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THE TOXICITY IN MAN OF HEXAVALENT URANIUM FOLLOWING INTRAVENOUS ADMINISTRATION*

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THIS investigation was undertaken for the dual purpose of deriving an intravenous tolerance dose of hexavalent uranium for man and the procurement of data pertinent to the distribution and excretion of this ion in man to aid in evaluating exposures in uranium processing and fabrication plants. The findings relative to this latter purpose have been described in a previous publication.[8]

The determination of an intravenous tolerance dose is an initial step in evaluating the possible application of U^{235} to the neutron capture therapy of brain tumors. The recent studies of Javid, Brownell and Sweet,[4] and Sweet and Javid[9] pointed out the possible use of the neutron capturing isotope boron 10 for the radiation therapy of these tumors, and the more recent publications of Farr *et al.*,[1] and Godwin *et al.*[2] have described the initial results in a group of 10 patients with glioblastoma multiforme treated with this isotope. The theoretic possibilities of using lithium 6 as the neutron capturing isotope have been explored by Luessenhop, Sweet and Robinson.[4]

The rationale of this form of therapy stems from several well established physiologic observations. The normal operation of the "blood-brain barrier" tends to limit the passage of certain substances into the extracellular space of the brain. In glioblastoma multiforme, one of the most malignant forms of brain tumor, the barrier phenomenon is much less pronounced in that a more rapid exchange of a substance between the blood and extracellular space of the tumor occurs than between the same compartments in the normal surrounding brain. Accordingly, the intravenous administration of a neutron capturing isotope leads to its greater concentration in the brain tumor, and because this isotope becomes the chief determinant of the evolved radiation energy following exposure to a flux of thermal neutrons, it presents the possibility of selectively destroying the tumor without significantly damaging the normal brain.

In the quest for isotopes that might be employed in this scheme, the use of certain fissionable isotopes was suggested. Following careful theoretic evaluation of the clinical use of U^{235}, it was concluded that its possibilities in neutron capture therapy were worthy of investigation. Although the capture cross section of U^{235} for thermal neutrons is substantially less than that of boron 10 and lithium 6, the fission energy following capture is considerably greater. In addition, the tremendous size of the uranium atom suggests a more complete exclusion by the blood-brain barrier and hence a more efficacious differential concentration between tumor and normal brain.

The preliminary steps necessary, before the therapeutic possibilities of this isotope can be evaluated, consist of: (1) finding a tolerance dose for man, and (2) determining the differential concentration of uranium in normal brain and brain tumor and the time interval over which it persists.

Prior to this study there were considerable data concerning the toxicity of urani-

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TABLE I

Patient	Age	Sex	Weight	Uranium Injected	Mg. U/kg.	Duration of Life After Injection
I	26	M	55.9 kg.	5.5 mg.	0.097	2.4 days
II	47	M	57.4	5.9	0.13	60
III	34	M	60.0	4.3	0.07	566
IV	63	F	67.7	11.2	0.17	42
V	39	M	55.9	15.8	0.28	63

um in animals.[11] This information revealed a considerable species difference in tolerance to intravenously administered hexavalent uranium; *e.g.*, mice tolerated 100 times more than rabbits, and certain strains of mice, 200 times more. Thus there was no clue to man's tolerance, but the data furnished valuable information concerning the distribution and mechanism of toxicity and suggested laboratory methods by which the initial signs of toxicity in man might be discerned.

METHODS

Selection and Care of Patients. The important information concerning the 5 patients included in this study is shown in Table I. In all cases the diagnosis of glioblastoma multiforme or of astrocytoma of the dominant hemisphere had been established by a craniotomy. At the time of injection all but Patient I were in generally good physical condition and all were without definite evidence of pathologic processes other than their cerebral disease. With the exception of Patient III, who lived for seventeen months following injection, all were in coma or semicoma and remained so until their demise. They received the usual hospital care for comatose patients, consisting of indwelling catheters, feeding by gastric tube when necessary, frequent turning, antibiotics and tracheal suction when necessary. The gastric feeding was given every two hours and the volume of each feeding adjusted to prevent too great a gastric residuum. At times it was necessary to augment the tube feedings with intravenous fluids. Patients II and III were taking some oral feedings during the time of injection.

