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THE USE OF CHELATING AGENTS FOR ACCELERATING EXCRETION OF RADIOELEMENTS

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June 14, 1951

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THE USE OF CHELATING AGENTS FOR ACCELERATING EXCRETION OF RADIOELEMENTS*

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Although the deleterious effects of exposure to ionizing radiation were first recognized and described over fifty years ago, the adequate treatment of these effects still remains a therapeutic challenge. At the present time, when increasing numbers of our population are being exposed to radiation because of the great increase in availability and use of radioactive isotopes and because of the potential exposure of much greater numbers of people to radiation following a possible atomic bomb burst or from disseminated radioactivity, the need for development of adequate therapy is becoming an increasingly pressing medical problem.

In a consideration of possible approaches to therapy, one must distinguish between radiation from sources external to the body and radiation which results from radioactive materials which by some means or other have gained access into the body. Internally deposited radiation emitters can be particularly insidious since so many of them become fixed in the skeleton and are eliminated at very slow rates. While it is possible to remove external sources of damaging radiation once the hazard is recognized, the internal radiation emitters often are not readily displaced and the body remains exposed to prolonged continued radiation. Where long-lived elements, such as plutonium with a biological half-life of the order of 100 years[1] or radium with one of 45 years,[2] are involved, the body can be subject to continuous radiation for the remainder of its lifetime. Moreover, because the radiation persists for such long periods of time, only minute amounts of certain radioelements, i.e., plutonium and radium, need have entered initially to produce considerable

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injury. The effects of this type of chronic exposure to radiation are well documented in the case reports on radium poisoning in workers in the luminous dial industry.[3] The damage is manifest in various forms, i.e., severe anemia, osteitis, and osteogenic sarcoma.

In the past, therapy to check injury from internal radiation emitters had been directed to attempts to hasten the elimination of the noxious agent. These have included such methods as low calcium diets, parathormone, viosterol, ammonium chloride, calcium gluconate,[4,5] and low phosphorus diets.[6] Of these the decalcifying type of treatment was reported to have some measure of effectiveness. The results of the other types of therapy were equivocal.

The most successful approach was reported in the work of Schubert.[7] Using zirconium citrate complex, administered 3 hours after the injection of radioyttrium and plutonium into rats, he was able to increase the urinary excretion of the injected radioelements many times over that of the excretion in the untreated rats, in some instance up by a factor of 50 for the first day of excretion. However, when used at later time periods, i.e., in a dog at 150 days, the increase in urinary excretion was only a factor of 2 to 3 over the control period. The fecal excretion of the radioelements was not influenced by the treatment.

The present study reports a different approach for accelerating the excretion of radioelements, namely the use of chelating agents. Many of the rare earth and actinide series of elements form water-soluble chelates with various organic compounds. This consideration suggested the possibility that this property of chelating agents might be used "in vivo" to mobilize radioelements fixed within the body. Of the many compounds considered, ethylenediamine tetracetic acid (EDTA) was chosen for this study. The EDTA was selected because it forms a very stable chelate with many metal ions and hence has a strong tendency to remove such ions from insoluble combinations, i.e., it will

dissolve such salts as calcium oxalate, barium sulphate, and lead phosphate in neutral and alkaline solutions.[8] Moreover, it has suitable characteristics for "in vivo" application. It forms serum soluble chelates which are not readily broken down in the body but are rapidly excreted intact via the kidney. It is readily absorbed through the digestive tract. It has a very low level of toxicity when used as described in this study, namely as the calcium complex. A dose equivalent to 3 grams per kilo of body weight, injected intraperitoneally into rats, will result in death in approximately 50 per cent of the injected animals in one day. When administered as a neutral salt, the EDTA combines avidly with serum calcium and produces death in hypocalcemia with relatively small doses, i.e., approximately 200 milligrams per kilo of body weight in rats. However, when administered combined with an equivalent weight of calcium ion, this negative calcium balance is prevented and the compound is rendered relatively non-toxic. Under these conditions the EDTA will still chelate a large number of metals, namely the metals which displace the calcium from combination with the EDTA because they form more stable chelates. Fortunately, plutonium and many of the elements formed in fission fall in this category.

