Role of the Spleen in Acclimatization to Hypoxia

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ABSTRACT

In order to determine quantitatively the participation of the spleen and the bone marrow separately, five groups of splenectomized and non-splenectomized mice totalling 87 individuals were exposed to a simulated altitude of 15,000 feet continuously for periods of 30–58 days. RBC counts and hematocrit determinations were made at various intervals. It was found that about two-fifths of the increase in red cells could be referred to a tonic contraction of the spleen and the remaining three-fifths to the production of red cells by the bone marrow.

There has been extensive investigation of the effect of chronic hypoxia on the blood, centering around the almost invariable increase in red cell count under these conditions. The bibliography by Stickney and Van Liere (1) provides a comprehensive survey of the work which has been done in this field.

Thirty years ago Barcroft demonstrated the function of the spleen in increasing red blood cells under stress. By comparing the blood from the pulp of the spleen with that of the circulation, Barcroft and Poole (2) showed that there is a much higher concentration of erythrocytes in the spleen. Using photographs and computing the size of the spleen in the normal and dead animals, Barcroft and Stephens (3) estimated the capacity of the spleen to be 15% of that of the circulating blood volume. De Boer and Carroll (4) had previously shown that decrease in spleen volume is independent of blood pressure and is due to active contraction of spleen musculature. Binet and Fournier (5) demonstrated the immediate reaction of the spleen in throwing an increased amount of red cells into the circulation after hemorrhage and acute mechanical asphyxia.

The effect of splenic contraction is most clear when the stress is acute, as in the cases mentioned above. At the same time a chronic, or long-term influence has been described. Thus Barcroft and Stephens (6) observed shrinkage during the later phases of pregnancy and Shen (7) found a similar phenomenon to accompany repeated small hemorrhages in the rabbit. Nevertheless, we know of no attempt to segregate quantitatively the increased erythropoiesis of sustained moderate hypoxia from a possible continuous, or tonic splenic contraction which would in turn produce a rise in red cell count.

Due to the desirability of working with an organ of maximum dimensions, most of the previous research has been done on man and large mammals. However, this has had the effect of limiting certain types of experimental work. With small mammals (mice and rats) a large number can be employed and the results treated statistically. However, mice are preferable to rats because many splenectomized rats contract a fatal infection almost immediately after the operation.

MATERIAL AND METHODS

Primarily Swiss-strain male mice were used in this study, although a few animals of the DBA strain were used for preliminary work. Each animal weighed between 20 and 25 gm. All were fed a standard laboratory maintenance diet known as ‘green feed.’ Hypoxic exposure was effected by keeping the animals continuously in a low pressure chamber at a simulated altitude of 15,000 feet.

Splenectomy was performed through the lateral dorsal approach, Nembutal being used for anesthesia. The incision was made through the muscles of the left side, and the spleen was exposed. The splenic vein and the splenic artery were tied separately and the spleen excised without hemorrhage. Muscles and skin were...
sewed separately to avoid hernia. A period of 6 weeks was allowed after the operation, during which recovery was complete. There was no mortality. This procedure achieved results similar to those obtained by Webster and Liljegren (8).

Before and during exposure to hypoxia, blood samples were taken from the tail vein and the red cells counted by the usual method. For hematocrit, fine heparinized capillary tubes were employed and centrifuged at 5,000 rpm for 30 minutes. The amount of blood lost by the animal per test was usually about 25 lambda and did not exceed 50 lambda. This is approximately 1-2% of the circulating blood volume.

One-tenth per cent epinephrine was diluted with distilled water (1-100 cc) and injections administered intraperitoneally were 1/5 cc each. Commercial Nembutal was diluted in the ratio of 1 cc to 5 cc saline solution and injections were 1/5 cc/mouse.

Variability was tested for all means cited in the section on results. With the groups containing 12 mice the standard errors ranged from 2.0-3.7% of the corresponding means; for the groups containing 18 or more mice, the standard errors ranged from 1.0-2.8% of the mean. Hence it follows that differences between means of more than 10% may be regarded as highly significant, and differences of from 5-10% slightly to moderately significant. Differences of less than 5% are without significance.

RESULTS AND CONCLUSIONS

Series 1. The red cell count of 10 normal Swiss mice was determined. The average was 9.8 millions. Approximately 1 hour afterwards 1/5 cc of epinephrine was injected into each animal and the cell count was again taken. This interval subsequent to injection was found by serial counts to represent the peak of the epinephrine effect. The average was 12.0 million, a change of 22.4%. One day later 1/5 cc of Nembutal was injected. During the narcosis, the average red cell count was 8.5 million. This amounts to a decrease of 13.2% from the normal value due to the Nembutal injection. The hematocrit fell from a normal value of 47 to 39.4 or 16.1% decrease. Thus the count can vary between the limits of 12 and 8.5 million. This is a range of 3.5 million, equivalent to 35.6% of the lower level. The wide possibility for variation in the red cell concentration in the mouse blood is evident, and the value obtained at any given time is clearly a function of the stress to which the animal is subjected. The mediating mechanism is undoubtedly the capacity of the spleen to relax and store red cells (Nembutal narcosis) and to contract with the full expulsion of the content (epinephrine).