Preparation of Injection Solution. Pure uranium oxide (U_3O_8) was converted to nitrate ($UO_2(NO_3)_2$) by dissolving the oxide in an excess of nitric acid and evaporating to dryness. The resulting nitrate crystals were dissolved in distilled water and repeatedly evaporated to dryness to eliminate final traces of nitric acid. The crystals were then dissolved and diluted to volume with distilled water. The solution was assayed colorimetrically and by alpha counting. The desired quantity of the nitrate was placed in a rubber sealed container and autoclaved for sterility. A sterile 0.4M sodium acetate solution was mixed with the nitrate solution in equal volumes shortly before injection to adjust the pH to 5.5 to 6.0.

Injection Procedure. In all patients the uranium solution was injected into an antecubital vein over a period of ten to fifteen seconds. To preclude the possibility of extravasation, a continuous intravenous injection of normal saline was started and observed for subcutaneous leakage. The uranyl nitrate solution was taken up in a graduated syringe and injected into the intravenous tubing. With the first patient a metal threeway stopcock was used, but this resulted in the loss of a small but significant amount of solution by leakage. In the second patient a glass threeway stopcock was employed, but near the end of the injection the glass arm broke and again a small amount of the solution was lost. The remainder of the injections were made by inserting a No. 22 needle on the syringe

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directly into the lumen of the rubber intravenous tubing. The syringe was irrigated four or five times with saline from the intravenous bottle prior to its removal from the tubing.

Following the injection, the syringe employed in the administration was used to deliver replicate volumes of the injection solution to several flasks for determination of uranium content. This afforded an accurate check on the amount of uranium injected.

Collection of Specimens. The methods of collection and analysis of blood, urine, feces and bone specimens for uranium content have been described in a previous publication.[8]

Urine specimens, collected from indwelling catheters, were taken at hourly intervals during the first twenty-four hours and thereafter at twelve hour intervals for pH, specific gravity, volume and uranium content. Specimens for glucose, acetone, albumin, catalase, and microscopic examination were taken directly from the catheters. For calculation of clearances, samples of the urine collections were taken for creatinine, urea, and chloride determinations.

Blood specimens for hematologic tests, uranium content, liver function tests, electrolytes, total protein, nonprotein nitrogen and amylase were drawn by phlebotomy at varying intervals both before and after injection. Blood samples for urea chloride and creatinine were drawn at twenty-four hour intervals. Intravenous glucose tolerance tests were performed on 3 of the patients.

Analytical Methods. The presence of glucose in the urine was determined by Benedict's qualitative test and, if present, subjected to Benedict's quantitative test. The nitroprusside test was used to detect acetone bodies. The sulphosalicylic acid method of Kingsbury, Clark, Williams and Post (*J. Lab. & Clin. Med.*, 1926, *11*, 981) was employed for the quantitative determination of urinary protein. For microscopic urinalysis, 15 ml. of fresh urine was centrifuged at 2,000 rev./min. for five minutes and the supernatant discarded. The BSP test was performed by injecting 5 mg. of bromsulphalein per kilogram body weight and procuring a blood sample forty-five minutes later, as described by Mateer, Baltz, Marion, and Macmillan (*J.A.M.A.*, 1953, *121*, 723).

Urinary catalase was determined by a manometric method employing the Warburg apparatus as described by Wills (Voegtlin and Hodge: Pharmacology and Toxicology of Uranium Compounds, McGraw-Hill, 1949, p. 270). In the cases where urinary content of catalase was sufficiently high to approach the limitation of maximum reading on the manometers, separate determinations were made on samples of urine diluted 1:5 and 1:20. This procedure consistently resulted in significantly higher values. The final results of each determination were recorded as cc. C_2/0.2 ml. of urine.

