Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 111 NOVEMBER 1962 No. 2

SECTION MEETINGS

SOUTHEASTERN
University of Florida Medical Center November 10, 1962

Metabolism of the Chelating Agent Diethylenetriamine Pentaacetic Acid (C^{14}DTPA) in Man.* (27752)

ESTELLE STEVENS, BETTY ROSOFF,† MURRAY WEINER† AND HERTA SPENCER
Division of Neoplastic Diseases, Montefiore Hospital, New York, N. Y. and Veterans Administration Hospital, Hines, Illinois

Chelating agents of the polyamino acid type are now considered an effective method of therapy for removal of stable and radioactive metals in man(1-6). The metabolic effects of the sodium and calcium salt of EDTA given intravenously or orally to man have been described(7). A series of newer chelating agents had become available in the past few years. One of these, diethylenetriamine pentaacetic acid, DTPA, has a high affinity to yttrium. Following intravenous injection of YDTPA, yttrium was shown to be quantitatively excreted in man in 24 hours(8). This chelating agent has also been proven to be effective in removal of plutonium, iron, scandium and zinc(4,9-12). In these studies the excretion of the metal or its retention in tissues was determined, while the fate of the chelating agent was not investigated. In the present study the excretion and absorption of the chelating agent DTPA, labelled with C^{14}, was investigated in man and the results were compared to those obtained with C^{14} EDTA.‡

Materials and method. C^{14} labelled DTPA‡ solutions were prepared by dissovling the chelate in 1/10 N NaOH to form the sodium salt. Doses for intravenous use were prepared in a volume of 5 ml and adjusted to physiological pH. Each dose contained 10-15 mg DTPA with a C^{14} activity of 15-20 μc. Doses for oral administration were prepared in 10 ml water and contained either 3 mg DTPA with a C^{14} activity of 5-10 μc or 50 mg DTPA with C^{14} activity of 75-100 μc.

Dose standards were prepared by injecting a dose identical to the intravenous dose

* This study was carried out under contract with U. S. Atomic Energy Commission.

† Present address: N.Y.U., Research Division, Goldwater Memorial Hospital, New York City.

‡ C^{14}DTPA and C^{14}EDTA were either obtained through Dr. Murray Weiner, Geigy Chemical Corp., Ardsley, N. Y. or prepared in our laboratories under the direction of Dr. Harry Kroll.

with the same syringe and needle into a volumetric flask and diluting to an appropriate volume with water. Standards for the orally administered dose were prepared by pipetting an aliquot of the dose into a volumetric flask and diluting with water to an appropriate volume.

Single doses of C^{14}DTPA were administered intravenously to 4 patients, and orally to 7 patients. The renal and intestinal functions of all patients were normal. Complete urine collections were made daily for as long as significant counts could be obtained with the equipment available.

Blood samples were obtained at 1, 4, 8 and 24 hours following intravenous injection of the tracer and at 1, 2, 4, 8, 24, 48 and 72 hours following oral administration of the dose.

Aliquots of the dose standard solutions, urine, stool and plasma were pipetted onto weighed planchets and dried under a heat lamp with constant rotation. The dried planchets were weighed and the β rays were counted in an automatic Q-gas flow counter. Appropriate corrections were made for self-absorption. C^{14} excretions in urine and stool and C^{14} plasma levels were expressed in percent of the administered dose.

Comparative EDTA studies were performed in a similar manner using equivalent amounts of C^{14} labelled EDTA.

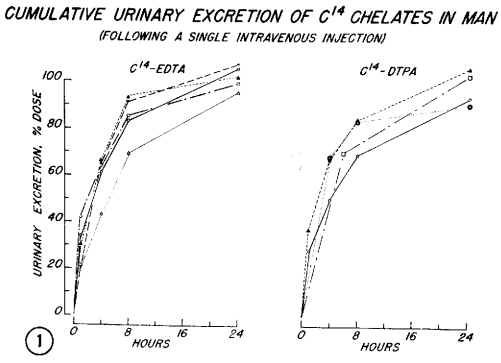
Results. The urinary excretion of C^{14}-DTPA and C^{14}EDTA following intravenous injection of the tracer is graphed in Fig. 1. The kidney was found to be the main pathway of DTPA excretion. Thirty to forty percent of the dose was excreted 2 hours after the injections, 50 to 70% after 4 hours, and an additional 15 to 20% in the subsequent 4 hours. At the end of 24 hours, 90-100% of the dose could be accounted for by urinary excretion of C^{14}. The excretion pattern of C^{14}EDTA is similar to that of C^{14}DTPA. Radioassays of stool did not reveal the presence of any C^{14} activity. Plasma levels of C^{14} averaged 10% of the dose at one hour following intravenous injection of C^{14}-DTPA and decreased rapidly thereafter. Radioactivity could not be detected in plasma at 2 hours.

