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Reticulocytogenic Effects of Liver Extract and Congo Red in Guinea Pigs under Reduced Oxygen Tension.

ARTHUR P. RICHARDSON AND WILLIAM DOCK.

From the Departments of Pharmacology and of Pathology, Stanford University School of Medicine, San Francisco.

Some investigators have claimed significant rises in reticulocytes after administration of potent liver extracts to guinea pigs,[1-6] while others have denied these claims.[7-10] Jacobson[11] suggested that the discrepancy in results could be explained by differences in diet. He believes that a diet of lettuce, carrots, and oats lacks some factor which is contained in liver extract. In support of this view, Gall[12] reported that the bone marrow of some of Jacobson's guinea pigs on this limited diet showed a densely cellular marrow with a preponderance of immature cells. Following the administration of liver to some of these animals, there was a marked depletion of the cellular content and accelerated maturation of the marrow elements. Inasmuch as the usefulness of guinea pigs for the bioassay of antipernicious anemia substances is controversial it appeared desirable to explore the reticulocytogenic action of liver in these animals under various conditions.

If, as some observers[1-6, 12] believe, the guinea pigs which have been on restricted diets and are "reactive" to liver extract have a retarded rate of maturation of the red cell series, they should respond slowly and inadequately to demands for increased rates of hematopoiesis. Guinea pigs in such a state should therefore have a less marked response to low atmospheric pressure than animals

1 Jacobson, B. M., *Science*, 1934, **80**, 211.

2 Landberg, J. W., and Thompson, M. R., *J. Am. Pharm. Assn.*, 1934, **32**, 964.

3 Miller, D. K., and Rhoads, C. P., *New England J. Med.*, 1935, **213**, 99.

4 Clark, G. W., and Coene, A. M., *Proc. Am. Soc. Biol. Chem.*, 30th Annual Meeting, 1936, p. 16.

5 Mermod, C., *J. Clin. Invest.*, 1936, **15**, 559.

6 Barrie, M. M. O., *J. Pharm. Exp. Ther.*, 1937, **60**, 235.

7 Lassen, H. C. O., Jacobsen, E., and Nielsen, A. K., *Acta Path. Microbiol.*, 1936, **13**, 543.

8 Goodman, L. S., Geiger, A. J., and Klumpp, T. G., *J. Clin. Invest.*, 1936, **15**, 435.

9 Ege, R., and Hagens, E., *Acta Path. Microbiol.*, 1937, **14**, 597.

10 Bachrach, W. H., and Fogelson, S. J., *J. Lab. Clin. Med.*, 1937, **22**, 925.

11 Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 665.

12 Gall, E. A., *Am. J. Path.*, 1937, **13**, 575.

treated with liver extract containing the so-called "hematopoietic" fraction. Mermod, in some preliminary and unpublished experiments, observed no difference in the reticulocytosis and rise in red count of untreated reactive guinea pigs and those treated with liver when both groups were subjected to 10-12 days of 360-400 mm barometric pressure. This report confirms and extends her observations, and deals with the effect of Congo red and the bone marrow histology in such animals.

Methods. Twenty adult guinea pigs were used. All were fed on a diet of lettuce, oats, and carrots, or of lettuce, oats, and turnips,[9] during an experiment and for at least one month prior to injection of liver extract. Reticulocytes were counted at least every other day by the usual wet technic, using brilliant cresyl blue and sodium citrate. Blood was obtained by puncturing a small ear vein. Concentrated Liver Extract, Lilly (N.N.R.), was injected intramuscularly, using 1.0 cc per kg on 3 successive days, total 3.0 cc per kg. Congo red in 5% dextrose solution was injected intraperitoneally, using 50 mg per kg daily for 4 successive days, total 200 mg per kg.

Following a 3-day period of control counts of reticulocytes, the guinea pigs were put into a specially constructed steel tank (30 x 30 x 50 cm). Vacuum was produced by a Nelson vacuum and pressure pump. Pressure was maintained by a Saad valve so that air flowed through the chamber at an absolute pressure of 380 to 400 mm Hg., or about 76 to 80 mm partial pressure oxygen. The reduced pressure was maintained for 24 hours daily except for a short time each day for changing food and water, and making reticulocyte counts. The results obtained are presented as curves of average per cent changes in Figure 1.

Controls. When 5 normal animals, which had been on the experimental diet for one month, were subjected to such reduced pressure, the results obtained agreed practically with those of Gordon and Kleinberg.[13] In 2-4 days, the reticulocytes began to rise, reaching a peak in about 7-12 days, of from 8 to 14%. Then the percentage fell gradually and in 3-4 weeks the reticulocyte count approached the control (Fig. 1).