The remainder of the analyses were performed by the clinical laboratory of the Massachusetts General Hospital by methods described in the *New England J. Med.*, 1950, *243*, 748.

RESULTS

Clinical State. The parlous clinical state of the patients made interpretation of the vital signs difficult. In general, there were no consistent or marked changes in blood pressure, pulse, respiration, or body temperature that could be attributed to the uranium injection. There were no obvious dermal changes such as erythema, sweating, or eruptions. No change in gastric residua or stools was noted. The neurologic status of the patients remained unaltered.

Cardiac Status. Electrocardiograms were performed on all the patients preceding and one to three days following the uranium injection. No changes from the control readings were noted, with the exception of Patient IV. This patient had a sinus tachycardia for three days preceding the injection, and ten hours following the injec-

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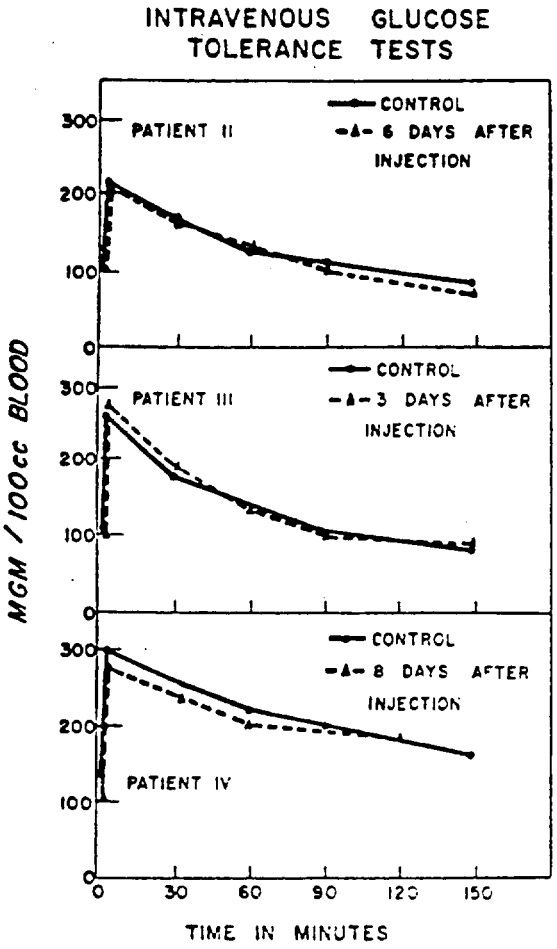

FIG. 1

tion began to have extrasystoles. The electrocardiographic tracing on the second day following injection was interpreted as showing a sinus tachycardia with occasional ventricular premature beat. The extrasystoles disappeared on the fifth day, but the tachycardia persisted for the remaining twenty-seven days of hospitalization.

Glucose Tolerance Tests. The preinjection and postinjection intravenous glucose tolerance tests, which were performed on Patients II, III, and IV, are shown in Figure 1. In all 3 patients the preinjection and postinjection curves closely coincide.

Liver Function Tests. The results pertinent to hepatic function are shown in Table II. Patient I showed definite evidence of hepatic damage both before and following the injection. To a large measure, this