Experimental

In the experiments to be described, the following elements were used, namely (a) yttrium^{91}, because it is a relatively abundant fission product; (b) cerium^{144}, because it is one of the lanthanide rare earths, and (c) plutonium^{239}. All three of these elements are potentially serious health hazards in that they are bone seekers with long biological half-lives.

In all the experiments, female Curtis-Dunning rats weighing approximately 250 grams were employed. The yttrium experiment was performed using 5 groups of 3 rats each. Each rat was injected intravenously with approximately 25 μc of carrier-free Y^{91} in 0.5 cc. of a solution of yttrium chloride in isotonic saline

freshly adjusted to pH 6. The first group, A, was used as control and received no further treatment. The other groups each received calcium EDTA by different routes, intraperitoneally, intramuscularly, and by stomach tube under a time dosage schedule as indicated in Table 1.

TABLE 1

Administration of Ca EDTA to rats injected with Y^{91}

Time after Y^{91} Administration	Group A (control)	B-C	D	E
2 hours	none	20 mgm I.V.*	none	none
48 hours	none	none	20 mgm I.M.*	20 mgm S.T.*
20th day	none	25 mgm I.P.*	25 mgm I.M.	25 mgm S.T.
21st day	none	25 mgm I.P.	25 mgm I.M.	25 mgm S.T.
22nd day	none	25 mgm I.P.	25 mgm I.M.	25 mgm S.T.
23rd day	none	25 mgm I.P.	25 mgm I.M.	25 mgm S.T.
48th day	none	25 mgm I.P., twice daily	25 mgm I.M., twice daily	25 mgm S.T., twice daily
49th day	none	25 mgm I.P., twice daily	25 mgm I.M., twice daily	25 mgm S.T., twice daily
50th day	none	25 mgm I.P., twice daily	25 mgm I.M., twice daily	25 mgm S.T., twice daily
51st day	none	25 mgm I.P., twice daily	25 mgm I.M., twice daily	25 mgm S.T., twice daily
52nd day	none	25 mgm I.P., twice daily	25 mgm I.M., twice daily	25 mgm S.T., twice daily

* I.V. - intravenous; I.M. - intramuscular; S.T. - stomach tube; I.P. - intraperitoneal.

The urine and feces were collected separately. The collection of 3 days excretion was lumped into groups except in the first 3 days, and the yttrium content assayed according to the method described by Scott et al.[9] The results were expressed on the basis of per cent of the originally administered dose per

gram of dry ash. The experiments with Ce^{144} and Pu^{239} were carried out together. Since cerium^{144} is a beta emitter and plutonium^{239} is an alpha emitter, they can be assayed in the presence of each other on the same sample. Because of this, it was possible to use the same groups of animals for both elements. A dose of approximately 20 μc of carrier-free Ce^{144} as $CeCl_3$ and 15 γ of Pu^{239} as sodium plutonyl acetate in 0.5 cc. of isotonic neutral solution containing 15 mgm of citric acid was injected into each of 12 animals. Six of the animals were set aside as controls and received no treatment. The other groups received different chelating agents at various time periods as indicated in Table 2.

TABLE 2

Administration of chelating agents to rats injected with Ce^{144} and Pu^{239}

Time after injection of radioelement	Amount and type of chelating agent
18 hours	25 mgm Ca EDTA, I.P.
13th through 17th day	50 mgm Ca EDTA, I.P., twice daily
35th through 38th day	100 mgm Ca citrate, I.P.
48th through 52nd day	100 mgm Fe-3*, I.P.

The feces and urine were collected separately daily. The excreta were assayed for plutonium and cerium by methods previously used in this laboratory and described by Scott and co-workers.[10] After 62 days the animals were sacrificed and tissues taken for assay. The results of the tissue assay will be presented in a future publication.

