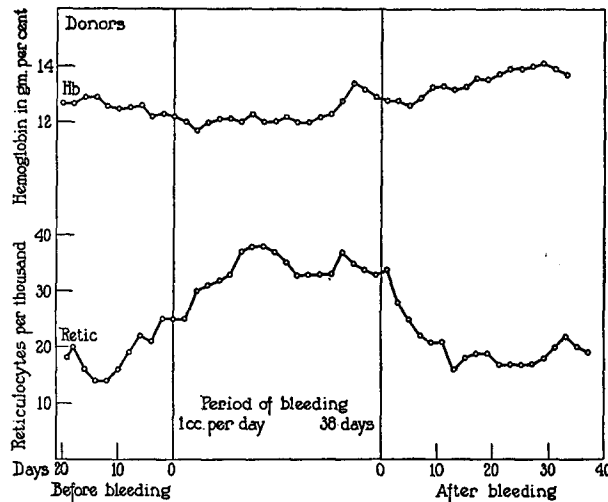but it was best studied, because best controlled, in the rabbits of the

CHART 9. Composite chart of animals deprived of small amounts of blood
daily.

third group that received compatible blood (Charts 3, 4, and 5). The
phenomenon occurred in all five of these animals. The high hemo-
globin level was maintained throughout some weeks of observation,
that is to say until expediency rendered it necessary to terminate the
experiments; and the induced marrow activity, though not so great as
after the hemoglobin first fell off, only gradually diminished to the
normal rate as indicated by the percentage of reticulocytes. Evi-
dently the organism had been so altered by the experimental proce-
dures that a much higher hemoglobin percentage than that obtaining
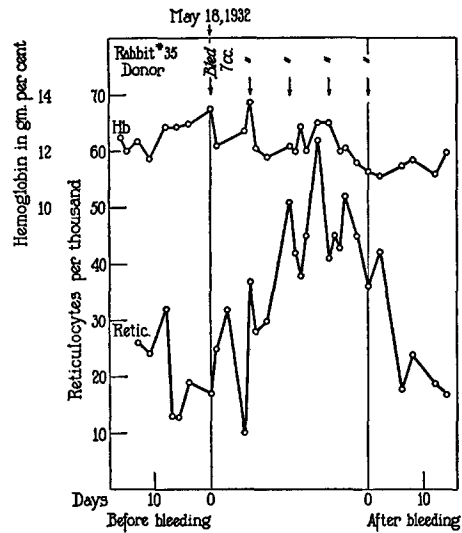prior to the transfusions was now normal to it.

CHART 10

DISCUSSION

The initial object of the experiments was to determine the rate of blood production. It was supposed, on inference from indications here and there in the literature, that this could be done by supplying compatible blood to the normal organism in precisely the amount daily produced by the erythropoietic tissue, whereupon presumably this tissue would cease work. What occurred was wholly different. The day to day introduction into the circulation of small amounts of blood was without perceptible influence on bone marrow activity and the hemoglobin percentage rose gradually yet markedly. Its activity continued unabated throughout the transfusion period and must have contributed in no small part to the rise in hemoglobin. The grounds for these conclusions have been considered in the text.

If, in some of the recipient animals, it is possible to suppose that the increase in hemoglobin represented merely blood betterment over a previous low normal as result of favorable conditions (Charts 1 and 2), in the case of others (Charts 3 and 5) one is forced to look upon the state of affairs brought about by the additions of blood as constituting a superabundance for reasons that have been given. The findings as

concerns marrow activity were similar in all of the recipients of small amounts of blood. They indicated that blood formation was practically unaffected by the gradually mounting hemoglobin (Charts 3, 4, and 5).

As already stated, Boycott and Oakley (2) transfused rabbits repeatedly with large amounts of blood to determine whether, as result of the provision of cells from without, the marrow would not atrophy or at least stop work. They found, as had Robertson before them, that the reticulocytes soon fell to zero; but although the transfusions were kept up this change did not endure but with every inter-
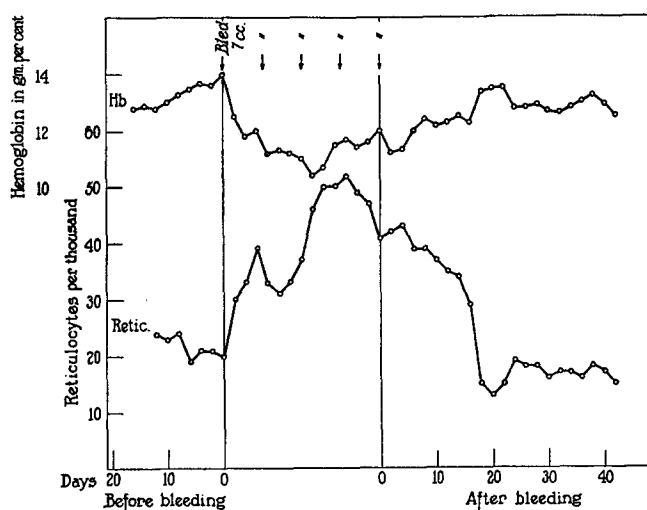

