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II

I. ~~THE TOLERANCE OF MAN FOR HEXAVALENT URANIUM. PART II: THE EXCRETION OF URANYL NITRATE GIVEN INTRAVENOUSLY TO HUMAN SUBJECTS~~

by

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THE TOLERANCE OF MAN FOR HEXAVALENT URANIUM, PART II. THE EXCRETION OF URANYL NITRATE GIVEN INTRAVENOUSLY TO HUMAN SUBJECTS

Abstract

Tracer studies employing ~~an~~ uranium enriched ~~mixture~~ ~~of~~ in the isotopes ~~of~~ U^{234}, U^{235} have been carried out in six human subjects: four males and two females. The uranium was given intravenously in the hexavalent state as uranyl nitrate in amounts ranging from 6 micrograms to 70 micrograms per kilogram of body weight.

Each individual of the series received a single injection of the metal except for Subject #6 who was given two widely spaced doses. The first of these was when his condition was normal and the second after an acidosis had been produced by ingestion of ammonium chloride. Renal function tests including urinary catalase, protein, amino N to Cretainine N ratio, and clearances of mannitol and p - aminohippurate were done before and after administration of uranium. Only at the 70 microgram per kilogram level in Subject #6 was there a slight rise in urinary catalase and protein suggesting that tolerance had been reached. All other tests were negative.

*1 The excretion of uranium was mainly in the urine where from 70 to 85 per cent ~~appeared in twenty-four hours~~. The production of an acidosis with ammonium chloride (Subject #6) decreased the rate of excretion.

~~Traces of uranium continued to be eliminated for at least two weeks after it had been administered. It is surmised that this represented metal retained in the bones.~~ Attempts at mobilization of the fraction retained by injection of citrate and by the use of a low calcium diet plus dihydrotochysterol were ineffective.

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VII Summary (Use also as Abstract)

Tracer studies employing an enriched mixture of the isotopes of U^{234}, U^{235} have been carried out in six human subjects, four males and two females. The uranium was given intravenously in the hexavalent state as uranyl nitrate in amounts ranging from 6 micrograms to 70 micrograms per kilogram of body weight.

Each individual of the series received a single injection of the metal except for subject #6 who was given two widely spaced doses. The first of these was when his condition was normal and the second after an acidosis had been produced by ingestion of ammonium chloride. Renal function tests including urinary catalase, protein, amino N & Creatinine N ratio and clearances of mannitol, and p-aminohippurate were done before and after administration of uranium. Only at the 70 microgram per kilogram level in Subject #6 was there a slight rise in urinary catalase and protein suggesting that tolerance had been reached. All other tests were negative.

The excretion of uranium was mainly in the urine where from 70 & 85 per cent appeared in 24 hrs. The production of an acidosis with ammonium chloride

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(Subject #6) decreased the rate of excretion.

Traces of uranium continued to be eliminated for at least two weeks after it had been administered.

It is surmised that this represented metal retained in the bones. Attempts at mobilization of the fraction retained by injection of citrate and by the use of a low calcium diet plus dihydrotachysterol were ineffective.

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in the urine, where from 70 to 80 per cent of administered dose appeared in the first 24 hours. Urine of the second 24 hours contained about 4 per cent, and the third 24 hours urine 1.5 per cent of the administered dose. Detectable amounts were excreted for at least two weeks. Negligible amounts of uranium were found in the feces.

The production of an acidosis with ammonium chloride (Subject 6) decreased the rate of excretion.

It is surmised that this late excretion represented uranium originally stored in the bones.

Attempts at mobilization of the fraction retained in the body by injection of citrate and by the use of a low calcium diet plus dihydrotachysterol were ineffective.

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Part II

Clinical Methods

The Excretion of Uranyl Nitrate Given Intravenously to Man

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I. Introduction

P 755 The toxicological work on uranium has conceived of the metal mainly as a chemical hazard, and it is along these lines that the present experiments have been directed. While uranium compounds are highly poisonous when they gain access to the circulation, there is little reason to fear that serious toxic exposures will occur during the ordinary course of industrial activities. Uranium salts are absorbed poorly when ingested and unless there is gross carelessness, the inhalation of contaminated dust can be controlled readily.