Results

Yttrium: The results for the urinary and fecal excretion of yttrium are presented in Figures 1 and 2. For undetermined reasons all of the control

* A commercial chelating agent manufactured by the Bersworth Chemical Company, Framingham, Massachusetts. The structure is not revealed.

animals except one died on the 12th day, hence data on the level of excreta for these animals are not available after that time period. Fortunately, the other groups could be used as their own controls in this type of experiment. This was arranged by delaying administration of the chelating agent until the time at which urinary excretion of the radioelement was occurring at a constant rate. Any changes brought about by the treatment were measured as compared to the pretreatment base line. The second course of treatment was again delayed until a steady excretion state had recurred.

All modes of administration of the calcium EDTA whether intragastrically, intraperitoneally, or intramuscularly, produced approximately the same effect in enhancing the urinary excretion of the radioyttrium. As would be expected, the effect produced when the compound was given by stomach tube tended to appear more slowly and was more prolonged. A single dose given at 2 hours resulted in a urinary excretion of 67 per cent as compared to a mean of the untreated groups of about 30 per cent. When 25 milligrams of calcium EDTA was administered in the 20 through 23 day period, the level of excretion during the time of administration rose 5 to 10 times over the level of excretion in the time periods before and after, i.e., from a level in the range of .07 per cent up to .7 per cent. At the 28 through 52 day period the administration of 25 milligrams of the chelate twice daily accelerated the excretion from base levels of .07 per cent up to as high as 1.75 per cent, a factor of 20 to 25 times over that in the time periods immediately preceding and shortly after the treatment. In this instance administration of the dose twice daily resulted in considerably more than twice as great an effect.

The effect of the treatment on fecal excretion was much more difficult to determine because of the wide day-to-day variation. However, it appears that there was some small effect when the chelating agent was administered in the 20 through 23 day time period.

Plutonium: The results for plutonium are presented in Figures 3 and 4. The administration of the calcium EDTA at the time periods of 1 day and 13

through 18 days resulted in enhanced urinary excretion of the injected radioelement to levels which were approximately 10 times greater than that of the control animals. The effect lasted for several days after the cessation of the treatment. Calcium citrate administered under the same conditions but at the time period of 34 through 38 days did not significantly alter the urinary excretion. Fe-3 administered at the time period of 48 through 53 days increased the urinary excretion by the factor of 6 to 7 each day of administration and by somewhat smaller amounts for the 3 days following cessation of the treatment. The fecal excretion of plutonium was not affected by the chelating agents except when the calcium EDTA was administered during the first 24 hours. At this time there was a marked effect.

Cerium: The results for cerium are presented in Figures 5 and 6. The administration of the calcium EDTA did not influence the urinary excretion of the cerium except when administered during the first 24 hours when an effect of approximately 3 times as great as the control level was obtained. The fecal excretion of cerium was also not affected by the chelating agents except when administered during the first 24 hours and here again as in the plutonium a pronounced effect was noted. Calcium citrate did not affect the cerium excretion levels. Fe-3, on the other hand, given in the 48 through 53 day period, increased the urinary excretion of the cerium by a factor of as much as 6 times over the control group.

Discussion:

The marked enhancement of urinary excretion of the injected plutonium and radioyttrium which followed the administration of calcium EDTA and Fe-3 to rats strongly indicates that these agents are worthy of further investigation as a possible means of mobilizing internally deposited radioelements.

It is apparent from a consideration of the above data that for effective therapy involving long-lived elements, prolonged continued treatment will be necessary even when as profound an alteration as a tenfold increase in the

excretion rate is effected. In the rat, at the time when plutonium is well-fixed in the body, the urinary-fecal ratio is approximately 1 to 10. Hence a tenfold increase in urinary excretion will not effect the overall excretion of the radioelement very markedly. In the human being the situation is more favorable. The urinary-fecal ratio when the plutonium is well-fixed in the body is approximately one to one[1] and here a tenfold increase in urinary excretion will result in an approximately tenfold increase in the total excretion of the plutonium. With an increase in the excretion rate of a factor of 10 the turn-over time in the body would be decreased to 1/10 of that in untreated individuals, assuming that the treatment continues effective throughout the entire therapy period. As was indicated above, with elements having very long turn-over times the treatment may have to be extended for a period of months or even years. In this connection it is of interest that repeated day-to-day administration of chelating agents in this experiment continued to bring forth high levels of urinary radioelement excretion. It still remains to be seen, of course, whether this effect could be brought about in humans.

