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The Journal of Laboratory and Clinical Medicine

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VOLUME 43
JANUARY—JUNE, 1954

ST. LOUIS
THE C. V. MOSBY CO.
1954

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FILE BARCODE
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THE METABOLISM OF C^{14} LABELED ETHYLENEDIAMINETETRA-ACETIC ACID IN HUMAN BEINGS

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LOS ALAMOS, N. M.

ETHYLENEDIAMINETETRAACETIC acid is a recently developed therapeutic agent used in the treatment of lead[1, 2] and radioactive metal poisoning,[3] as an anticoagulant in blood used for transfusion,[4] and in other instances where control and investigation of metal ion concentration is desired.[5] A study of the metabolism of the compound was undertaken to gain information which might be used for more effective application of the material, in particular for establishing optimum dosage schedules, and to reveal any possible deleterious effects which might result from prolonged use of the drug. The calcium chelate of the compound was studied since this is the form in which the drug is most commonly employed.

METHODS

Young adult healthy men were used as subjects. Studies were carried out after intravenous, intramuscular, and oral administration and after application on the skin. Three subjects were used in each group.

In the intravenous studies, the injection solution was prepared by mixing 2.2 mg. of Ca ethylenediamine-2-C_2^{14} acetic acid, yielding approximately 2.5×10^7 disintegrations per minute (d/m) with 2 Gm. of "cold" (unlabeled) $CaNa_2$ EDTA* in a total of 10.5 ml. of water solution. The C^{14} labeled material was prepared by Murray and Ronzio.[6] The "cold" $CaNa_2$ EDTA was prepared as a 20 per cent neutral solution in water, especially for drug use. The injections were given over two- to three-minute periods into the antecubital arm vein. Blood samples were drawn at appropriate intervals indicated in Fig. 1, and in one case a spinal fluid sample was obtained. Urine was collected as voided for twenty-four hours, at which time the activity had fallen below detection levels. In two of the intravenous studies blood pressure readings and four-lead electrocardiogram tracings were made every fifteen minutes starting one-half hour before the administration of dose and continuing for an hour after. This was done to determine any cardiovascular changes brought about by the drug.

The dose for intramuscular studies consisted of 2.2 mg. C^{14} labeled Ca EDTA mixed with 1 Gm. "cold" Ca EDTA and 2 ml. of a 1 per cent procaine solution, all made up to 10 ml. with water. Injections were made into the buttocks, 5 ml. on each side. Blood and urine samples were collected as in the intravenous studies. Feces were not collected since pilot studies indicated 85 to 98 per cent of the activity could be recovered in the urine.

For oral administration, 1.5 mg. of C^{14} labeled Ca EDTA yielding approximately 1.6×10^7 d/m was dissolved in one-half ml. of water and given in a gelatin capsule. Urine and feces were collected for assay. Blood samples were drawn in the first study but it was found that the level of activity was too low to measure and hence this was not done in subsequent studies.

The dose used in skin absorption studies was prepared in a thin paste of Aquaphor containing 2.0 mg. C^{14} labeled Ca EDTA yielding 2.2×10^7 d/m and 1 Gm. of "cold" Ca EDTA. In one study KY jelly was the water-soluble base and Na EDTA was used in the

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Work done under the auspices of the Atomic Energy Commission.
Received for publication, Dec. 9, 1953.
*Obtained from Riker Laboratories.

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...ace of Ca EDTA. The paste containing the dose was spread over an area of 100 cm.² on ... sheet of thin latex and taped firmly to the lateral chest wall just inferior to the axilla. It ...as kept there for twenty-four hours. The activity appearing in the urine was used as the ...easure of the absorption which had taken place.

Assays were carried out by plating the samples on stainless steel circular disks having ... surface area of 15 cm.² and counting in a windowless, methane-argon gas flow proportional ...ounter. Most urine samples were plated directly since the activity was sufficiently high ...hat small aliquots, i.e., 100 to 500 microliters gave satisfactory counts. In the oral studies, however, it was necessary to concentrate the urine by evaporation before plating. In these samples, mass absorption corrections were necessary. Because of the very low activity, the urine from skin absorption studies required special treatment. To the urine, which was made slightly alkaline, was added 100 to 200 mg. of "cold" Na EDTA as carrier. The sample was then brought to pH 1-2 with a methanol hydrochloric acid solution and the entire EDTA fraction, including the tracer, was precipitated out. The precipitate was separated by centrifugation and dissolved in 1 ml. of concentrated NH_4OH. An aliquot of this solution was plated and counted.