It is possible, however, that long continued exposure to the dust of concentrates of U^{234}, U^{235} may, because of more intensive alpha emission, constitute a radiological hazard particularly for lung and bone.

Heavy Uranium forms complexes with bicarbonate, citrate, phosphate, and protein, and when given in small amounts, is ~~excreted~~ filtered rapidly ~~in the urine complexed~~ through the glomeruli combined with bicarbonate. A fraction of the total dose, from 10 to 20 per cent, becomes fixed to the phosphate groups of bone,(1) (2) from which it is released gradually. Because of the manner of its elimination, the chief toxicity of uranium is for the kidney, where it produces a severe tubular nephritis (3). It has been found recently that rabbits and dogs often show responses in increased urinary catalase and protein to injections of uranyl acetate given intravenously at a level of 0.01 mg. per Kg. of body weight (0.006 mg. of uranium metal).(4) This dose is well below the level that will produce gross symptoms of toxicity. ~~and is about the smallest amount that can be employed profitably.~~ The daily urinary excretion of uranium ~~reported~~ for some workers in uranium plants ~~is of the same order of magnitude~~ has been reported to be about 0.3 mg in 24 hrs.(5) Obviously it is of interest to

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Factors include drop in pH & HCO_3^- resorption which break up the complex & water resorption which concentrates the U

& amino acid intrag

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1 1/2 inches

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determine whether man will react with catalasuria and proteinuria to such small quantities of uranium. From animal studies we find ~~increasing~~ different degrees of tolerance; the most susceptible animal that has been investigated is the rabbit, while the toxic effects of uranium are produced less readily in the cat, dog, and rat in approximately the order mentioned. The experiments which we have been able to carry through ~~lead us to the conclusion~~ suggest to us that the chemical toxicity for adult humans as measured by catalase and proteinuria is more nearly the order of that for the rat and suggests that genus homo occupies a position in the more resistant group of mammals.

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II. Purpose of Experiment and Methods

The investigation was undertaken with these objectives in mind.

A. To find that dose of a soluble uranium salt which, when introduced intravenously as a single dose, would produce just detectable renal injury. The tests employed to ascertain renal damage were urinary catalase (Dounce),(4) urinary protein,(6) ratio of amino nitrogen to creatinine nitrogen in the urine,(7) glomerular filtration (mannitol),(8) renal plasma flow, maximum tubular excretory capacity (para aminohippuric acide)(9) and urea clearance ~~(VanSlyke and Kugel)~~.(10) Not all of the more complicated tests of renal function were done in every patient; however, urinary catalase and protein which are very sensitive indicators of renal injury were followed carefully in each case.

Most and done by H. Berk

B. To measure the rate at which soluble uranium compounds are eliminated from the human body once they have entered the circulation. Both urine and fecal specimens were obtained from the experimental subjects. Urine was collected in individual voidings on the day of the injection and thereafter in pools of twenty-four hours. The excretion was determined by the method of electroplating and alpha α counting since in part one of this report. It was soon found that the amount of uranium appearing in human feces after intravenous administration of salts of U_6 was

α alpha γ gamma μ μ
β beta

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negligible and the fecal analyses were discontinued.

C. To observe the effect of measures aimed at increasing or decreasing the rate of excretion of uranium.

Three experiments of this nature were performed. Mobilization of the small fraction of the dose which was apparently retained was not affected by the intravenous administration of citrate, nor by placing one of the subjects on a low calcium diet together with large doses of A.T 10.* Production of an acidosis with ammonium chloride, on the other hand, clearly delayed the excretion of uranium in the urine. The details of these experiments appear in ~~Part~~ Section V

III. Selection of Subjects and Size of Dose.

The experimental subjects were chosen from a large group of hospital patients. Criteria of importance in making the selection were reasonably good kidney function with urine free from protein and with a normal sediment on clinical examination. The probability that the patient would benefit from continued hospitalization and medical care was also a factor in the choice. When higher levels of dosage were contemplated, individuals from the older age groups were preferred in view of the remote possibility that late radiation effects might occur. and the enriched isotope mixture was diluted with ordinary uranium

The initial dose was set at approximately 6 ~~mc. gm.~~ micrograms of the metal per kilo of body weight and was chosen because this is the amount which will occasionally produce just detectable temporary renal damage in the rabbit, a highly susceptible animal.(4) The plan called for a slowly increasing dosage provided that no signs of injury to the kidney were detected. Until it was established that the rate of, and total excretion were of the same order as that for experimental animals, no subject received more than a single dose. When the amount given approached 50 ~~mc. gm.~~ micrograms per kilo, the enriched mixture of isotopes of U^{234}, U^{235} was diluted

alteration (Damage sounds bad)

* Dihydrotachysterol

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1 - 4 using uranyl acetate of the ordinary isotopic ~~mixture~~ composition. It was estimated that this would decrease the radioactivity to a point where any late radiation effects would be highly improbable.