It is a reasonable assumption that the mechanism of the mobilization of radioelements is the formation of water-soluble chelates in the body, similar to the formation of chelates of metals in the test tube. It is significant that the postulation from "in vitro" data that plutonium and yttrium would displace the calcium from combination with the EDTA was verified "in vivo". One can predict that strontium would not be mobilized from the body by this treatment because of the fact that calcium forms a much stronger chelate with EDTA than does strontium. From similar consideration, one would not expect radium to be removed from the body with EDTA. Cerium is evidently a border-line case in that the relative affinity of EDTA for this element is only slightly greater than that of calcium and hence cerium is only sparingly removed "in vivo". The increase in fecal excretion only on the first days of treatment is probably related to the high plutonium content in the liver at this time period.

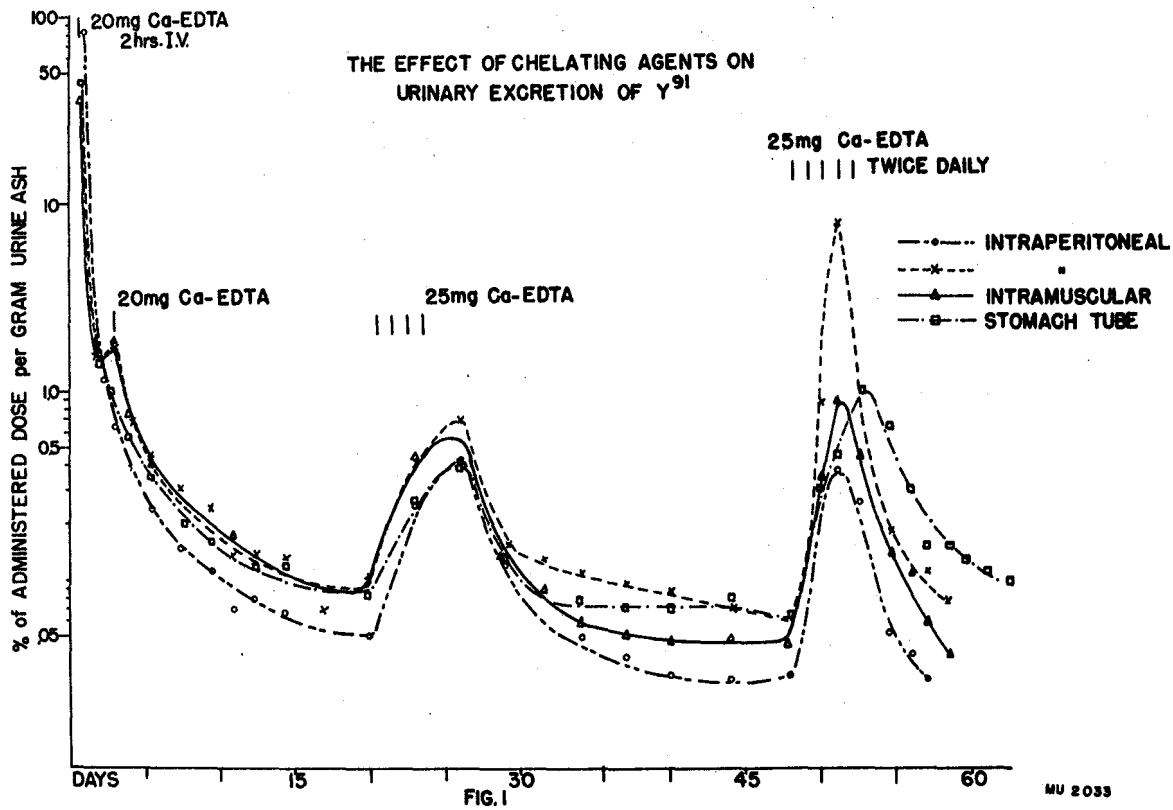
THE EFFECT OF CHELATING AGENTS ON
URINARY EXCRETION OF Y91
20mg Ca-EDTA
2hrs. I.V.
20mg Ca-EDTA
25mg Ca-EDTA
25mg Ca-EDTA
TWICE DAILY
INTRAPERITONEAL
INTRAMUSCULAR
STOMACH TUBE
% of ADMINISTERED DOSE per GRAM URINE ASH
100
50
10
1.0
0.5
0.05
DAYS
15
30
45
60
MU 2033