CHART 11. Composite chart of animals bled at weekly intervals.

current fall in hemoglobin from the abnormally high level,—and these were frequent,—the reticulocytes reappeared in greater or less number. Boycott and Oakley did not ensure compatible blood by preliminary tests; and the difficulties they mention of maintaining a superabundance of hemoglobin, difficulties evident in their charts, indicate that now and again the alien corpuscles must have been abruptly destroyed in quantity, a fact which they themselves recognized, doing control experiments on the possible effects of blood destruction to cause marrow stimulation. They could not bring the marrow completely to a standstill, though, as they remark, the abundance of transfused cells might be presumed to have saved the organ from the necessity of making any fresh ones.

Evidence was obtained in all of the recipient groups of the present work that habituation develops to an increased amount of circulating

hemoglobin when this increase is brought about gradually. Soon after the daily transfusions were stopped, the hemoglobin percentage began to decline from its high level, but long before it had progressed far in the direction of the previous normal, the marrow became unusually active, as shown by a pronounced increase in the number of reticulated cells, and the high pigment level was restored and maintained (Charts 3, 4, and 5). The possibility that the increased erythropoietic activity resulted from the stimulation due to intercurrent blood destruction can be dismissed for reasons already given. Unfortunately the animals had to be sacrificed within a few weeks after the transfusions had been stopped, so the late consequences of these remain unknown.

Robertson showed that when a superabundance of hemoglobin had existed for some time the withdrawal of an amount of blood insufficient to bring the amount of pigment to the pretransfusion level resulted nevertheless in an increase in bone marrow activity. In doing this experiment he abruptly and considerably reduced the blood bulk. That considerable blood reductions stimulate the marrow of the normal animal, as repeated small losses to the same total amount do not, is shown by the large increase in the reticulocytes in our rabbits that were bled 7 cc. once a week as compared with the relatively slight increase in those bled 1 cc. *per diem*; and it might be inferred that Robertson's finding was the result merely of a drastic change in the blood bulk. This possibility was controlled in the transfused rabbits of the present work. Hemorrhage was not the cause of the post-transfusion fall in hemoglobin which resulted in marrow stimulation, nor, for that matter, was the reduction in the pigment percentage a great one.

Subsidiary evidence on the insensitiveness of the marrow to hemoglobin increases when these occur gradually can perhaps be found in the data on the donors for the third group of transfused rabbits. Although these lost 1 cc. of blood per day the hemoglobin percentages had returned to the initial level when the losses were stopped, and soon thereafter they rose above it. Thoughout the period of the bleedings the marrow had been unusually active as shown by the increased percentage of reticulocytes; but when they were discontinued, the number of these cells declined to the previous normal. Below this normal they did not fall despite the fact that the hemoglobin percentage continued to mount.

The conception of habituation to superabundance of hemoglobin would seem on first consideration to be excluded by the numerous observations which attest to the fact that induced alterations in the oxygen supply to the body cause changes in the activity of the erythropoietic tissue. But the contradiction is only an apparent one. True, when an animal is suddenly injected with a large amount of compatible blood, or is abruptly placed in an atmosphere abnormally rich in oxygen, its marrow slows in the production of red cells. Any abrupt and sustained large increase or decrease in the opportunity for oxygen evokes an alteration in the rate of erythropoiesis. But it does not follow that such an alteration will be elicited when the change is small or gradual. Interpolated between the supply of oxygen and the needs of the tissues are a number of physiological mechanisms, circulatory and respiratory in the main, which act to minimize or ward off completely the stimulus to the marrow of incidental changes in oxygen demand or supply. Were it not for the working of these mechanisms the erythropoietic system would be having to respond to constantly recurring buffets in the form of demands, quickening its activity after an individual had played tennis, slackening when he had spent a day or so in bed. But because of the protection afforded by intermediate mechanisms, transient changes in oxygen demand or supply, those normal to ordinary life, must scarcely be felt as stimuli by the erythropoietic tissue, if felt at all.

Boycott and Oakley draw attention to some of the resources of the organism, other than erythropoiesis, that are utilized in coping with changes in oxygen supply or demand and they provide an unique example in which all such resources were overborne. Rabbits were transfused excessively, with result in so great an increase in the viscosity of the blood that it could not be circulated well enough to prevent anoxemia of the tissues. Despite the immense quantity of hemoglobin present in circulation the erythropoietic tissue became unusually active, just as if there were a pigment deficiency.

Granting that the physiological mechanisms mediating between the available oxygen supply and the needs of the tissues suffice to protect the red marrow from having to respond to transient emergencies, is it possible that gradual changes in oxygen supply or demand are ever coped with over a long period by these mechanisms alone? The literature does not provide a comprehensive answer to this question.