Subject 46 received the largest amount of uranium, about 71 mc. gm. per kilo and was given a second injection after acidification with ammonium chloride. No renal toxic action was noted.

IV. Nature of Material and Technique of Administration.

Portions of the sample of uranium oxide (U_3O_8) enriched in U^{234} and U^{235} and described in Part I of this report were weighed out and used in the preparation of solutions of uranyl nitrate for intravenous injection. When the subject had been selected and the size of the dose agreed upon, enough of the ~~uranium~~ uranyl nitrate solution to contain several times the quantity of metal required was diluted with a 1.15 per cent solution of sodium acetate, the pH of which had been adjusted to 4.5 by addition of a little acetic acid. The dilution was arranged so that the quantitiy of uranium to be injected would be present in 5 to 8 ml. After autoclaving to insure sterility about 20 ml. of the buffered uranium salt was transferred ~~aseptically~~ aseptically to a sterile rubber capped vial. Trial analyses were run on the remaining solution by the method of plating and alpha counting to serve as a check on the accuracy of the initial weighing and dilution. If the amount of uranium present as calculated from these two procedures was found to be in agreement, the sterile solution was considered ready for use.

Technique of Administration

The necessary amount of sterile, buffered uranyl solution was drawn from the vial into a 10 ml. all-glass syringe and the needle removed. The syringe was handed to an assistant and held so that none of the contents would escape.

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A number 20 needle attached to another syringe partly filled with physiological saline was used to cannulate the median basilic vein of the patient and enough saline injected to insure that none of the liquid was leaking into the adjacent tissues. The needle was left in place and the syringe containing the solution of uranium was attached. A few drops of blood were drawn into the barrel to indicate the continued patency of the needle and the injection was made promptly. The syringe was rinsed once by withdrawing the plunger slowly until blood reached the mark of the previous filling, and the blood was reinjected into the vein.

Preparation of Dummy Solutions

The same syringe used for the injection was rapidly cleaned and dried. The solution of uranium was again drawn from the vial to the proper mark and the needle removed. A second 20 gauge needle filled with water was attached and the solution ejected through it into a 200 ml. volumetric flask. Water was now drawn through the needle into the syringe to the same volume as that of the uranium solution and this too discharged into the flask. Two such dummies were prepared from the solution used for the injection. The average value of the uranium metal found on analysis of the two dummy solutions was taken as the amount injected. As a rule, these did not differ from each other by more than 5 per cent. This was sufficiently good agreement for the purpose of the clinical study.

V. Cases Studied

Four male and two female patients were studied. A resumé of the experimental work appears in table 1. Subject #6 received the uranium in the experiment designated (A) prior to taking ammonium chloride. In experiment (B) he was given about 8 gm. of ammonium chloride daily for three days and then 12 gm. daily for two days. The CO_2 content of the serum decreased from 64 volumes per cent to

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32 volumes per cent. He received the second injection of +6 uranium seventeen days after the first dose and when 90 per cent of the latter had been excreted. The effect on the rate of excretion is seen best on inspection of Figure 1. The production of acidosis in man decreases the ~~speed~~ rapidity of excretion of uranium in the urine just as it does in the rabbit (11).

Excretion of Intravenously Administered Hexavalent Uranium. Details of the Experiments.

The analyses of the excreta after the intravenous administration of hexavalent uranium were carried out by the methods for plating and alpha counting described in Part I of this report. It was soon found that when the metal was given i.v., little could be recovered in the feces, while the major portion was excreted rather promptly in the urine. The injected uranium also disappeared from the blood stream very rapidly. While we have no positive information on this point, ~~menial~~ animal experiments suggest that it becomes fixed in the kidney and and particularly in the bone and that it is slowly released from the latter site. (1) The details of the human experiments are as follows:

Subject No. 1 - White male, aged 32 years, Ht. 171.5 cm., Wt. 60.5 Kg., B.P. 110/75.