TABLE II

Patient	I		II			III			IV				V				
Day After Injection	Control	1	2	Cont.	1	6	Cont.	1	5	Cont.	2	8	22	29	Cont.	2	6	19	26
BSP, % Retention 45 min.	32%		36%	8%		8%			0%	12%		12%			8%				
Cephalin Flocculation 24 hours	3+	2+		±	2+	0	2+	2+	1+	0	0	0	0	0	0	0	0	0	0
Cephalin Flocculation 48 hours	3+	3+		1+	3+	2+	2+	3+	2+	0	1+	0	0	0	1+	1+	0	0	0
Thymol Flocculation				0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymol Turbidity		8.5		2.4	1.5	2.5	3.5	2.6	3.0	3.5	2.5	2.5	3.0	2.0	1.0	1.5	1.5	3.0	2.0
Cholesterol, mg.%	288	194		224	208	234	249	237	218	248	251	237	249	291	232	245	240	250	250
Cholesterol Esters, mg.%	149	153		215	125	135	160	147	141	126	161	154	167	175	119	114	130	163	174
Serum Bilirubin, mg.%	0.6	4.4		0.3	0.3	0.5	0.3	0.3	0.2	0.5	0.5	0.2	0.2	0.3	0.4	0.2	0.2	0.2	0.3
Prothrombin Time, % of Normal	75%		55%	100%	100%	100%	100%	100%	100%			100%	70%		60%	60%	60%	50%	
										(day 14)					(day 1)			(day 12)	
Serum Albumin, gm.%	2.7	2.7	2.7	3.3	3.2	3.0	3.8	4.0	3.3	2.8	2.8	3.2		3.7	3.3	3.3		3.2	3.5

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TABLE III

MICROSCOPIC URINALYSIS

Patient Day	I			II						III			
	−1	1	2	−2	2	4	7	10	23	−3	1	3	6
WBC./HPF.	8–12	10–15	1–2	15–20	8–10	4–6	Loaded	20–30	10–15	Occ.	Occ.	15–20	Many
RBC./HPF.	1–2	1–2	Occ.	10–15	Rare	10–12	Occ.	1–2	0	0	0	4–8	Occ.
Epi. Cells	Occ.	Occ.	0	0	0	0	0	0	0	0	0	0	0
Crystals	0	0	Amph. Phos.	Ca Ox.	Amph. Phos.	Amph. Phos.	Triple Phos.	Triple Phos.	Triple Phos.	0	0	0	Triple Phos.
Casts				0	0	0	0	0	0	0	0	0	0
Hyaline	Occ.												
Gran.	Occ.		Occ.										
Cells		Occ.	Occ.										

may be accounted for by the fact that his blood pressure was at shock levels during and preceding the time the testing was performed. Patients II and III each had an elevation of cephalin flocculation above the normal range on the first postinjection day, but this returned to normal and the other tests remained within the normal range. Patients IV and V failed to show any changes consistent with hepatocellular damage.

Hematologic Studies. Patients I and V had elevated leukocyte counts during the preinjection period, and the counts were not appreciably altered following injection. All the patients with the exception of Patient II had an increased number of polymorphonuclear cells on differential smear which remained unchanged during the postinjection period. No changes in the morphologic characteristics of the leukocytes were observed.

A drop in the hemoglobin was observed in all the patients. However, this was consistent with blood loss of 300 to 400 cc. necessary for the numerous chemical tests and blood uranium determinations.

As anticipated, the sedimentation rate was markedly elevated prior to injection. Patients IV and V had some increase in sedimentation rate, but this was consistent with the fall in hematocrit. Reticulocyte counts on Patients II and III showed no important changes. Since there were no changes in the apparent abundance of platelets on the blood smear, actual counts were not carried out.

Microscopic Urinalysis. The results of these studies are shown in Tables III and IV. The presence of abnormal numbers of

TABLE IV

MICROSCOPIC URINALYSIS

Patient Day	IV							V						
	−1	2	3	4	7	9	12	−2	1	2	3	6	9	12
WBC./HPF.	Occ.	40–50	20–30	10–20	Many	8–10	10–15	4–6	10–15	10–15	5–8	Many	4–6	1–2
RBC./HPF.	0	3–5	10–20	Occ.	Occ.	0	10–15	0	Rare	1–2	Rare	Occ.	0	0
Epi. Cells	0	Occ. Squam.	0	0	0	0	0	Few	0	0	0	0	0	0
Crystals	0	0	Urate	0	0	0	0	0	0	Urate	Urate	0	0	Triple Amph. Phos.
Casts	0	0	0				0	0						0
Hyaline									Occ.					
Gran.				Rare Coarse	Many Coarse and Fine	Occ. Fine				Many Coarse	Occ. Fine	Occ. Fine	Rare Fine	
Cells									Occ.	Many				