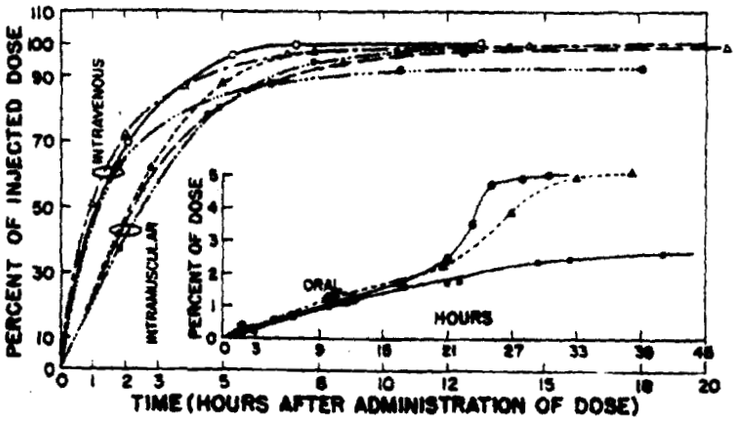

Fig. 1.—Cumulative urinary excretion after administration of C^{14} Ca EDTA.

Feces and blood samples required special processing before assaying. The active material in the feces was removed by repeated extraction with 1.5 per cent NH_4OH. The extraction solutions were concentrated to a small volume, 5 to 10 ml., by evaporation and an aliquot taken of this for assay. Mass absorption corrections were necessary. In some of the samples the concentration of the extraction fluid resulted in the formation of a floc of protein which had been carried over during the NH_4OH extraction. In these instances 5 ml. of 5 per cent zinc sulfate solution was added to insure complete precipitation and the precipitate was separated by centrifugation. It was necessary to keep the solution alkaline with Na_2CO_3 to prevent loss of tracer via the precipitate.

At first, assays were carried out on both whole blood and plasma, principally to determine the partition of the activity between the two compartments. In subsequent studies, after this ratio had been established, assays were run only on the plasma. Assays were carried out on protein-free filtrates. Protein-free filtrates were prepared by the barium hydroxide-zinc sulfate technique. Five milliliters of a saturated phenol solution were added to insure complete precipitation. The protein precipitate was washed with a 1.5 per cent NH_4OH solution to remove last traces of activity. The filtrate and washes were concentrated by evaporation and a suitable aliquot was taken for counting.

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The identification of the active material in the urine was done by filter paper chromatography. The method of Consden and co-workers[5] was followed with slight modification. Aliquots of 0.1 ml. of urine yielding approximately 6×10^4 d/m were used. The chromatograms were developed for twelve to fifteen hours in a descending column at approximately 25° C. with a mixture of 1:1 isopropyl alcohol and 0.2 N HCl. The final location of the activity on the strip were determined by the use of autoradiographs.

RESULTS

Except when urine or feces samples were missed, almost all of the parenterally-administered material could be accounted for in the urine within twenty-four hours. In the oral studies it was two to three days before all of the dose appeared in the excreta. The average recovery in the intravenous studies was 98.1 ± 4.6 per cent. Following intramuscular injection 98.8 ± 1.6 per cent was used in the urine and after oral administration an average of 91 ± 2.0 per cent appeared in the feces and 4.2 ± 2.0 per cent in the urine. Cumulative urinary excretion plotted against time is presented in Fig. 1. Fifty per cent of the dose was excreted by one hour after intravenous injection and by two and a half hours after intramuscular injection. By seven hours at least 90 per cent of the dose was excreted after either mode of parenteral administration. The material continued to be absorbed for at least eighteen hours after oral administration, although the total amount was low: a maximum of 5 per cent, as judged by the amount appearing in the urine. Excretion continued in the feces for two to three days. Table I presents the cumulative fecal excretion.