In hospital because of rheumatoid arthritis and uretheral strictures. At the time of study his disease was quiescent. The urine contained neither protein nor casts and the catalase activity was low. Control analyses of urine and feces did not disclose the presence of uranium. On 8/9/46 he was given 385 ~~mc. gm.~~ micrograms of uranium metal intravenously as the nitrate diluted to 5 ml. with sodium acetate buffer. Collections of urine and feces were started immediately. Blood was drawn four hours later and found to be without detectable alpha activity. The pertinent clinical laboratory data are to be found in table 2. Tables 3 and 4 present the values found on analyses of urine and feces repectively for uranium.

dose in µg/kg.

Daily tests of a fresh specimen of urine did not reveal increased catalase activity in the post injection period; there was no proteinuria; the ratio of amino N/Cretainine N did not show a significant variation from the control values and as may be seen from table 2 urea clearance, blood counts and blood chemistry were not affected by the injection.

In summary the intravenous injection of hexavalent uranium prepared from a mixture of isotopes enriched in U^{234}, U^{235} at a level of 6.3 ~~mc. gm.~~ micrograms (of metal) per kilogram did not produce demonstrable renal damage.

Subject No. 2 - White female, aged 40 years, Ht. 165.5 cm., Wt. 72.8 Kg., B.P. 136/90.

Hospitalization was required because of acute alcoholism, hallucinatory state, cirrhosis of the liver with congestive splenomegaly and a neuropathy probably due to deficiency of the vitamin B complex. At the time of this study the more acute symptoms had subsided and while still a bed patient, she was able to give adequate cooperation in the collection of specimens. On 9/18/46 she was given an intravenous injection of the enriched isotopic mixture of U^{234}, U^{235} similar in composition to that received by Subject No. 1. In this instance the dose was equivalent to 476.7 ~~mc.~~ micrograms gm. of the metal and was contained in 5 ml. of the buffer. The amount given per kilogram was thus 6.5 ~~mc. gm.~~ micrograms Collections of urine were begun immediately but the feces were not saved. Clinical laboratory data and the results of analyses of the urine for uranium may be found in tables 5 and 6. Table 7 gives the ratios observed for amino nitrogen to creatinine nitrogen in the urine before and after the uranium injection. It is evident that these did not change.

As in the first patient, the excretion of U_6 in urine was very rapid during the first twenty-four hours and accounted for 84 per cent of the amount given. The total excretion by this route was 87 per cent at the end of six days. Again there was no increase in urinary catalase or protein or other indication

of renal injury.

Subject No. 3 - White female, aged 24 years, Ht. 155.5 cm., Wt. 37 Kg., B.P. 100/60.

This young woman was in fairly good physical condition except for mild chronic undernutrition which was thought to be secondary to an emotional maladjustment.

Five ml. of acetate buffer containing uranium nitrate equivalent to 584 mc. gm. of the metal in the form of the enriched mixture of isotopes of U^{234} and U^{235} was injected into an arm vein on October 1, 1946. This dose represented 15.5 mc. gm. of +6 uranium per kilogram.

Urine and feces were analyzed for uranium ~~both by the fluorescence method of Neumann (12) and~~ by the electroplating and alpha counting method. The quantity of uranium excreted in the feces was too small to measure accurately and no tabulation of the results is shown. Table 8 gives the urine values obtained by the alpha counting method. The excretion appeared to be somewhat less complete at the end of five days than in the first two instances but the curve of elimination followed the usual pattern. The clinical tests are recorded in table 9 and the urinary amino N/Creatinine N ratios in table 10.

Although the dose of uranium on a kilogram basis had been more than doubled, there was no increase in urinary catalase or protein; the renal clearances remained unchanged and the urinary amino nitrogen/creatinine nitrogen ratio was not altered.

Subject No. 4 - White male, aged 42 years, Ht. Wt. 64 Kg., B.P. 100/72.

