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 technic 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.

The authors wish to thank Miss Jean Greiner and Mr. Joseph Negri for valuable technical assistance.

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Effect of Chemical Agents on Skeletal Content and Excretion of Injected Strontium^{89}.* (20414)

S. H. COHN, AND J. K. GONG. (Introduced by R. W. Brauer)

From the U. S. Naval Radiological Defense Laboratory, San Francisco, Calif.

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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TABLE I. Experimental Outline.

Part of study	No. of rats	Chemical agent used in treatment	Time of treatment with reference to Sr^{89} administration	Sacrifice time post-Sr^{89} administration (day)
I	10	NaEDTA (10 mg) followed in 15 min. by CaEDTA (50 mg)	One hr before	3
	10	None (controls)	——	3
II	5	Zirconium citrate (25 mg Zr), see Schubert(2)	One hr before	5
	9	*idem*	" " after	5
	8	*idem*	15 min. before and twice daily for 3 days after	5
	10	None (controls)	——	5
III	7	BAL (0.04 ml of 10% sol. in peanut oil) followed in 30 min. by NaEDTA (10 mg) and CaEDTA (50 mg)	BAL given 15 min. before, followed by EDTA salts and both given twice daily for 3 days after	5
	14	Zirconium citrate (25 mg Zr), followed in 2 hr by NaEDTA (10 mg) and CaEDTA (50 mg)	Zirconium citrate given 1 hr before, followed by EDTA salts, then both given twice daily for 3 days	5
	14	None (controls)	——	5

BAL. BAL was used in combination with EDTA in this study in an attempt to remove bone-fixed radiostrontium. Strontium was selected as the radioactive material to be removed because of its high fission yield(7) and further, because it is a prototype of a large number of fission products, the alkaline earths.

Experimental. The experiment was performed in 3 parts, using Sprague-Dawley rats as test animals. All the rats were injected with 15 μc of carrier-free $Sr^{89}Cl_2$ and treated with various chemical agents as indicated in Table I. All injections were administered by the intraperitoneal route with the exception of BAL, which was given intramuscularly. Animals were kept in individual metabolism cages and fed *ad libitum* with Purina laboratory chow. The metabolism cages were suspended in large glass jars and the excreta collected in plastic bags fitted over the bottom of the cage. All animals were sacrificed at intervals of either 3 or 5 days as indicated in Table I. Both tibiae were removed, ashed, weighed, and dissolved in 1 N HCl. Aliquots were analyzed for their Sr^{89} content by use of a Geiger-Müller counter and standard scaling circuit, in a manner previously described(1). Excreta for each animal were ashed, dissolved in 1 N HCl, and analyzed similarly.

Results and discussion. Part I. No significant differences were found between the mean values of the body weight and tibia ash weight in the EDTA treated and control groups. It was observed that pretreatment with EDTA salts did not prevent injected Sr^{89} from depositing in bone, as these treated animals had the same level of bone Sr^{89} as did the controls. The mean strontium content of both the tibiae of the control and EDTA-treated animals 3 days post-Sr^{89} injection was equal to 1.59% of the injected dose. From these data, an estimate of the total skeletal Sr^{89} content was calculated by using a factor of 27 as the ratio of total skeletal Sr^{89} content to the Sr^{89} content of one tibia. This method was described in a previous study(1). The mean percentage injected dose in the skeleton was 42.9 $\pm$ 5.2% in the control group and 42.7 $\pm$ 3.5% in the pretreated group 3 days post-Sr^{89} injection. In previous experiments (1) it had been found that injection of EDTA prior to injection of the fission product Y^{91} is the most effective time for EDTA administration as far as ionic complexing ability is concerned. Thus, even in the most favorable situation, the salts of EDTA were without

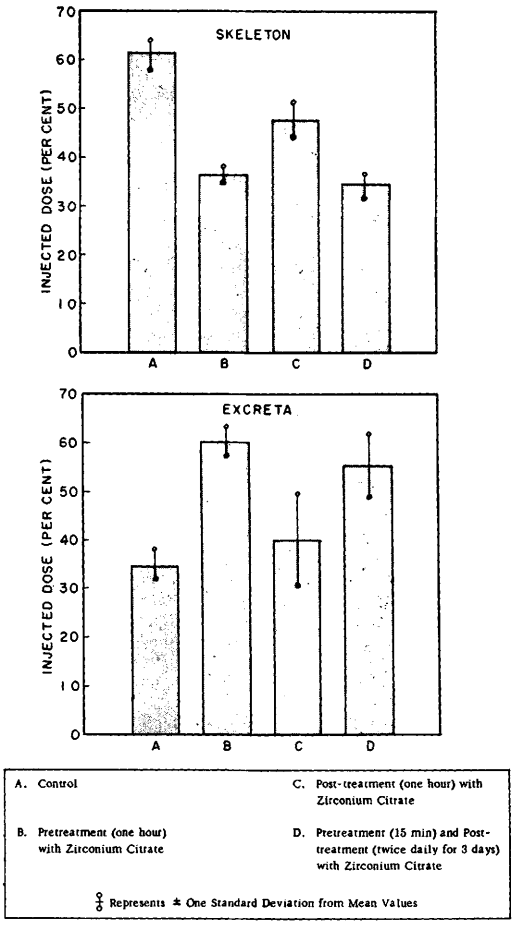

FIG. 1. Effect of zirconium citrate on skeletal content and excretion of injected radiostrontium.

