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Reticulocytosis Induced by Serum from Hypoxic Animals.* (22921)

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It has been demonstrated that whole plasma and serum from animals with blood loss anemia will induce a reticulocytosis when injected into normal animals(1-5). When large amounts of such plasma are injected over a prolonged period of time, increases in number of red blood cells, in hematocrit and in bone marrow normoblasts have been observed(3). The following experiment was carried out to investigate whether the serum of animals exposed to low oxygen tension of inspired air would induce a similar erythropoietic response.

Methods. To utilize the bioassay technic previously described(3) white New Zealand rabbits, weighing 2-3 kg, were used both as donors and recipients. Normal serum was obtained from normal animals kept at room air, and "hypoxic serum" from normal animals kept in 10% oxygen for 48 hours. The latter animals were kept in a bank of 8 cages, enclosed in an air tight envelope of plastic yard goods sealed around inlet and outflow tubes. Sufficient food and water was placed in the cages to provide for the animals until the end of the hypoxic period. Trays of calcium hydroxide and calcium chloride were used to absorb excess carbon dioxide and water. The air within the envelope was circulated by a small electric fan. Its temperature was checked and never rose above 31°C. The oxygen level in the envelope was kept constant at 10% by admitting compressed room air and pure nitrogen at appropriate flow rates. The total mixed air flow was 0.75-1.0 liter per minute per animal. The actual oxygen level was checked frequently by use of Beckman electromagnetic oxygen analyzer. Four 48 hour runs were made with above equipment with 8-12 animals in each run. After each run 2 animals were kept alive in room air as "tracers." The others were bled to death by intracardiac puncture within 1½ hours after end of hypoxic exposure. The "hypoxic serum" so obtained was pooled and frozen or refrigerated until used. Normal serum was obtained and stored in a similar fashion. Normal recipient rabbits each received 50 ml of serum i.v. once a day for 4 days. Daily hemoglobin determinations and reticulocyte counts were carried out on recipient animals and on tracer animals from the hypoxic group of donors. Reticulocyte counts were made on cover glass smears prestained with brilliant cresyl blue and counterstained with Wright's stain. Reticulocyte smears were counted "blind" and a minimum of 1000 red cells was examined—except on days 2, 3, 4, 5 when a minimum of 2000 cells was examined.

Results. Tracer animals. (Fig. 1). Eight rabbits, 2 from each of 4 runs, were followed after exposure to 10% oxygen for 48 hours. Although there was a wide variation in response, the mean reticulocyte level rose from a base line of 1.9% to maximum of 6.7% on day 3. Hemoglobin levels remained unchanged. *Normal recipients of normal serum.* (Fig. 2). Six normal rabbits received 200 ml of serum from normal donors. The reticulocyte levels rose slightly from a mean base

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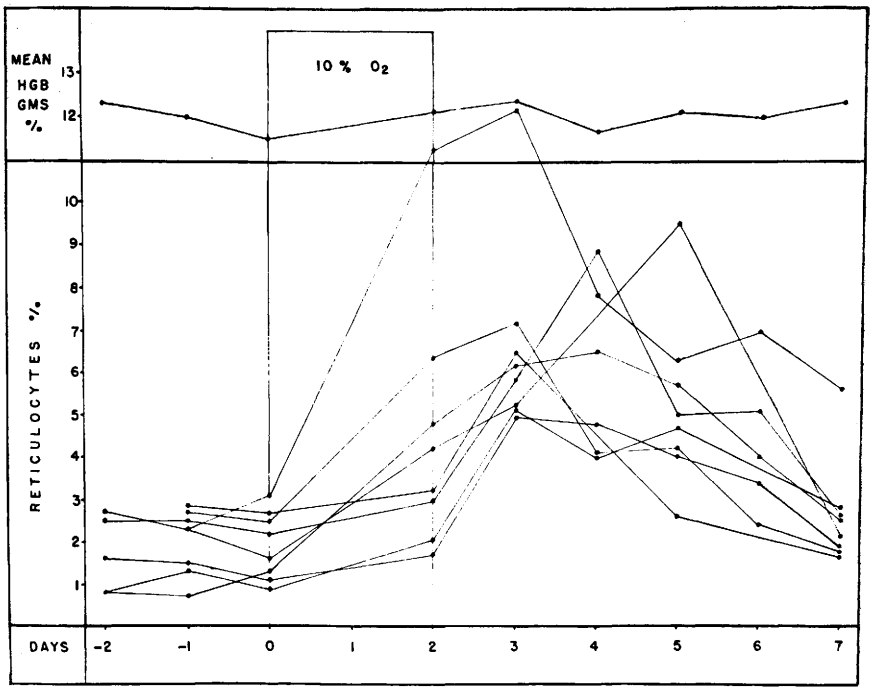