Entered hosptial because of chronic alcoholism and bleeding from the gastrointestinal tract. His acute symptoms subsided after ten days and he was transferred to the Metabolism Unit. Stools continued to show positive tests for occult blood for a week but were normal thereafter. X-ray studies with barium sulphate of the espphagus, stomach and intestinal tract were negative

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except for coarse gastric and upper small bowel mucosal patterns. The urine was normal. However, during tests of urea clearances, the patient experienced difficulty in voiding at the times requested. There was no evidence of urethral stricture or enlargement of the prostrate as a contributing factor.

Urea Clearances

Date	Type of Clearance	Per cent of Normal
10/14/46	Maximum	61
10/16/46	Standard	43
10/18/46	Maximum	71
10/30/46	Technician #1	
	Maximum	60
	Standard	53
10/30/46	Technician #2	
	Maximum	56
	Standard	50

The reduction of urea clearance was not felt to be a contraindication to continuance of the experiment, for the urine was normal to clincial examination and had a low catalse activity. The injection of +6 uranium was given on October 23, 1946. In this case equal parts of the enriched isotopic mixture and of the natural mixture of isotopes were given. The total dose of uranium metal was 1918 ~~mc. gm.~~ micrograms or 29.9 ~~mc. gm.~~ microgram per kilogram.

~~Urine and feces were analyzed both by the fluorescence and electroplating methods.~~ No significant amount of uranium could be detected in the feces. Table II gives the excretion of uranium in the urine ~~as determined by electroplating and alpha counting.~~ It is to be noted that the rate and percentage

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excretion was much the same as in Subject No. 3. Urinary catalase and protein did not increase in the post injection period.

At the end of twelve days the excretion in the urine per twenty-four hour period was of the order of 1 ~~mc. gm.~~ microgram and there was an apparent retention of ? 535 ~~mc. gm.~~ micrograms presumably in the bones. In view of the fact that hexavalent uranium complexes readily with citrate (13) it was decided to determine the effect of i.v. citrate on mobilization of the residual metal.

On November 4, 1946 the patient was given 2000 ml. of a solution containing 4.6 gm. sodium citrate and 1.1 gm. citric acid per liter. Unfortunately this solution was so hypotonic that although given slowly by intravenous drip over a three hour period, the next two voidings of urine contained homoglobin and his temperature rose to 39.5 C. As may be noted from inspection of table 11, this maneuver did not affect the excretion of uranium.

Blood One sample of blood was drawn eight minutes after completing the injection and was found to contain 0.043 ~~mc. gm.~~ micrograms of uranium per ml. If the blood volume of this man be estimated at 5000 ml., then 11 per cent of the dose was still within the vascular system.

The citrate excretion in the urine was measured on two control days during the administration of sodium citrate, citric acid solution and in a follow-up period. There was comparatively little increase in the urinary excretion of citrate (table 12) which suggests that much of the citrate administered must hage been metabolized rather than excreted.

Blood counts and blood chemistry may be found in table 13.

The range of dosage had now been reached where mild toxic effects on the kidney were to be anticipated.(14) There was no evidence of such damage in this subject. The level of urinary catalase and protein remained unaltered as did the amino N/Creatinine N ratio. Subsequent follow-up examinations in the clinic revealed steady improvement in his general condition.

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Subject No. 5 - Male negro, aged 51 years, Ht. 160 cm., Wt. 56.2 Kg., P.P. 120/60 T. 36.8 C.

Chronic cough had been present for about one and a half years and there was a history of rather high comsumption of beer and ale. Three weeks prior to his hosptialization on October 30, 1946, his cough became worse. The sputum was streaked with blood, he had fever at night, he become anorexic, dyspneic and complained of marked weakness. Physical examination revealed signs of consolidation of the right upper lobe of the lung. Type 14 pneumococcus was recovered from the sputum by direct typing and by injection into a mouse. The temperature was 40.4 C, leukocyte count 20,750, icterus index 24, cephalin flocculation 2+, CO_2 content of serum 39 volumes per cent, chlorides of serum 98 meq./liter, total serum protein 4.8 gm. per cent, non protein nitrogen 44mg. per cent. His illness ran a stormy course, but gradually responded to combined therapy with penicillin and sulfadiazine. These medications were discontinued on November 19, 1946. After he had received a total of 8,765,000 units of intramuscular penicillin and 87 gms. of sulfadiazine orally. He remained afebrile from this point on but his convalescence was slow and signs of consolidation of the right upper lobe, both by physical examination, and by X-ray, persisted. Bronchoscopy was performed on November 11, 1946 but no obstruction to the right upper lobe bronchus was discovered.