effect on the skeletal content of radiostrontium. This could have been predicted largely on the basis that the stability constant of the strontium-EDTA complex at pH 7.4 is lower than that for the complex formed with the biologically competitive calcium ion(8).

Part II. The effects of zirconium citrate on the skeletal content and excretion of injected radiostrontium are presented in Fig. 1. In the animals pretreated with zirconium citrate and then injected one hour later with radiostrontium, the mean percentage injected dose of Sr^{89} in the skeleton after 5 days was 36.2% as compared with 61.1% for the untreated controls. The higher skeletal Sr^{89} content found in these controls as compared with that in Part I, is probably due to a difference in the age of the animals. It has been shown by Jones(9) that the uptake of injected Sr^{89} is an inverse function of age. The rats used in Parts I and III were mature animals 5 months old; the animals used in Part II were approximately 3 months old. Pretreatment in this experiment resulted in a skeletal Sr^{89} content 60% of that of the controls at 5 days post-Sr^{89} injection. Further, an enhanced Sr^{89} excretion of about 40% was observed when comparison was made with the Sr^{89} excretion of their controls. It is to be noted that the skeletal plus the excreta Sr^{89} content accounts for 95% of the total injected dose 5 days after Sr^{89} injection. It can, therefore, be concluded that most of the increased excretion was derived from that fraction of the injected Sr^{89} which would have ordinarily been fixed by the skeletal system.

In the animals treated with zirconium citrate one hour post-Sr^{89} injection, there was a less marked decrease in the skeletal Sr^{89} content (20%) and a smaller increase in Sr^{89} excretion than observed in the pretreated animals. In the animals pretreated with zirconium citrate and treated in addition twice daily for 3 days, the skeletal Sr^{89} was only slightly lower (56% of controls) than in the one-hour pretreated group. It would appear, therefore, that the effect of zirconium citrate in complexing and removing the Sr^{89} from the body is greatest when the zirconium citrate is administered before Sr^{89} injection. Continued injection of zirconium citrate for 3 days did not increase the excretion of Sr^{89} appreciably.

Strontium is chemically and metabolically very similar to calcium and is deposited in the bone salt, in contrast to yttrium which is deposited primarily in the uncalcified organic matrix of bone(10,11). Conditions which affect the metabolism of yttrium do not appear to influence the metabolism of strontium(9). Thus, as Schubert(2) points out, one would not necessarily expect zirconium administration (found to influence the metabolism of yttrium) to greatly affect the metabolism of bone-fixed strontium. Schubert did find, however, that after strontium uptake by bone is complete, the administration of zirconium citrate can apparently remove a small portion of the strontium from the body(5). The principal action of the zirconate complex is

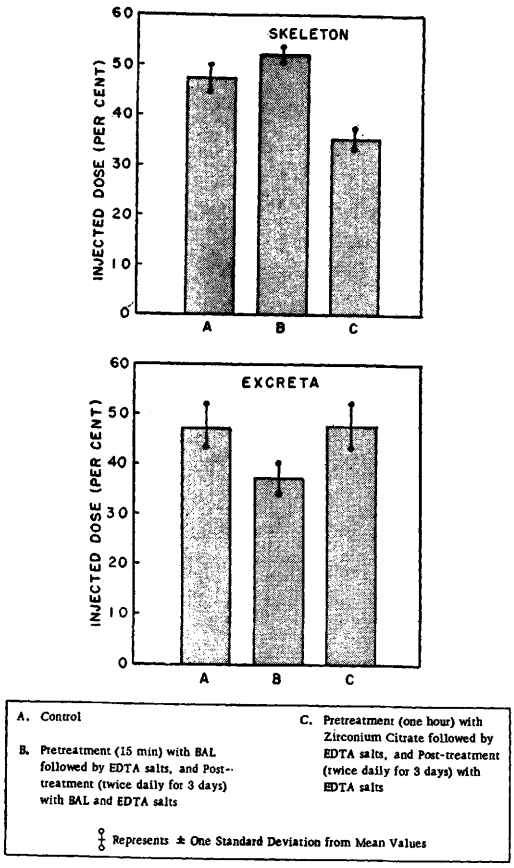

FIG. 2. Effect of various chemical agents on skeletal content and excretion of injected radiostrontium.