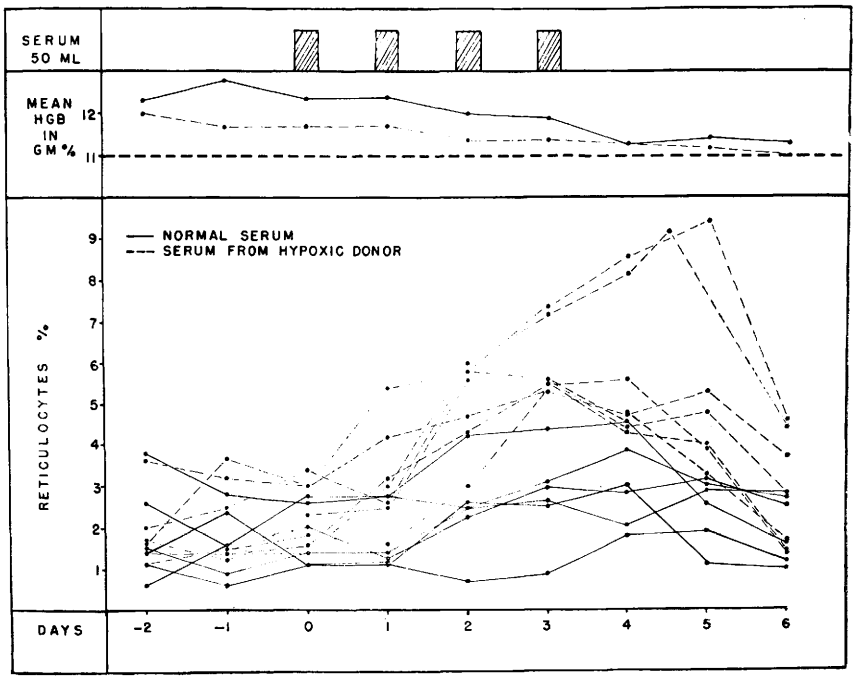

FIG. 1 (Top). Reticulocyte counts and hemoglobin concentrations of 8 rabbits exposed to 10% oxygen for 48 hours.

FIG. 2 (Bottom). Reticulocyte counts and hemoglobin concentration of six normal rabbits each receiving a total of 200 ml of serum from normal donors (solid lines) and of 7 normal rabbits each receiving a total of 200 ml of serum from donors exposed to 10% oxygen for 48 hours (stippled lines).

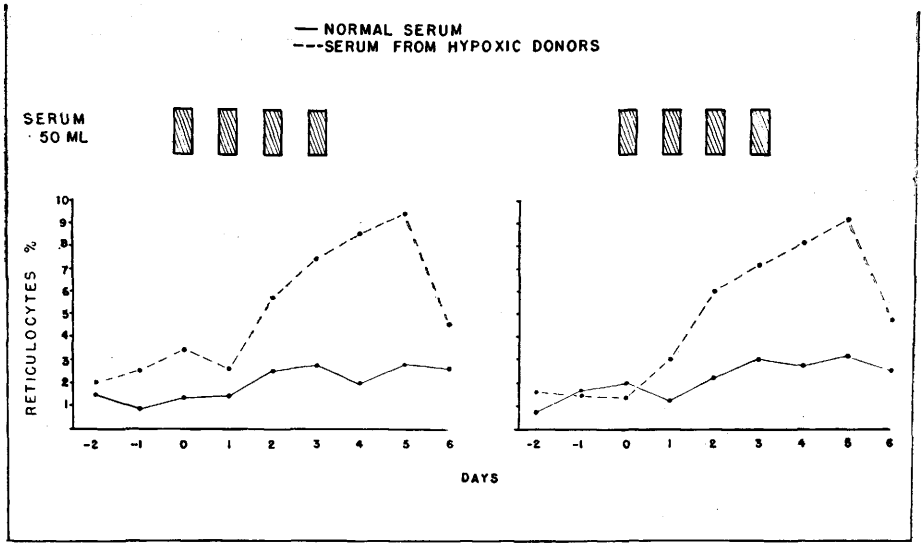

FIG. 3. Reticulocyte counts of 2 rabbits each receiving first 200 ml of "hypoxic" serum (stippled lines) and then, 2 weeks later, 200 ml of normal serum (solid lines).

line value of 1.9% to a maximum of 3.0% on day 4. Hemoglobin levels were essentially unchanged.

