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Erythropoietic Stimulation Induced by "Anemic" Serum.* (20413)

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Carnot and Deflandre(1) suggested that red cell production is governed by a factor present in blood serum. Since then numerous investigators have attempted to demonstrate this factor in serum and plasma from animals rendered anemic by bleeding or hypoxemic by exposure to air with a low oxygen tension(2). All the results were either negative or so equivocal that there has been a general refusal to accept the hypothesis of a humoral regulation of red cell production. Krumdiech(3) reported that 20 ml of serum from anemic rabbits induced a striking reticulocytosis when injected into normal rabbits. These results were not confirmed by Toha *et al.*(4) who subsequently injected 20 ml of "anemic" rabbit serum once a day for 3 days followed by 10 ml a day for 15 days into normal rabbits and were unable to demonstrate more than a slight rise in the number of reticulocytes. Erslev(5) was likewise unable to demonstrate a significant rise in reticulocytes when similar amounts of "anemic" plasma were used. However, when 200 ml of "anemic" rabbit plasma was administered to normal rabbits during a 4-day period a significant in-

crease in the number of reticulocytes was found. The bone marrow showed a concomitant rise in the relative number of normoblasts and when 650 ml of "anemic" plasma was given during a period of 18 days there was a definite rise in hematocrit values and red blood cell count. These experiments showed that in rabbits red cell production can be stimulated by a humoral plasma factor. Reissman's(6) study with parabiotic rats tends to indicate that a similar factor exists in plasma from anemic rats.

Before further investigation was carried out into the nature and production of this factor it was felt advisable to demonstrate its presence in an animal species more closely related to humans than rabbit or rat. *Macaca mulatta* monkeys were selected for this purpose.

Materials and methods. Adult *Macaca mulatta* monkeys were used as donors and recipients. Three monkeys were made anemic by bleeding. They were bled from the femoral artery or vein after the animals had been anesthetized with 2.5 ml 6.0% nembutal given intravenously. Two or 3 times a week 80-100 ml of blood was withdrawn and "anemic" serum was obtained and pooled after the hemoglobin had been reduced to below 7 g/100 ml. Part of the pooled serum was kept for 2 weeks at 4°C while the remainder was frozen in a dry ice box. Serum from normal monkeys was ob-

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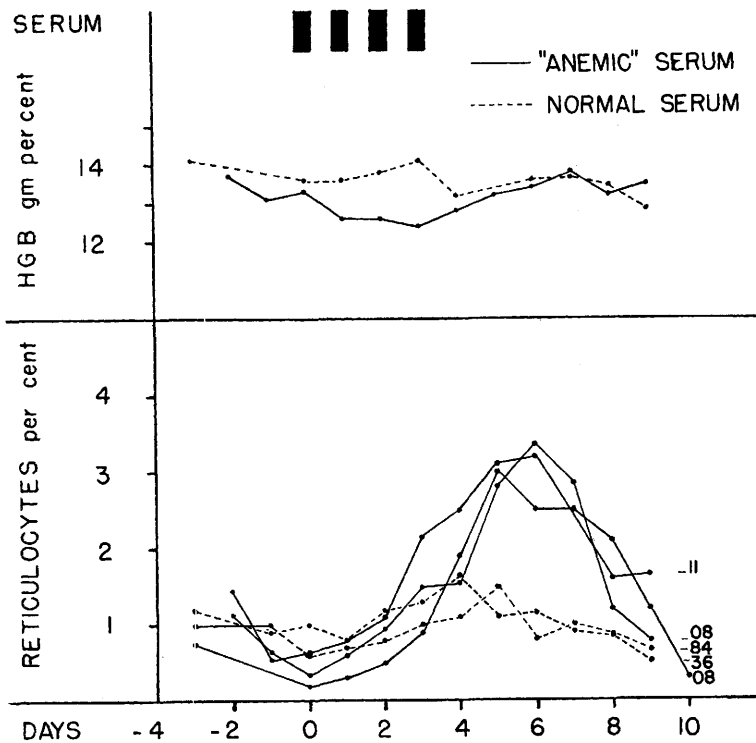


fig 1

FIG. 1. Upper part shows average hemoglobin of monkeys receiving "anemic" serum and monkeys receiving normal serum. Lower part shows the individual reticulocyte counts. Blocks at top of graph indicate the four days on which anemic or normal serum was injected intravenously.