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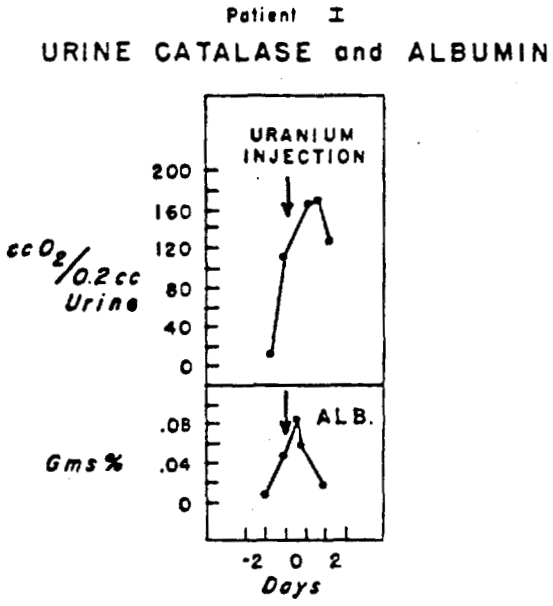

FIG. 2

leukocytes and erythrocytes was consistent with the indwelling catheters. Patient I had casts in his urine both before and after injection without change in abundance. Patients IV and V had casts only during the postinjection period. With Patient IV they made their appearance on the fourth day as a rare coarsely granular cast/high power [illegible] On the seventh day the number of coarsely granular casts was greatly increased, and in addition there were many finely granular casts. On the ninth day, only an occasional finely granular cast/HPF was noted, and on the twelfth day none could be found.

Patient V had occasional hyaline and cellular casts/HPF on the first day, which changed to many coarsely granular and cellular casts/HPF on the second day. On the third, sixth, and ninth days, only a rare finely granular cast/HPF could be found, and on the twelfth day there were none.

Urinary Catalase and Protein. The results of the urinary catalase and protein determinations are depicted in Figures 2 to 6. The rapid demise of Patient I permitted too few determinations for proper evaluation. Patient III, who received the smallest dose, showed a temporary rise in

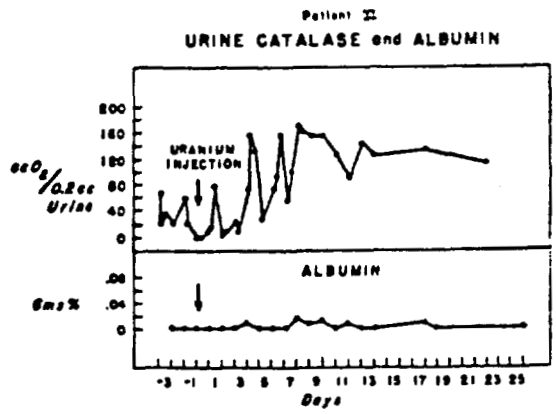

FIG. 3

catalase level on the first postinjection day, but this did not exceed a control determination three days prior to injection. Also this patient had a marked elevation in urinary protein on the day preceding injection which returned to normal during the first two days following injection. This obscured any slight effect of the uranium injection.