FIG. 1

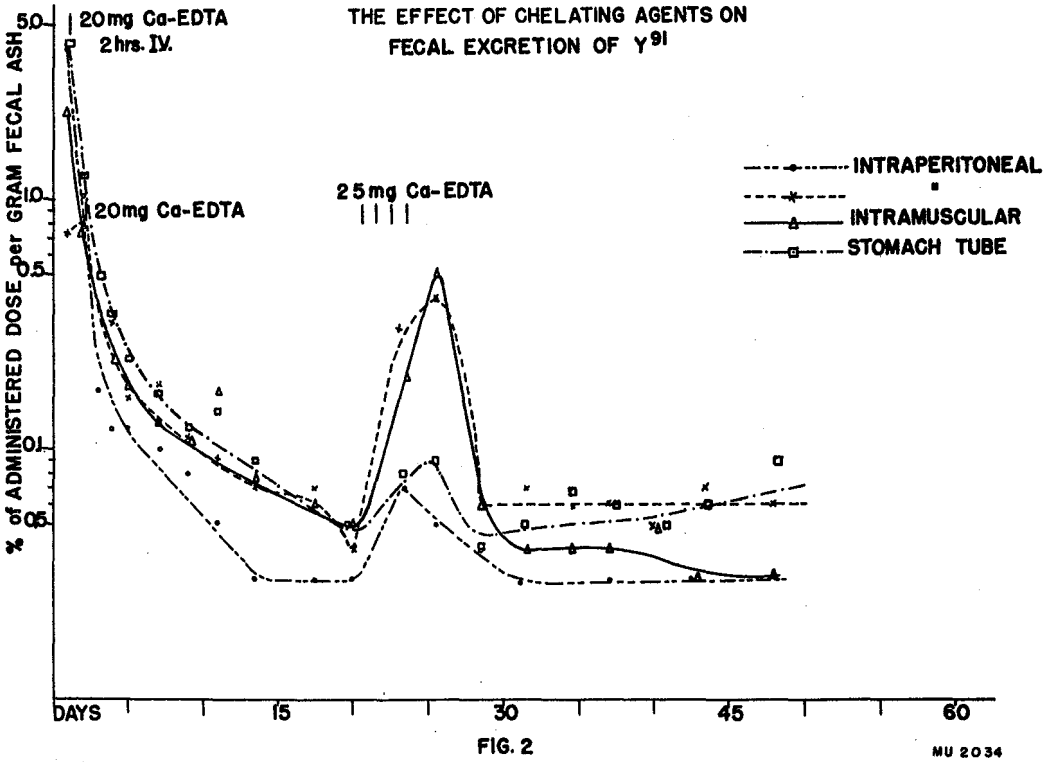
THE EFFECT OF CHELATING AGENTS ON
FECAL EXCRETION OF Y 91
20mg Ca-EDTA
2hrs. IV.
20mg Ca-EDTA
25mg Ca-EDTA
INTRAPERITONEAL
INTRAMUSCULAR
STOMACH TUBE
% of ADMINISTERED DOSE per GRAM FECAL ASH
5.0
1.0
0.5
0.1
0.05
DAYS
15
30
45
60