TABLE I. CUMULATIVE FECAL EXCRETION AFTER ORAL ADMINISTRATION, EXPRESSED IN PERCENTAGE OF ADMINISTERED DOSE

TIME (HOURS)	SUBJECT		
	I	II	III
0-24	48.3	57.2	60.0
24-48	77.0	83.7	92.8
48-72	89.6	90.0	93.3

The maximum of activity in the urine after application of the drug to the skin was 0.001 per cent, indicating a very low level of absorption.

The distribution of activity between cells and plasma was determined by separate assays of whole blood and plasma on several of the blood samples and calculated from the following relationship:

$$\% A_{rc} = \frac{A_{wb} - A_p\,(1\text{-hematocrit})}{A_{wb}} \times 100,$$

where A_{rc}, A_{wb}, and A_p represent the activity in the red cell fraction, the activity per milliliter of whole blood and the activity per milliliter of plasma, respectively. The hematocrit is expressed as a fraction. Direct determination of activity in the separated red cell fraction was undesirable because of the possible loss of activity during the washing process. As can be seen from Table II the activity resides principally, if not entirely, in the plasma. The small deviations in column four are probably due to experimental uncertainties.

TABLE II.

Subject I	
Hematocrit	.509
Subject II	
Hematocrit	.485

*A_{wb} = Activity
†A_p = Activity p
‡% A_{rc} = Per ce

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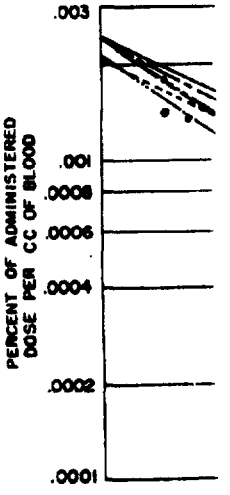

Fig. 2.—Blood disa

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TABLE II. PARTITION OF ACTIVITY BETWEEN RED CELLS AND PLASMA

		TIME AFTER ADMINISTRATION (HOURS)	[illegible]	[illegible]	PER CENT A_{rc}‡
Subject I		½	660	1,360	- 1.2
		1	144	[illegible]	-11.0
Hematocrit	.509	1½	333	663	- 2.3
Subject II		½	450	810	- 3.2
		1	222	483	-12.0
Hematocrit	.485	1½	160	340	- 9.4

*A_{wb} = Activity per ml. of whole blood.
†A_p = Activity per ml. of plasma.
‡% A_{rc} = Per cent activity in the red cell fraction.

The disappearance of the activity from the blood is illustrated in the blood clearance curves in Fig. 2. The half time for clearance was one hour and five minutes for the intravenous study, and one and one half hours after intramuscular administration. The level of activity in the blood never reached a level sufficiently high to be detected after oral administration or after application to the skin.

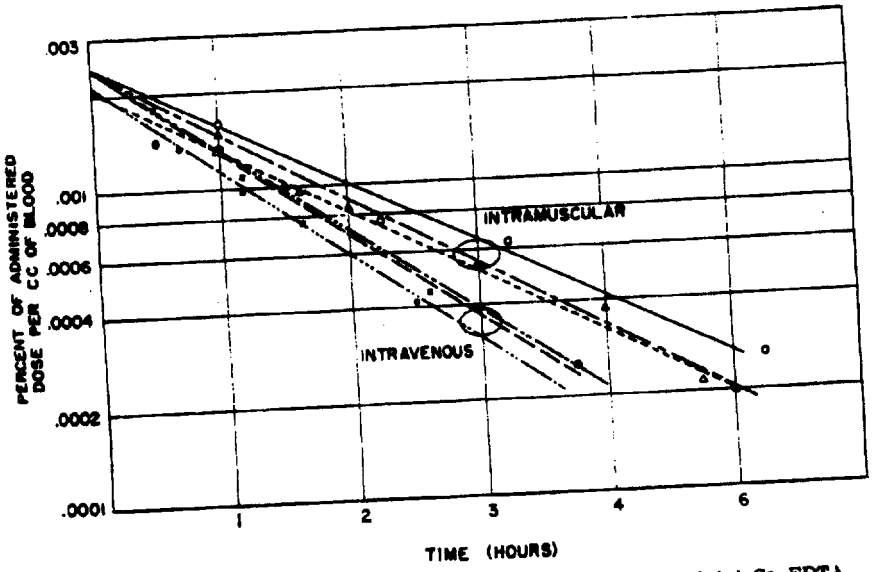

Fig. 2.—Blood disappearance curves following administration of C^{14} labeled Ca EDTA.