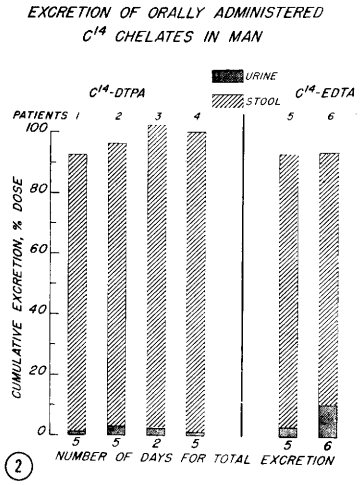
Fig. 2 shows the data of 4 patients who received tracer doses of C^{14}DTPA orally. The major portion of the ingested C^{14}DTPA passed *via* the intestine and from 95-100% of the dose was recovered in stool within 2 to 5 days. Urinary excretion of C^{14} was less than 3% of the administered dose in the 4 patients and from 3-8% in 3 other patients (not graphed in Fig. 2). The results obtained in 2 studies with orally administered C^{14}EDTA graphed for comparative purposes in this figure, are similar to those obtained for C^{14}DTPA.

Fig. 3 shows the irregularity with which C^{14}DTPA passed through the intestinal tract. The time interval for the passage of orally administered DTPA varied from 2 to 5 days in these 2 patients. Fecal C^{14} excretion of patient 1 was very low until the third day after ingestion of C^{14}DTPA when 35% was eliminated, and the total dose was passed by the fifth day. In contrast, in Patient 2, fecal excretion of the chelate was complete within 48 hours of oral administration of the dose. C^{14}DTPA was present for 4 days in the urine of Patient 1 and for 2 days in that of Patient 2. Blood samples taken at different time intervals from 1 hour up to 3 days after oral administration of the dose did not reveal the presence of any C^{14} activity. Similar results were obtained following oral administration of EDTA.

Discussion. The metabolism of chelating agents was not clearly established in the earlier studies since only the excretion of the metal ion was determined(1,3,13,14). Studies with C^{14} labelled chelates have shown that intravenously injected tracer doses of C^{14}-DTPA and C^{14}EDTA are quantitatively excreted in urine in 24 hours. Orally administered doses of C^{14}DTPA pass through the intestine almost completely unabsorbed. The presence of C^{14} in urine indicates some absorption of the chelate; however, this absorption is low. The urinary C^{14} excretion was also very low in 5 additional patients who received C^{14}DTPA orally. Failure to detect C^{14} in the vascular space may be due to inadequate methods of assay now available.

The C^{14} chelate excretion in excess of 100% obtained in some of the studies is prob-





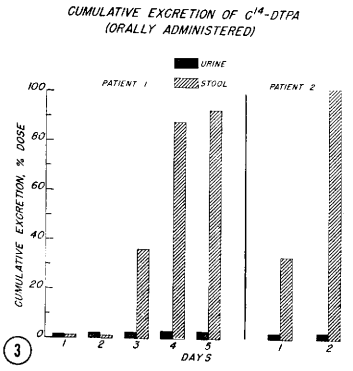


FIG. 1. A single intrav. inj. of C^{14} chelate was administered.

FIG. 2. A single oral dose of C^{14}DTPA was administered on Day 1 to each of 5 patients. Two patients received a single oral dose of C^{14}EDTA.

FIG. 3. A single oral dose of C^{14}DTPA was administered on Day 1 to 2 patients.

ably due to analytical counting errors. The recovery of less than 100% of the dose following intravenous injection might be due to the excretion of small amounts of the chelate in urine for several days, since some C^{14} activity could be detected on the second and third day of the study. In agreement with this assumption are observations made in studies in which relatively large doses of DTPA were used for removal of plutonium, scandium, zinc and yttrium. The excretion of these metals was not only increased on the days of administration of DTPA but on several days thereafter(9,11,12,15).

The studies presented indicate that the metabolism of orally and intravenously administered DTPA is similar to that of EDTA. Since DTPA was shown to be more effective than EDTA for removal of some metals(6,9, 11,15,16), DTPA may replace EDTA in the treatment of metal and radioisotope poisoning.

Summary. 1. Intravenously injected C^{14}-DTPA in man is quantitatively excreted in urine in 24 hours. 2. Following oral ingestion of C^{14}DTPA, the major portion of the chelate passes unabsorbed through the intestine (90-100%). However, the presence of the C^{14} chelate in urine is evidence for absorption of some, although small, amounts of the chelating agents. 3. The metabolism of DTPA was shown to be similar to that of EDTA.