FIG. 2

MU 2034

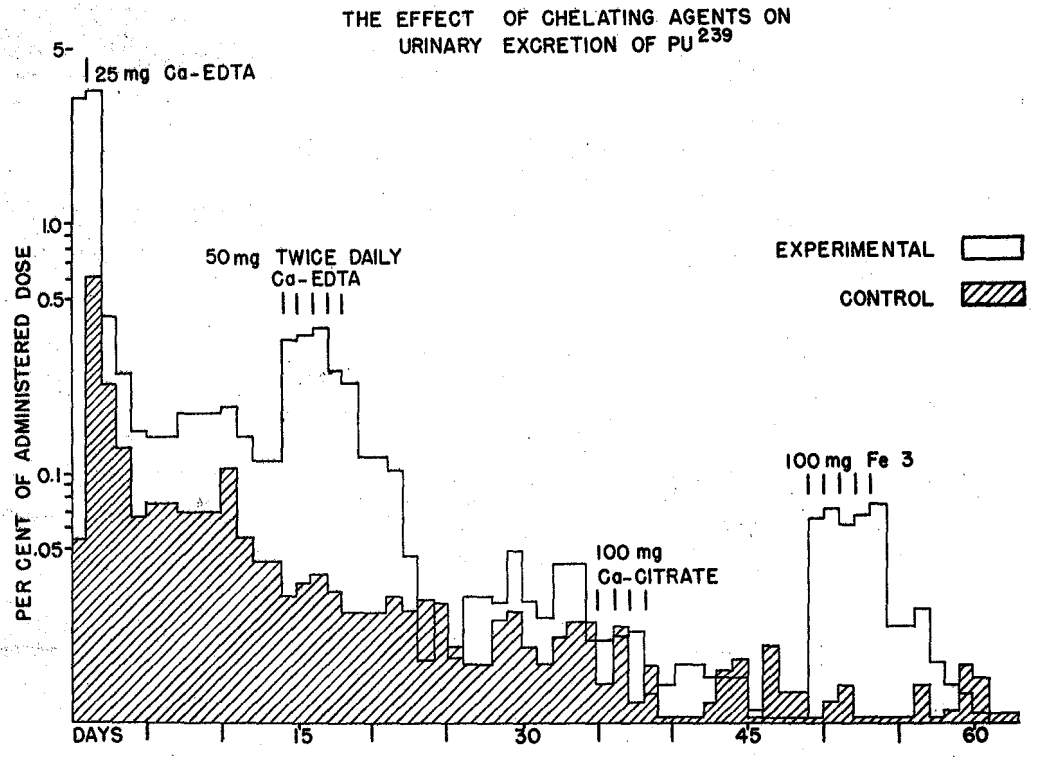
THE EFFECT OF CHELATING AGENTS ON
URINARY EXCRETION OF PU 239
5-
25 mg Ca-EDTA
1.0
0.5
0.1
.05
PER CENT OF ADMINISTERED DOSE
50 mg TWICE DAILY
Ca-EDTA
EXPERIMENTAL
CONTROL
100 mg Fe 3
100 mg
Ca-CITRATE
DAYS
15
30
45
60
MU 2035

FIG. 3

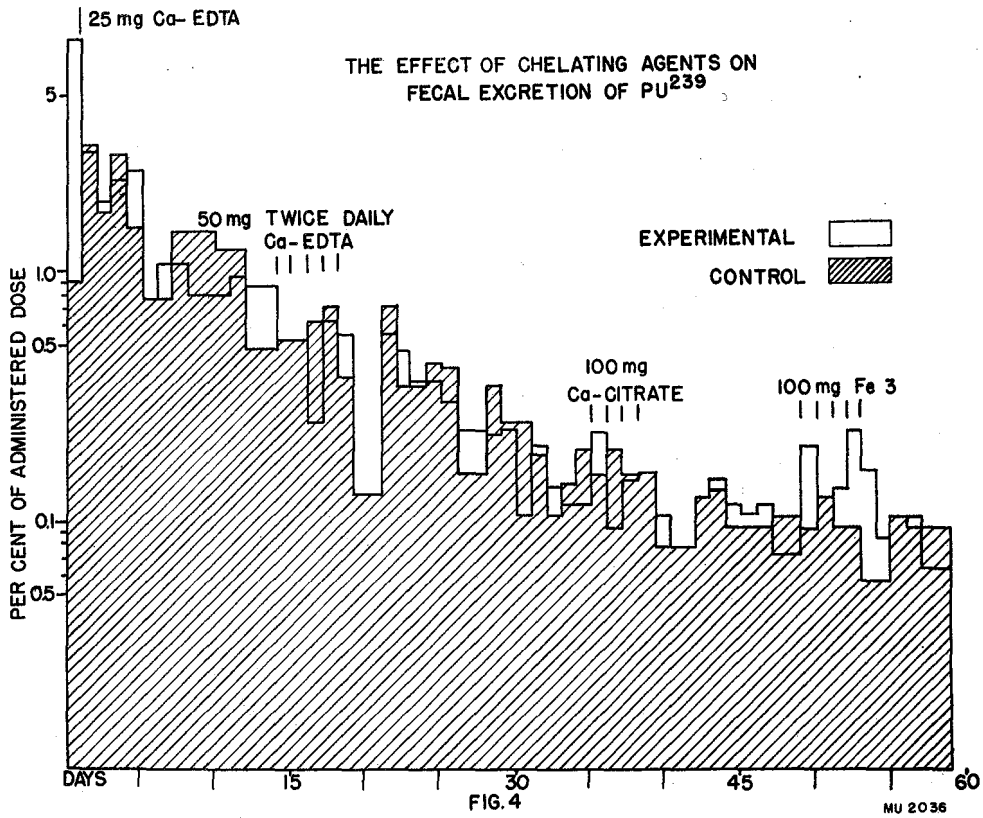
25 mg Ca- EDTA
THE EFFECT OF CHELATING AGENTS ON
FECAL EXCRETION OF PU239
5
50 mg TWICE DAILY
Ca-EDTA
EXPERIMENTAL
CONTROL
1.0
0.5
100 mg
Ca-CITRATE
100 mg Fe 3
0.1
0.5
PER CENT OF ADMINISTERED DOSE
DAYS
15
30
45
60
MU 2036