Liver Extract. After being one month on the experimental diet, 2 guinea pigs were injected with liver extract. One week after the first injection, when they should have given a reticulocyte response, they were put into the low pressure tank. The results in Fig. 1,

[13] Gordon, A. S., and Kleinberg W., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 507.

which are labelled "Liver before Reduced Pressure," represent the average of 2 guinea pigs. It is seen that, before the period of low oxygen tension, these animals showed a slight reticulocytosis of 2.7%, presumably due to liver. It is clear, however, that this treatment did not affect the response of the bone marrow to chronic anoxemia. The peak in one animal was 10.2% reticulocytes, while in the other animal, 11.1%.

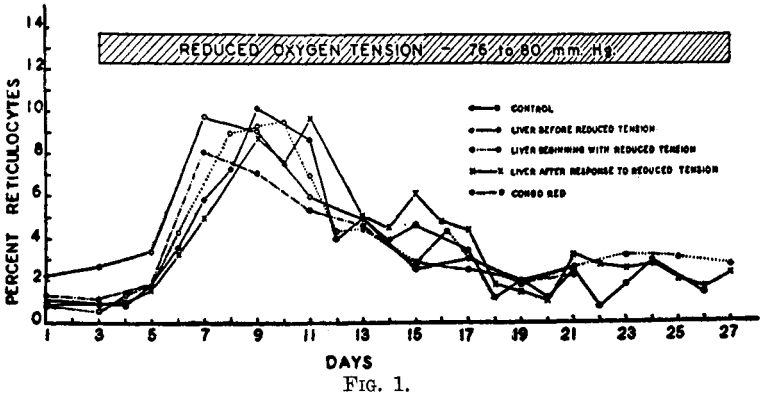

FIG. 1.

Comparative reticulocytogenic effects of liver extract and Congo red in guinea pigs under reduced oxygen tension.

Five guinea pigs were injected with liver extract and placed at once into the low pressure tank. The results in Fig. 1, labelled "Liver Beginning with Reduced Tension," represent the average of all 5 animals. The changes were practically the same as those in the controls, the peaks of individual responses varying from 9.5% to 11.4%.

Five guinea pigs were subjected to low oxygen tension for 2 weeks, or until the peak of the reticulocyte shower had passed, and the percentage of the reticulated cells had fallen nearly to the control level. At this point, the animals were injected with liver extract, and kept in the tank for 10 days. The average results labelled in Fig. 1 "Liver after Response to Reduced Tension," show that, under these conditions, liver extract was ineffective on the circulating reticulocytes.

Congo red. Since Congo red may cause remissions in active cases of pernicious anemia,[14, 15] the reticulocytogenic effect of this dye was tested under low oxygen tensions. Three guinea pigs were given, intraperitoneally, 200 mg per kg of Congo red, these injec-

[14] Massa, M., and Zolezzi, G., *Klin. Wochnschr.*, 1935, **14**, 235.

[15] Mermod, C., and Dock, W., *Science*, 1935, **82**, 155.

tions being started at the same time the animals were put into the reduced pressure tank. The peak responses varied from 7.8 to 8.2%, *i. e.,* were somewhat lower than those of the controls (Fig. 1), the differences probably being without significance.

Bone Marrow. At the conclusion of the experiments most of the animals were killed and bone marrow from the lower vertebræ and femur was examined histologically. All the animals exposed to reduced oxygen tensions showed hyperplasia with very few fat vacuoles. While there was a slight decrease in eosinophiles, primitive red cells, and in megakaryocytes in the treated as contrasted with control animals, there was so much variation from one animal to another in the same group, or even from the femoral to the vertebral marrow of the same animal, that it was not possible, from the cells of the marrow, to distinguish the treated animals from the others.

These experiments prove clearly that guinea pigs, placed on diets which presumably render them reactive to liver extract, have no defect in the power of the marrow to form red cells and mobilize reticulocytes when the marrow is stimulated by arterial anoxemia due to low barometric pressure. Such exposure to low oxygen tension does not heighten the reticulocyte response of liver-treated animals over that of the controls, and instead of making the response to liver extract more striking than that under normal conditions, it entirely conceals any effect the liver may have on these animals.

It seems obvious that if there is a blood disorder in guinea pigs on the lettuce, carrot and oats diet, this is extremely mild since it results neither in anemia, nor in diminished response to hematopoietic stimulus such as anoxemia. At present it seems improper to speak of such animals as having an arrest in maturation of erythropoietic cells.

Conclusions. Guinea pigs subjected to reduced oxygen tensions showed prompt rises in reticulated red cells which reached a peak (8-14%), in 7-12 days. Then the reticulocytes decreased and in 3-4 weeks approached the control level.

Intramuscular injection of liver extract prior to, simultaneously with, or following, exposure to reduced oxygen pressure, had no demonstrable effects on the reticulocyte response; and the bone marrow of liver-treated animals could not be distinguished from that of untreated controls.

Congo red was also without effect on the reticulocyte response of guinea pigs under reduced oxygen tensions.