1. Hart, H., Laszlo, D., *Science,* 1954, v118, 3053.

2. Cohn, S. H., Gong, J. K., Fishler, M. C., *Nucleonics,* 1953, v2, 56.

3. Bessman, S. P., Ried, H., Rubin, M., *Med. Ann. Dist. Col.,* 1952, v21, 312.

4. Rubin, M., Princiotto, J. V., *Ann. N. Y. Acad. Sci.,* 1960, v88, 450.

5. Foreman, H., *Ind. Med. and Surg.,* 1956, v24, 7.

6. Foreman, H., *Metal Binding in Medicine,* Lippincott, Philadelphia, 1960, p160.

7. Spencer, H., *Ann. N. Y. Acad. Sci.,* 1960, v88, 435.

8. Kroll, H., Korman, S., Siegel, E., Hart, H. E., Rosoff, B., Spencer, H., Laszlo, D., *Nature,* 1957, v180, 919.

9. Fried, J. F., Graul, E. H., Schubert, J., Westfall, N. M., *Atompraxis,* 1959, v1, 1.

10. Szot, Z. Z., Taylor, D. M., *Rad. Res.,* 1962, v16, 583.

11. Rosoff, B., Feldstein, A., Stevens, E., Spencer, H., *Fed. Proc.,* 1961, v20, 291A.

12. Feldstein, A., Rosoff, B., Sullivan, K., Spen-

cer, H., *ibid.*, 1961, v20, 291A.

13. Spencer, H., *Metal Binding in Medicine,* Lippincott, Philadelphia, 1960, p104.

14. Spencer, H., Greenberg, J., Berger, E., Perrone, M., Laszlo, D., *J. Lab. and Clin. Med.,* 1956, v478, 29.

15. Rosoff, B., Ritter, S., Sullivan, K., Hart, H., Spencer, H., Laszlo, D., *Health Physics,* 1961, v6, 177.

16. Norwood, W. D., *J. Occup. Med.,* 1960, v2, 371.

Received June 18, 1962. P.S.E.B.M., 1962, v111.

Influence of Intravascular Pressure on Vascular Response to Epinephrine.* (27753)

E. ASCHHEIM,† B. W. ZWEIFACH‡ AND M. B. ENGELBERG
Department of Pathology, New York University Medical Center, New York City

The increased peripheral resistance which accompanies the hypertensive syndrome has been ascribed to an increased reactivity of the smooth muscle elements of the terminal arterioles. In this context, the data have been interpreted as indicating the formation and systemic distribution of various vasoactive agents which either are vasoconstrictor by themselves(1,2), or potentiate the vascular response to adrenergic stimuli(3,4). Some workers believe that structural changes in the vessel wall can account for the heightened vasoconstrictor response(5,6,7), while others maintain that the distending force of the abnormal blood pressure places the smooth muscle elements under greater stretch, a factor which by itself may lead to a more forceful response to a given stimulus(8,9,10).

The procedures which have been used to investigate this aspect of the problem involve for the most part either plethysmography to assess changes in blood flow following intravascular infusion of vasoconstrictor agents (6,8,9,10), or the measurement of changes in tension of arterial strips exposed *in vitro* to a variety of experimental situations(5,7,11,12, 13). Although *in vitro* studies of isolated muscle preparations provide more quantitative data, information of this kind may be misleading, since it is tacitly assumed that conclusions based on changes in larger vessels are directly applicable to the resistance vessels. *In vivo* studies are difficult to interpret because of compensatory constrictor nervous influences. Perhaps the most direct approach to the problem under consideration would be to perfuse an isolated structure, such as the rabbit ear, at selected pressures so as to make it possible to relate the initial pressure and the flow decrement which is induced by vasoactive agents.

The perfusion data reported here show that epinephrine causes a greater reduction of flow at higher perfusion pressures when measured in absolute values, but that the per cent decrement of flow actually remains approximately constant.

Materials and methods. New Zealand albino rabbits (1500-2000 g body weight) were anesthetized with pentobarbital (35 mg/kg body weight), the ear removed with a sharp scalpel and the central artery cannulated. The ear was perfused with Tyrode's solution containing 1% (v/v) of citrated human plasma. Perfusion solutions were prepared a day earlier and filtered to avoid mechanical interference with flow because of the presence of small clots. Perfusion pressure was maintained at a given level by a suspended Mariotte's bottle. A water manometer inserted close to the cannulated ear indicated the perfusion pressure. The effluent was collected and weighed continuously on the pan of a suitable balance.

The isolated preparation was perfused at a constant pressure for 15 minutes to allow

* Supported by a grant from Life Insurance Medical Research Fund.

† Postdoctoral Fellow, U. S. Public Health Service.

‡ Investigatorship of Health Research Council of the City of New York.