then largely on that fraction of injected Sr^{89} deposited in the soft tissue. The fact that pretreatment with zirconium did have an effect on the disposition of subsequently injected strontium may possibly be explained in the following manner. Uptake of small amounts of strontium by bone is usually interpreted as a result of adsorption or ionic exchange(12). If injected zirconium citrate is adsorbed on the surface of bone salt crystals, so that free surface-bonds are saturated, the subsequently injected strontium would not be fixed by the bone and would then be excreted. This same explanation would hold for the animals treated with zirconium citrate at one hour after strontium injection. At one hour post-intraperitoneal injection, strontium is still largely in the blood and soft tissue.

Part III. The results of EDTA treatment used in combination with zirconium citrate and with BAL are presented in Fig. 2. Treatment with BAL prior to sodium and calcium EDTA administration had no significant effect on the skeletal Sr^{89} or on the amount found in the excreta 5 days post-Sr^{89} injection. The total percentage recovery of the injected dose in the experimental animals was somewhat lower than that found in the controls. The rationale for pretreatment with BAL was the observation that BAL is able to shift polonium from the skeleton to the soft tissue(6). It is apparent that BAL does not have this ability to shift strontium from the bone to the soft tissues where it would be available for complexing with administered EDTA salts.

If the removal of strontium from the skeleton following the combined administration of EDTA and zirconium citrate is compared with the results of the experiment in which zirconium citrate alone was administered, it is seen that the combined use not only fails to enhance the effect of zirconium citrate, but on the contrary lowers it. This latter effect may be due to the complexing of zirconium by EDTA with subsequent removal from the body.

It can be concluded, then, that the salts of EDTA administered before or after Sr^{89} injection, either alone or in combination with zirconium citrate or BAL, have no effect on the Sr^{89} content in the skeleton or on the concomitant Sr^{89} excretion.

Summary. 1. The effect of chemical agents ethylene diamine tetra-acetic acid (EDTA) and zirconium citrate on skeletal content and excretion of injected radiostrontium was studied in rats. BAL was also administered in combination with EDTA in an effort to increase the excretion of injected strontium. 2. Administration of salts of EDTA under the most favorable conditions for its action had no effect on skeletal distribution of injected carrier-free Sr^{89}. Zirconium citrate administrated prior to injection of Sr^{89} resulted in a level of Sr^{89} in the skeleton at 5 days 40% lower than that found in controls. A corresponding increase in Sr^{89} excretion was also noted. Combined administration of EDTA with zirconium citrate and with BAL are without effect on skeletal distribution of injected Sr^{89}. Possible

explanations for the effect of the various chemical agents employed are discussed.

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Safety of Immune Serum Globulin with Respect to Homologous Serum Hepatitis.* (20415)

RODERICK MURRAY AND FRANK RATNER (Introduced by S. E. Branham)

From the Laboratory of Biologics Control, National Microbiological Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare, Bethesda, Md.

Immune serum globulin is prepared commercially at the present time by two general processes: (a) salting-out, *e.g.,* by means of ammonium sulphate, and (b) by the cold-ethanol process(1,2) or a variation thereof. Blood obtained either by venipuncture or by extraction from placentas is used as the source material. *The Minimum Requirements of the National Institutes of Health for Immune Serum Globulin*(3) have stipulated that pools consist of at least 500 individual contributions.† In view of the fact that approximately one individual out of 300 receiving blood transfusions may develop hepatitis(4-7), it might be expected that many of the pools of plasma used in the manufacture of immune serum globulin would be infected with the agent(s) of homologous serum hepatitis. General experience indicates that the incidence of homologous serum hepatitis following administration of immune serum globulin must be low. According to Ordman *et al.*(8), one of 400 individuals in one series receiving immune serum globulin developed jaundice. The fact that 74 other children in the series received material from the same lot without untoward sequelae makes it unlikely that the immune serum globulin was the cause of the hepatitis in this one individual. During 1943-44 a follow-up by Janeway of 869 individuals who received immune serum globulin revealed no cases of jaundice(9). Hammon, Coriell, and Stokes, in a follow-up of field studies on poliomyelitis in Utah, found that immune serum globulin injected into 2,800 children was not icterogenic(10). Material prepared by the cold-ethanol method was used in these studies. Cockburn(11) has reported the development of one case of hepatitis among 58 subjects inoculated with immune serum globulin produced by ether fractiona-

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† A recent revision (April 9, 1953) of these Minimum Requirements has increased the figure to 1,000. This is also the figure laid down by the Minimum Requirements for Poliomyelitis Immune Globulin (Apr. 9, 1953).