During the period of observation on the metabolism ward his appetite was excellent and he gained 1.5 Kg. in weight.

Since the tolerance for uranyl nitrate appeared good in Subject No. 4 at a level of about 30 micrograms ~~mc. gm.~~/kilogram (as uranium metal), a further moderate increase in the dose was given Subject No. 5. The mixture of isotopes enriched in U^{234}, U^{235} was diluted 1 - 4 with ordinary uranium and injected in the amount

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of 2746 ~~mc. gm.~~ micrograms or 48.8 ~~mc. gm.~~ micrograms per kilogram on December 17, 1946.

The analyses of the urine appear in table 14. Again no significant amount of uranium could be detected in the feces by the method of analysis employed. Although there was no increase in urinary catalase (table 15), a trace of protein appeared in the urine on the fourth post injection day. While it is suspected that this was a chance observation and without significance as far as the uranium was concerned, it is unfortunate that the patient could not have been followed longer. He was feeling much improved at the time and insisted on being discharged from the hospital. There was no follow-up.

Other clinical data and the amino N/Creatinine N ratios in the urine may be found in tables 16 and 17.

Subject No. 6 - White male, aged 61 years, Ht. 163 cm., Wt. 55.2 Kg., P.B. 150/84. T. 36.5 C.

The fifth admission of this patient was on October 14, 1946. He remained in the hosptial continuously from this date until completion of the metabolic studies on April 1, 1947. His transfer to the metabolism unit for the purpose of the studies recorded here was effected on January 3, 1947.

Some of the previous diagnoses were: arteriosclerosis general, hypertension secondary, heart disease arteriosclerotic and hypertensive Class IIa; alcoholism chronic; spontaneous pneumothroax; pneumonia right upper lobe, pneumococcus Type II; tuberculosis pulmonary. The last diagnosis was probably based upon insufficient evidence for after three months observation in a tuberculosis sanatarium, he was discharged with a diagnosis of chronic pulmonary fibrosis secondary to his previous Type II pneumonia. The present admission was for symptoms suggesting a gastric lesion. This was confirmed by X-ray and gastroscopy and was thought to be a benign ulcer. He improved promptly on medical treatment

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and the ulcer appeared to heal. Further pulmonary studies including concentration of the sputa, bronchoscopy and X-ray failed to reveal evidence of active pulmonary tuberculosis. As he had no home, he agreed willingly to enter the metabolic unit for special studies.

Two experiments were set up. In the first of these the patient served as his own control for the second experiment.

Experiment 1 - Condition Normal

Laboratory Data:

Blood: Hb. 13.8 gm./100 ml.
RBC. 4.62 million/cu. mm.
WBC. 8,300

Differential Count:

Neutrophiles 75, Lymphocytes 22, Monocytes 3.

Urine:

Specific Gravity 1.024, Albumin 0, Surgar 0.
Acetone 0. Microscopic 2-3, W.B.C. per high power field

Stool:

Negative quaiac reaction for blood on admission to metabolism.

X-ray of Lungs:

Resolving pneumonic process in right upper lobe with residual fibrosis.

X-ray of Stomach:

Decrease in size of the ulcer on the lesser curvature.

Renal Function: 1/9/47 (Condition normal)

Renal plasma flow	482 ml/min.	(Normal 697 ± 130)
Glomerular Filtration Rate	99 ml/min.	(" 131 ± 22)
Maximum Tubular Excretory Capacity	95mg/min.	(" 77.5 ± 12.9)
Filtration Fraction	0.205	(" 0.19 ± 0.02)

Blood Chemistry 1/6/47

NPN 30 mg. per cent
Total Serum Protein 6.5 gm. per cent
Albumin 4.2 gm. per cent
Globulin 2.3 gm. per cent
Chloride 98 m. eq./liter
CO_2 64 vols. per cent

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Uranium (as +6 metal) in the amount of 3910 ~~mc. gm.~~ micrograms was injected intravenously at 10:25 A. M. on January 10, 1947. As in the case of subjects No. 4 and 5, the enriched mixture of the isotopes U^{234}, U^{235} was diluted by addition of uranyl acetate ~~in the~~ of natural isotopic ~~mixture~~ composition. The total activity of the dose was computed as 0.03 microcuries, and the metal injected per kilogram of body weight was 70.9 ~~mc. gm.~~ micrograms. The uranium analyses for the urine are given in table 18 by the method of electroplating and alpha counting.