Patients II, IV and V, who received the largest doses, showed striking changes from the control values. In all 3 patients there was a tendency for double peaking of the catalase and protein elevations. The initial peak appeared during the second to fourth day and the second peak during the sixth to eighth day. There

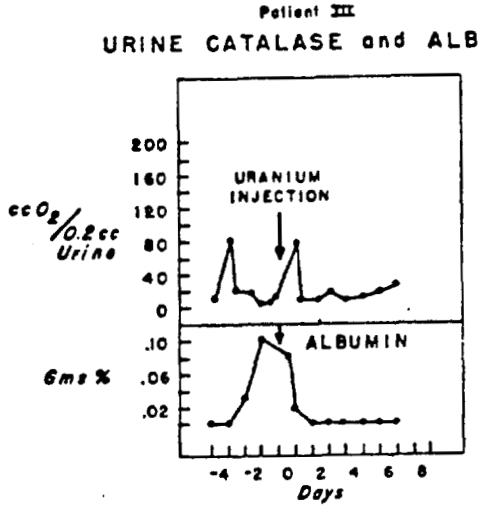

FIG. 4

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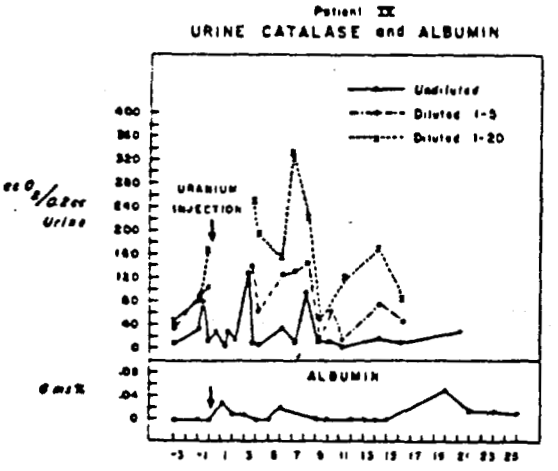

Fig. 5

appeared to be a tendency for the peaks to develop earlier as the dosage was increased. The greater catalase values obtained by diluting the urine sample tested are clearly apparent in the figures for Patients IV and V.

Renal Function Studies. The parameters employed to test renal function are shown graphically in Figures 7 to 10. The bar graphs at the bottom of each of these figures indicate the total twenty-four hour fluid intake and output. The possible