Observations on the effects of changes in altitude upon the blood have in general been conducted upon persons whose environment in such respect has been changed suddenly; while animals studied with relation to the effects of changes in the amount of oxygen in the inspired air have as a rule been suddenly placed in atmospheres poor or rich in this gas and as abruptly removed therefrom. There can be no doubt that a prolonged sojourn in rarefied air is attended by an increase in circulating hemoglobin and that persons living all their lives at high altitudes have in general a greater hemoglobin percentage than natives of the lowlands. In most instances the amount of the pigment varies directly with the availability of oxygen. That this is not always the case is indicated by the observations of Somervell upon two Tibetans who had lived most of their lives at a height of 16,500 feet. Their hemoglobin percentages were only 92 and 82 respectively, yet they could race up steep slopes "twice as fast" as the English climbers whose hemoglobin percentages had mounted to an average of 120 as result of the change in altitude. The average hemoglobin quantity in Hurtado's large series of individuals living at 14,900 feet was only 15.93 gm. per cent, as compared with 15.75 gm. per cent for those at sea level whom he studied. Campbell found the oxygen tension in the tissues of a number of normal rabbits to be identical despite wide individual variations in the hemoglobin percentage,—though abrupt, great changes in this percentage, brought about by experimental means, resulted in changes in the tension.

These various findings indicate that the organism does not necessarily make more blood or less blood when the opportunity for oxygen is large or small throughout a protracted period, but may on occasion have recourse for adjustment to its other resources.

There are many clinical reports of obdurate anemia resulting from repeated slight hemorrhages which might be taken to indicate that the organism can become habituated to a smaller amount of circulating hemoglobin than the normal, just as it becomes habituated to a larger one, according to the findings here reported. Campbell's observations, above referred to, on the identical oxygen tension of the tissues in normal rabbits with widely differing hemoglobin percentages provide good evidence of habituation to a relative anemia. Yet in the present experiments the organism did not adjust itself to repeated small hemorrhages to such extent that the erythropoietic tissue failed to be stimulated by them. At the end of many daily losses of 1 cc. of blood,—about 1/100 of the total quantity,—it was still responding as actively as at first, and this although the hemoglobin percentage had returned to the initial level. Very possibly the marrow was re-

sponding to the repeated slight diminutions in blood bulk as such. The great response to bleeding 7 cc. once a week, as compared with 1 cc. per day, and the differing course of the hemoglobin curves, illustrate a point which deserves reiteration; namely, that the consequences of considerable and abrupt changes in blood volume and hemoglobin percentage provide no sufficient basis upon which to predict the outcome of repeated small ones to the same total amount.

The facts do not enable one to say whether the rabbits manifesting habituation to an increased amount of hemoglobin would have continued to keep this amount in circulation for a long period. Plainly the change in their circumstances had greatly altered their case. But whatever this case the hemoglobin percentage maintained by them would, in the absence of abnormal blood destruction, necessarily have been, in last analysis, the resultant of the forces making for depression and stimulation of the marrow respectively, just as in normal animals. According to Boycott and Oakley "The normal animal is evidently working about a nice level of delicate balance, which is presumably the reason for the constant presence of a few reticulated cells." A balance there certainly is, but hardly a nice one. For the present work has disclosed the fact that the erythropoietic tissue is insensitive to the effects of repeated, small blood accretions, though very sensitive to blood losses of the same magnitude. This is what one would expect if, throughout the course of age-long differentiation and selective survival, body needs have had the effect of determining body capabilities. The normal organism has always had to cope with accidental losses of hemoglobin, if it was to survive, but almost never with a superabundance of the pigment.

SUMMARY

The effects of very gradually increasing or diminishing the amount of circulating hemoglobin have been studied in rabbits. Contrary to expectation it was found that when the pigment was increased by the injection of a small quantity of compatible blood every day during some weeks the erythropoietic tissue did not lessen its activities. The hemoglobin percentage mounted gradually yet considerably when even as little as 1/100 of the amount of blood initially possessed by the animal was injected each day; and the figure it finally attained must in

some instances at least have been expressive of a superabundance. To this superabundance the animal itself evidently contributed through its persisting erythropoietic activity.

The results were very different when rabbits were bled daily to the same small amount that was injected into their fellows. The marrow became abnormally active, and this activity continued undiminished throughout the long period of the bleedings. The organism is evidently far more susceptible to blood losses than to blood gains, a fact which is scarcely surprising when one considers that throughout its differentiation as a going concern it has had to cope with exigencies of the first sort only.

Rabbits in which the hemoglobin is very gradually increased by the injection of strange blood become so accustomed to the abundance of pigment that even a slight falling off causes the erythropoietic tissue to become abnormally active to maintain the new *status quo*. Good reasons exist for referring the habituation thus manifested to readjustments in the functioning of the physiological mechanisms which mediate between oxygen demand and erythropoietic response. Too little recognition has been given to the rôle of these mechanisms in such relation. No evidence was obtained of any effective readjustment to protect the erythropoietic tissue from the stimulus of daily small blood losses.

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