In the study in which the transfer across the blood spinal fluid barrier was measured, it was found that the level of activity in the spinal fluid was approximately one-twentieth that of the plasma, namely 45 c./m./ml. in the cerebrospinal fluid as compared to 870 c./m./ml. of plasma. The measurements were made one hour after intravenous injection of the dose. In the two

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studies in which blood pressure, pulse, and electrocardiograms were followed before and after intravenous injection of the drug, no unusual cardiovascular changes could be detected.

The chromatographed urine samples yielded a single band of activity with an R_F value of 0.8. This corresponded to the band which was identified to be EDTA in urine obtained from rats subjected to a similar metabolic study.[4]

DISCUSSION

The appearance of over 95 per cent of the injected activity in the urine and the identification of this activity as labeled Ca EDTA indicate that the metabolism of the compound is singularly uncomplicated; namely, that it passes through the body apparently unaltered and is excreted almost entirely through the kidney. It appears that the only interaction in the body is combination with certain di- and tetravalent metal ions and that any therapeutic or deleterious effects occur solely via this mechanism. Because of the rapid and complete elimination of the drug, the only likely long term deleterious effect from prolonged administration one might consider, is a slow depletion of the body of an essential ion, perhaps iron, cobalt, or manganese, etc. This possibility is not answerable by the present study.

Consideration of the blood clearance curves shows that shortly after parenteral administration the drug leaves the blood and mixes with a large volume of tissue fluid, namely from 40.8 to 47.6 liters* in the various individuals studied. These figures represent from 58.2 to 68 per cent of the body weights of the subjects, which is approximately the entire body water. The material, however, must not mix with all of body water initially since, as seen from Table II, it does not enter the red cells, and, as seen from the spinal fluid study, it passes very slowly across the blood-spinal fluid barrier.

Renal clearance, determined from the equation $C = \frac{0.693}{T\ 1/2} V$, where C = renal clearance, T 1/2 = half time of blood clearance, and V = the dilution volume (see Foreman, Vier, and Magee[8]), gives a value for the intravenous study of 680 ml. per minute which indicates the material is cleared by tubular excretion as well as by glomerular filtration. The rapid turnover time suggests that frequently repeated doses will be required to maintain blood levels, unless some device for slow continuous release of the material into the blood stream is used.

The low absorption after oral administration is very surprising in view of the finding that the material is effective by that route in accelerating the excretion of yttrium[2] and lead.[1] There is no satisfactory readily apparent explanation of this at present.

The lack of absorption across the skin likewise was surprisisg. It was expected this would be appreciable in view of the findings of Popovici and co-

*The "initial" concentration (obtained by extrapolation back to zero time on the curves) were 0.0021, 0.00215 and 0.00245 per cent of dose, indicating that the material must have been diluted with large volumes, namely 47.6, 46.5 and 40.8 liters, respectively, in order to give such concentrations.

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workers[9] which indicated there was a fall in serum calcium levels after application of the drug to the skin.

SUMMARY

This study in human beings using C^{14} labeled material indicates that Ca EDTA passes through the body unchanged. It is excreted via the kidney by both glomerular filtration and tubular excretion. The turnover time from the blood is approximately one hour after intravenous administration and one and a half hours after intramuscular injection. It quickly mixes with almost all of the body water except that it does not pass into the red cells and passes relatively slowly into the spinal fluid compartment. It is poorly absorbed from the gastrointestinal tract (a maximum of 5 per cent) and practically not at all through the skin. The use in therapeutic doses did not bring about any unusual cardiovascular changes in the cases studied.

We wish to express thanks to Ernest Anderson, Samuel Rothermel, and Thomas Moore for their aid in this work.

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