Normal recipients of hypoxic serum: (Fig. 2). Seven normal rabbits received 200 ml of serum from hypoxic donors. The mean reticulocyte counts rose from a base line level of 2.2% to maximum of 5.8% on day 4. Hemoglobin levels were essentially unchanged. Reticulocyte responses on day 2-5 differed from responses to normal serum in a statistically significant manner with a p value of less than 0.01.

Due to wide individual variation in recipients receiving the same serum, it was decided to use as recipients for normal serum the 2 animals which had the highest response to hypoxic serum. The normal serum was injected one week after the reticulocyte counts had retuned to normal base line values. The results are shown in Fig. 3. There was no rise in reticulocyte levels during the second series of injections.

Discussion. A number of previous studies (see review by Grant and Root) (6) have indicated that serum from animals kept at low oxygen tensions is capable of inducing an erythropoietic reaction when infused into normal recipients. However, serious doubts have been raised as to the validity of the bioassay technics used(7) and a re-examination of this problem was felt to be justified.

The studies reported here do strongly suggest that serum of rabbits exposed to 10% oxygen for 48 hours contains a factor capable of stimulating erythropoietic activity in normal rabbits. Similar results in rats have been reported by Stohlman and Brecher(8). It is conceivable that the reticulocytosis seen in this study represents not an increase in red cell production, but a non-specific release of preformed reticulocytes from bone marrow. However, the response is very similar to that seen in tracer animals after exposure to 10% oxygen for 48 hours, and also to the reticulocytosis induced by serum from animals made anemic by bleeding. Both of these responses have been shown to reflect true erythropoietic activity.

A surprising finding was that the reticulocyte responses of the rabbits to hypoxia itself, and to "hypoxic serum" were essentially the same. At first glance it would seem hard to understand why the recipients should have as great and in some cases greater response than animals exposed directly to low oxygen. Certainly, the concentration of any factor would be higher in the donor than in the recipients, where it is diluted immediately 1:3 in the plasma volume. The explanation for this

phenomenon is perhaps, that although the stimulating factor or factors were necessarily diluted in the recipients' plasma, they exerted their effect over a longer period of time, causing a sustained and additive stimulation.

Conclusions and summary. 1. Once a day for 4 days 7 normal rabbits each received 50 ml of serum obtained from hypoxic rabbits, and 6 normal rabbits each received the same amount of serum obtained from normal rabbits. The hypoxic serum induced a reticulocytosis which was significantly different (p value<0.01) from the response induced by injection of normal serum. At the level of hypoxia used (10% oxygen for 48 hours) the response in the recipient animals was as great as that seen in the animals directly exposed to hypoxia. 2. These results bolster the hypothesis that the erythropoietic response to hypoxia, or to blood loss anemia involves the production of a humoral factor.

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Fate of 2-Keto-L-Gulonic Acid in Rat and Guinea Pig.* (22922)

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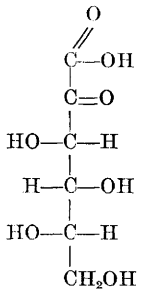
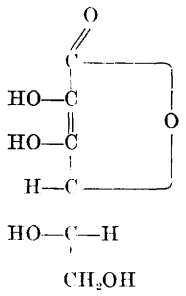
The close structural relation between 2-keto-L-gulonic acid and L-ascorbic acid has prompted studies of 2-keto-L-gulonic acid as a possible precursor of L-ascorbic acid(1-6).



2-keto-L-gulonic acid



L-ascorbic acid

The recent finding that L-gulonolactone can be transformed by the rat to L-ascorbic acid(6,7) suggests that 2-keto-L-gulonic acid could be an intermediate in this transformation. For these reasons experiments were carried out in guinea pigs and rats with uniformly C^{14} labeled 2-keto-L-gulonic acid. The results of this study indicate no appreciable conversion of this compound to L-ascorbic acid in either species.

Methods. 2-Keto-L-gulonic acid C_6^{14} was synthesized from L-sorbose C_6^{14} according to modification of the method of Reichstein and Grüssner(8-11). The compound had a specific activity of 0.2 μc/mg and melted at 171°C. Titration of a 5 mg sample of the labeled substance with indophenol dye(12) showed that no more than 0.25% of L-ascorbic acid could be present. Radioactive purity was established by 2 criteria. The first consisted of finding no change of activity upon recrystallization with carrier from a solvent mixture consisting of methanol, acetone and ligroin. Secondly, L-ascorbic acid prepared from the labeled compound(10) had the same molar specific activity when counted as

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