FIG. 4

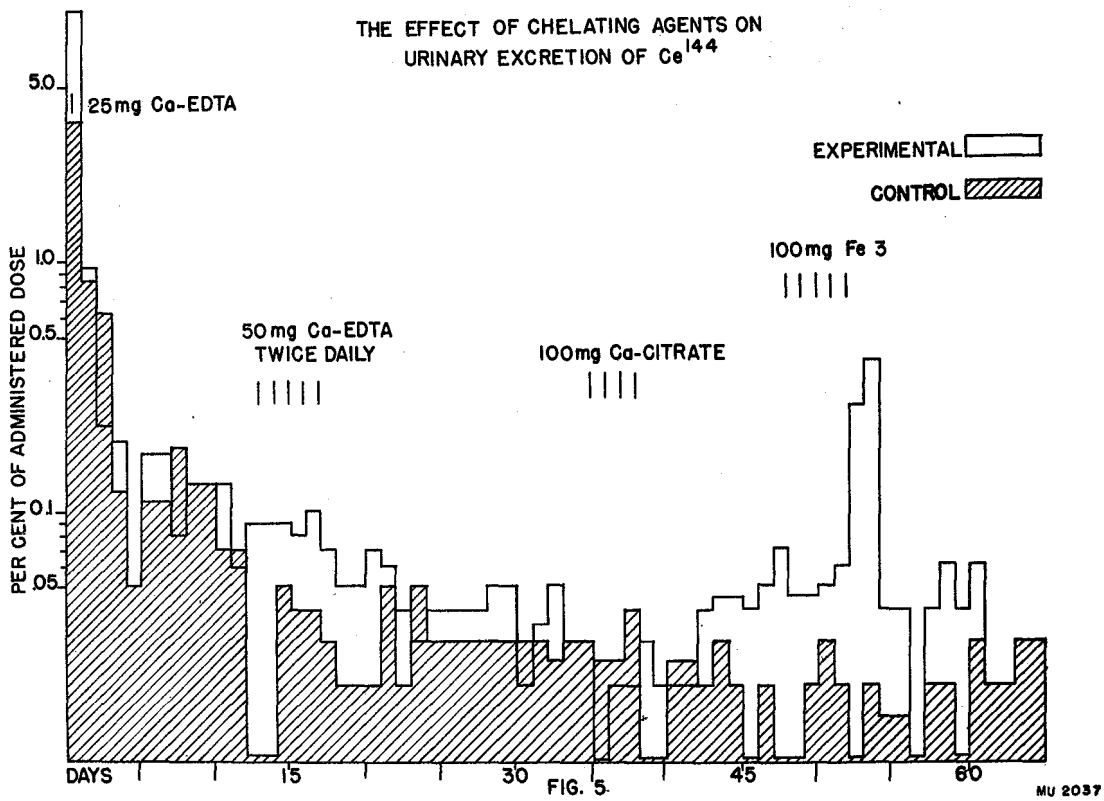
THE EFFECT OF CHELATING AGENTS ON
URINARY EXCRETION OF Ce144
5.0
25mg Ca-EDTA
EXPERIMENTAL
CONTROL
100mg Fe 3
1.0
0.5
50mg Ca-EDTA
TWICE DAILY
100mg Ca-CITRATE
PER CENT OF ADMINISTERED DOSE
0.1
.05
DAYS
15
30
45
60
MU 2037