Series 2. Twenty-four normal DBA strain mice were divided into two groups. Twelve mice were exposed continuously to a simulated altitude of 15,000 feet; the others were kept at sea level. Before exposure the red cell count for the experimental group averaged 11.0 million, and the hematocrit was 45.6. After 30 days exposure to the simulated altitude the animals were removed from the chamber and samples taken within 30 minutes. The red cell count of the experimental group was 15.3 million, and the hematocrit average had increased to 64.1. These figures correspond to 39.0% increase in red cell count and 40.5% increase in hematocrit. Upon injection of Nembutal into these animals, the count dropped to 10.7 million, or about the level prior to exposure to altitude. Meanwhile the equivalent values for the control group at sea level were 11.2 million and 11.3 million.

Series 3. The difference in response to high altitude between splenectomized and nonsplenectomized animals was demonstrated in the following manner.

First, as a preliminary step, DBA strain mice were splenectomized and 6 weeks was allowed for recovery. The average red cell count was 10.5 million. Upon injection of 1/5 cc of Nembutal, the average became 10.4 million. This represents no significant change from the nonnarcotized state, and demonstrates that the reduction in red cell count in nonsplenectomized mice (see series 1) is due to splenic relaxation.

Subsequently, the same 12 splenectomized DBA's, with an average of 10.5 million red cells, were placed in the decompression tank at a simulated altitude of 15,000 feet for a period of 60 days. After 16 days of exposure, the red cell count rose to 12.4 million, an increase of 18.1%. After 42 days of exposure, the count was 12.5 million, an increase of 19.0%. After 60 days exposure, the count was still 12.5 million. At 60 days, the mice were taken out of the decompression tank. Four days thereafter, the average red cell count dropped to 10.6 million which represents only 9.9% increase above the original value. Eleven days after being taken out of
the tank, the average count was 10.4 million. This corresponds to a 1.0\% decrease from the original value, and differs from the latter by an amount which has no statistical significance.

**Series 4.** Thirty-six male Swiss mice were splenectomized and a recovery period was allowed. They were then divided into two groups of 18 mice each: IA and IB. Thirty-eight male Swiss mice, nonsplenectomized, were divided into two groups: IIA and IIB, with 20 and 18 mice, respectively.

A control group of 13 splenectomized animals (designated IIIA) was maintained at sea level throughout the experiment. Hematocrit values indicated that there was no change in the red cell level. The groups of animals described for series 1 and 2 served as controls for the nonsplenectomized mice, in which there was likewise no alteration in the blood picture.

At the beginning of the run, the four experimental groups (IA and IB; IIA and IIB) were placed in the decompression tank and subjected to a simulated altitude for 30 days. During this period examination was made of the red cell picture at frequent intervals as shown in table 1. At the end of the 30 days groups IA and IIB were removed from the tank. Groups IB and IIA, values for which are shown in the table for 34 and 35 days exposure, respectively, were retained in the decompression chamber. Groups IA and IIB were tested, as shown in the table, at the end of 4 days subsequent to removal from the tank.

As may be observed in table 1, during the first 48 and 96 hours there is an increase of red cells in all groups amounting to a minimum of 10\%. This very rapid increase may be ascribed at least in part to hemoconcentration, although we cannot yet strictly evaluate either the extent or the time course of this factor. The problem is still under investigation. By 16 days exposure time the maximum value has been reached and thereafter tends to remain substantially constant. At this point the splenectomized mice showed a red cell count approximately 19\% higher than the initial value whereas the equivalent increase in the nonsplenectomized animals was nearly 32\%. In a parallel manner the hematocrit increased by factors of 20\% and 31\%, respectively. The conclusion is warranted that 19\% of the increase in count is referable to accelerated red cell formation and that the difference, 13\% may be ascribed to a tonic reduction in the size of the spleen. This finding is in accord with the fact that 12 of the nonsplenectomized mice, when tested with epinephrine, now failed to show a significant increase in cell count.

The last column in table 1 shows that when altitude-adapted mice are brought back to sea level the original red cell level is very quickly restored. In this instance the counts after 4 days at sea level had fallen to approximately 5\% of the pre-exposure values. These results confirm those already mentioned for series 3.

The rapid reversion to normal can be ascribed to a possible hemodilution, or to an accelerated red cell destruction. A splenic relaxation with storage of erythrocytes does not seem to constitute an important factor since there was an equally rapid fall in cell count observed in the splenectomized animals, as in the normal animals.

With reference to adaptation to chronic hypoxia the results cited here as a whole indicate that the increase in red cells observed in mice may be ascribed to at least three processes: hemoconcentration, erythropoiesis, and tonic contraction of the spleen. The quantitative relationships between these three
factors would have to be evaluated for each set of environmental circumstances.

REFERENCES