tained in a similar manner and frozen. Four monkeys weighing between 3.5 kg and 5.3 kg were used as recipients. Serum was administered intravenously in the smaller saphenous vein once a day for 4 days. The total volume of serum given corresponded to from 6.1 to 10% of the body weight. This is the dosage which was found effective in rabbits(5). Determination of hemoglobin (electrophotometric) in duplicate, red blood cell count in duplicate, white blood cell count and platelet count were made daily on capillary blood from the ear. Reticulocyte counts (dry method) were determined by averaging the per cent obtained by two observers who counted 1000 red blood cells each.

Results. Monkey Mm-08, weighing 4 kg, received a total of 290 ml (7.3% of body weight) of "anemic" serum which had been kept at 4°C for 2 weeks. Three weeks later it received a total of 400 ml (10% of body

weight) of "anemic" serum, which had been kept in a dry ice box for 5 weeks. Monkey Mm-11, weighing 5.2 kg, received 350 ml (6.7% of body wt) of "anemic" serum which had been kept at 4°C for 2 weeks. Fig. 1 shows that the "anemic" serum induced a significant rise in the number of reticulocytes in both animals.

Monkey Mm-84, weighing 4.5 kg, and Monkey Mm-36, weighing 4.7 kg, received 285 ml (6.4% and 6.1% of body weights) of normal serum which had been kept in a dry ice box for 2 weeks. Only a slight rise in the number of reticulocytes occurred (Fig. 1). The hemoglobin (Fig. 1), red blood cell count, white blood cell count and platelet count did not change significantly during or after the administration of normal or "anemic" serum.

Discussion. Serum obtained from anemic monkeys induced a definite rise in the number of reticulocytes when injected intravenously

into normal monkeys but did not cause any other change in the peripheral blood count. Normal serum had no significant effect on the reticulocyte count or peripheral blood count. The reticulocyte count rose to approximately 3 times the normal values. This corresponds very closely to the relative increase observed in rabbits receiving "anemic" rabbit serum (5). Due to the crude semiquantitative technique and the small number of animals used it is impossible to evaluate the effect of freezing or refrigeration on the activity of "anemic" serum. However, the activity is not destroyed by these procedures. The above mentioned limitations also make impossible an evaluation of the effect of age of serum and of dosage on the reticulocyte response.

This experiment was designed to investigate the possible presence in the serum of anemic primates of a humoral factor capable of stimulating red cell production similar to the factor demonstrated in "anemic" rabbit plasma.

The findings reported here strongly suggest that this is the case.

Summary. Serum from monkeys made anemic by bleeding induced a significant reticulocytosis when large amounts (from 6 to 10% of the body weight) were infused into normal monkeys. Serum from normal monkeys did not have a similar effect.

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Effect of Chemical Agents on Skeletal Content and Excretion of Injected Strontium⁸⁹* (20414)

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It has been previously demonstrated(1) that administration of the sodium and calcium salts of ethylene diamine tetra-acetic acid (EDTA) results in the removal of substantial amounts of bone-fixed radioyttrium from rats. Removal effectively reduces the chronic radiation hazard associated with the internal deposition of this biologically long-lived fission product. Pretreatment of rats with the salts of EDTA also essentially prevented the deposition in bone of subsequently injected radioyttrium(1). The salts of EDTA are effective decontaminants because they are

strong chelating agents which form a soluble undissociated complex with yttrium that is readily excreted through the kidney. Another chemical compound, zirconium citrate, has previously been found effective in reducing the skeletal concentration of yttrium and plutonium and in increasing their urinary excretion (2-4). Zirconium citrate also has a limited ability to increase strontium excretion from the body, if injected soon after the administration of the radioelement(5). The chemical agent, 2,3-dimercaptopropanol, (BAL) has been shown to increase the excretion of polonium and to mobilize it from bone into muscle tissue(6).

The object of the present experiment is to attempt the removal of injected radiostrontium from the bodies of rats by administration of the salts of EDTA, zirconium citrate, and

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