FIG. 5.

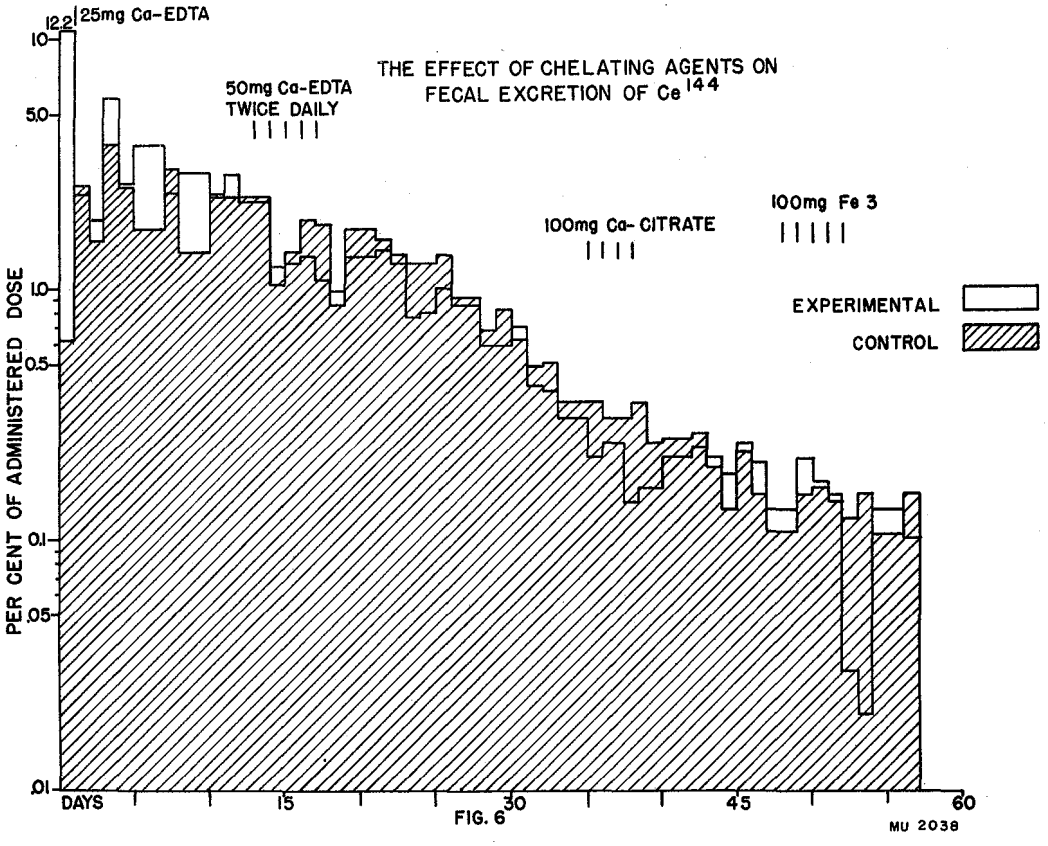
THE EFFECT OF CHELATING AGENTS ON
FECAL EXCRETION OF Ce 144
12.2
25mg Ca-EDTA
50mg Ca-EDTA
TWICE DAILY
100mg Ca- CITRATE
100mg Fe 3
EXPERIMENTAL
CONTROL
PER CENT OF ADMINISTERED DOSE
10
5.0
1.0
0.5
0.1
0.05
0.01
DAYS
15
30
45
60
MU 2038

FIG. 6

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