The catalase and protein tests for tubular damage ennumerated in table 19 for the first time are slightly higher on the fifth and sixth days, after the injection. While it cannot be stated that either test is unequivocally positive, it would seem advisable that any further increase in the dose should be made in small increments. There was no change in the ratio Amino N/Creatinine in the urine (table 20).

Renal clearance tests were repeated six days after the injection at a time when, according to the animal work, injury would be apt to be manifest. No significant difference from the control values is evident.

Date of Tests, January 16, 1947 - ~~Dr. C. Waterhouse~~

Renal Plasma Flow	499 ml./min.
Glomerular Filtration Rate	93 ml./min.
Maximum Tubular Excretory	
Capacity for para aminohippuric acid	83 mg./min.
Filtration fraction	0.185

Experiment 2 - Condition Acidotic

About 90 per cent of the first dose of uranium had been excreted in the urine by January 22nd. Acidification was begun by giving 8 gm. of ammonium chloride daily for three days and the dose was increased to 12 gm. a day on the 4th and 5th days. The second dose of uranium was given intravenously on Jan. 27

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It was somewhat smaller than the first dose, 3170 ~~mc. gm.~~ micrograms of U_6 metal, or 56.5 ~~mc. gm.~~ micrograms/kilogram body weight. At the same time the dose of ammonium chloride was reduced to 4 gm. a day and discontinued after January 31st. The effect of NH_4Cl on the blood chemistry was as follows:

Blood Chemistry

	Jan 27	Jan 28	Jan 31	Feb. 4
CO_2 content of Serum, Vols %	32	36	48	70
Chloride in serum, ~~Meq~~ mg./liter	116	112	108	104
Non protein nitrogen, mg. %	60	50	41	

It is evident that since the control values for CO_2 content of serum were above 60 vols. %, a considerable acidosis was achieved by the administration of the ammonium chloride.

Table 21 gives data on the rate and cumulative excretion of uranium during acidosis. The slower excretion is appreciated best by referring to Figure 1. The twenty-four hour urinary excretion of uranium had fallen to a very low level fifteen days after the second injection and analyses were discontinued. The amount retained in the body at this time may be estimated roughly as:

Total Uranium (U_6) administered (Sum of the two doses)	7080 mc. gms.
Less total Uranium eliminated in urine	5913 mc. gms.
possible retention in body	1167 mc. gms.

The final tests of kidney function were done on January 31, 1947 during the period of acidosis.

Renal plasma flow	555 ml./min.
Glomerular Filtration	102 ml./min.
Maximum Tubular Excretory capacity for para aminohippuric acid	92 mg./min.
Filtration Fraction	0.184

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The apparent retention of U_6 by this man offered the opportunity to test the effect of measures aimed at mobilization of the uranium presumably retained in the bones. To this end the dietary calcium was reduced to 0.2 gm. a day which is less than the amount required for equilibrium (15) and gradually increasing doses of dihydrotachysterol (A.T 10) were simultaneously administered.

It was hoped that this program would have a moderate decalicfying effect and perhaps liberate some uranium from its conbination with the phosphate groups of bone. We have assembled the data on serum Ca + P and urinary Ca and P for this experiment in table 22. The maximum dose of A.T. 10 was 7.5 mg. a day continued for eighteen days. While this about tripled the excretion of calcium in the urine, it did not affect urinary phosphorus and there was no increase in the excretion of uranium.

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VI. Mechanism of Excretion of Hexavalent Uranium

Dounce (13) has shown that uranyl ions complex readily with carbonate or bicarbonate, organic acids as citrate, malate and acetate, with protein and with phosphate. In blood where there is a large excess of bicarbonate about 60 per cent of U_6 is thought to form a firm complex of the type $UO_2(CO_3)_2$. Evidently from the studies we have been able to carry out, man behaves as does the rabbit and other animals with regard to excretion of uranium. A schema giving the probable mechanism of excretion of U_6 by the kidney is given in Figure 2 (modified from Dounce (14)) The diffusable bicarbonate complex is filtered at the glomerulus. Measures which cause acidification of the urine decrease the concentration of bicarbonate in it and hence its ability to complex uranium. Complexing of the metal with the cell protein of the renal tubules is thus favored. This mechanism is well illustrated in the animal studies of Wells (11). In Subject No. 6 it seems probable that the production of a highly acid urine through the administration of ammonium chloride is responsible for slowing the excretion of U_6. The lowered content of bicarbonate in the urine permitted uranium to complex with cell protein where it was held temporarily. Its removal from the cell ~~could be~~ was accomplished slowly through the presence of traces of strongly complexing amions such as citrate, malate and bicarbonate which when present even in very small quantities successfully compete with protein for uranium (16). If sufficiently damaged, these cells may be shed into the urine or at least become more permeable and leak catalase into it. The early proteinuria of uranium poisoning no doubt has a similar etiology.

The small and in our experiments inconsequential amounts of uranium(U_6) found in the feces are also in agreement with the animal work. Possibly a very

both organic and inorganic especially pyro-phosphate groups.

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minute fraction of the injected dose is excreted in the bile and traces probably appear in other intestinal secretions. The presence of dietary phosphorus and the reaction of the intestinal contents would favor fixation of any uranium entering the gut as a phosphate complex (16) or as a precipitate of uranyl phosphate.

The retention of 15 to 20 per cent of the amount administered ~~is apparently temporary and our longer experiments certainly suggest that it is eliminated slowly.~~ may be later significantly reduced by continued slow excretion ~~continuing to be~~ From the work of Neuman and collaborators (2) uranyl ions appear to be fixed to bone "by a slightly dissociated linkage with two phosphate groups adjacent to each other in the bone surface". The U_6 held in the skeleton without doubt represents that fraction of the total dose which is not eliminated within the first few days.

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Table 22

Subject No. 6

Daily intake:

Calcium 235 mg.
Phosphorus 1127 mg.

	Blood		Urine		A.T. 10 Daily	Uranium in Urine
Day	Ca mg.%	P mg. %	Ca mg. 24 hrs	P mg. 24 hrs.	cc	Micrograms in 24 hrs
1	2/5/47		49	1008	None	
2			31	812	"	
3			33	705	"	
4			31	655	"	
5			32	765	"	
6	9.7	3.4	22	711	"	
7			30	868	"	
8.			34	785	"	12.5
9			26	730	"	14.7
10			27	753	"	8.9
11			38	704	"	6.5
12			30	699	"	9.7
13	2/17/47		34	688	2 ml.	13.0
14			40	716	" "	7.2
15			43	730	" "	2.9
16			45	681	" "	8.2
17			39	722	" "	5.2
18			35	720	" "	
19			47	748		
20	9.5	3.5	36	702	4 ml.	9.2
21			43	758	" "	9.1
22			37	747	" "	5.4
23			38	797	" "	8.1
24	9.3	4.0	17?	529?	6 ml.	9.4
25			29	771	" "	5.2
26			28	782	" "	2.0
27			32	750	" "	11.3
28	10.0	3.6	44	726	" "	5.2
29			47	721	" "	1.0
30			44	753	" "	1.9
31			54	858	" "	8.4
32	8.7	4.1	61	832	" "	10.6
33			67	806	" "	7.4
34			84	881	" "	0.0
35	9.9	3.5	76	844	" "	0.0
36			86	798	" "	4.3
37			91	811	" "	2.2
38			85	710	" "	5.5
39			97	764	" "	7.1
40			97	730	" "	10.6
41			104	682	" "	0.0
42			98	728	None	3.2

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The Tolerance of Man for Hexavalent Uranium

Part II

The Excretion of Uranyl Nitrate Given Intravenously to Human Subjects

by

Nathan Cedaro
Samuel H. Bassett, Albert Frenkel, Helen E. Van Alstine
[redacted] Christine Waterhouse, and Katherine Y. Cusson

Submitted by: ~~Andrew H. Dowdy~~
Director

[redacted]

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Pharmacology Monograph

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