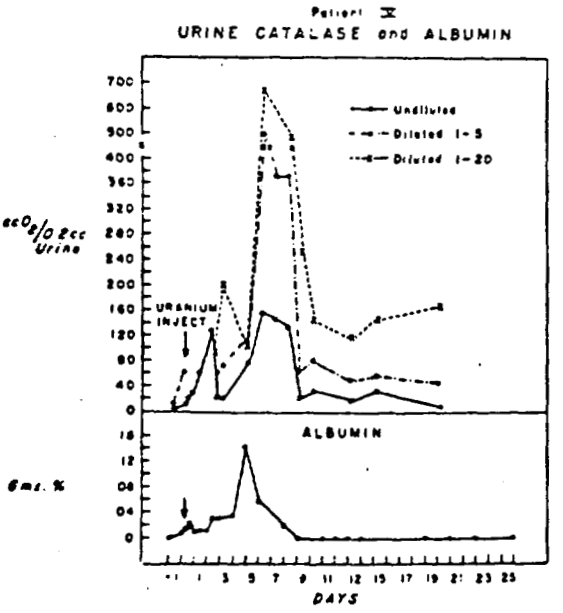

Fig. 6

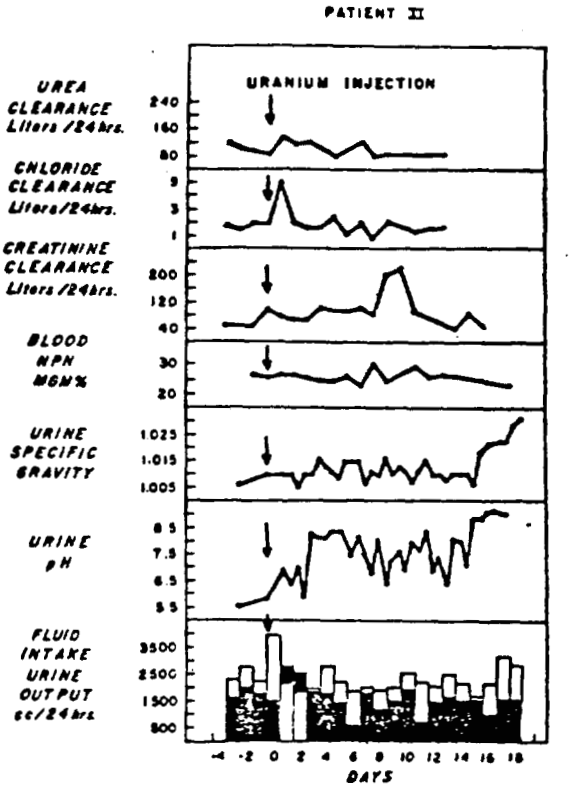

Fig. 7

effects of the uranium injection on the hourly urine output, specific gravity, and pH during the first twenty-four hours are shown in Figures 11 to 15. Prior to the uranium injection, the patients were in an adequate state of hydration as adjudged by control fluid balances and electrolytes.

Patient I failed to show a definite effect from the injection. The control output of 100 cc. of urine per hour fell to 20 cc. per hour by three hours following injection and remained at approximately this level for the next seventeen hours in spite of adequate intravenous fluids. This patient's blood pressure had a tendency to drop to shock levels for protracted periods of time, and no doubt this was an important contributing factor.

Patients II, III, IV, and V showed an immediate increase in urinary output following injection. However, at the same time they were receiving .85 per cent saline intravenously. Patients II and III had a sec-

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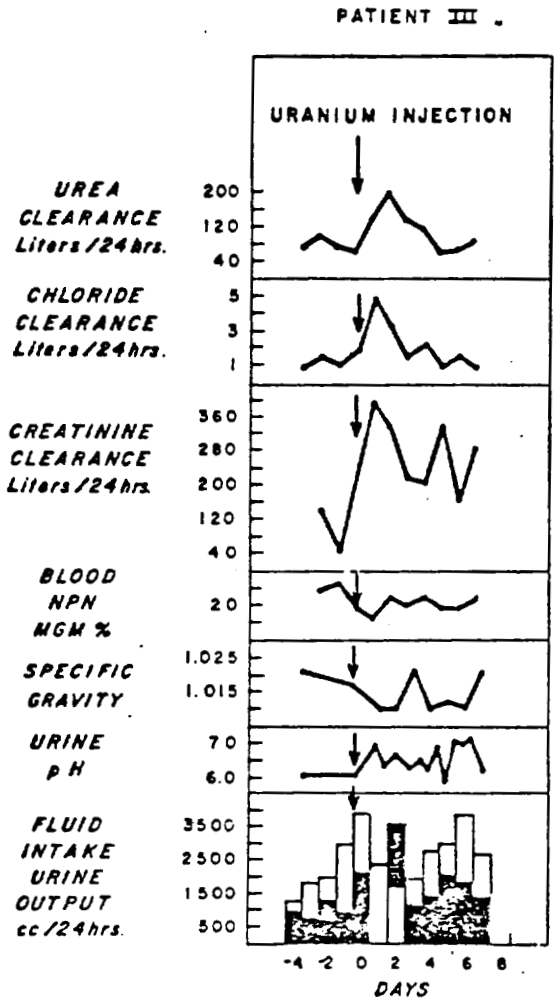

FIG. 8

ond diuresis in response to more intravenous fluids. In the case of Patient II this was delayed until over 2,000 cc. had been administered.

The values for pH and specific gravity on the hourly specimens fluctuated in accordance with the output. All the patients were able to acidify to below pH 6 and concentrate their urine with a specific gravity greater than 1.020 when the hourly output became low.

The twenty-four hour fluid balances revealed a greater output than intake in Patients II and III for two days following injection. Patients IV and V showed evidence of a slight decrease in output; the lowest output being 500 cc. on the second

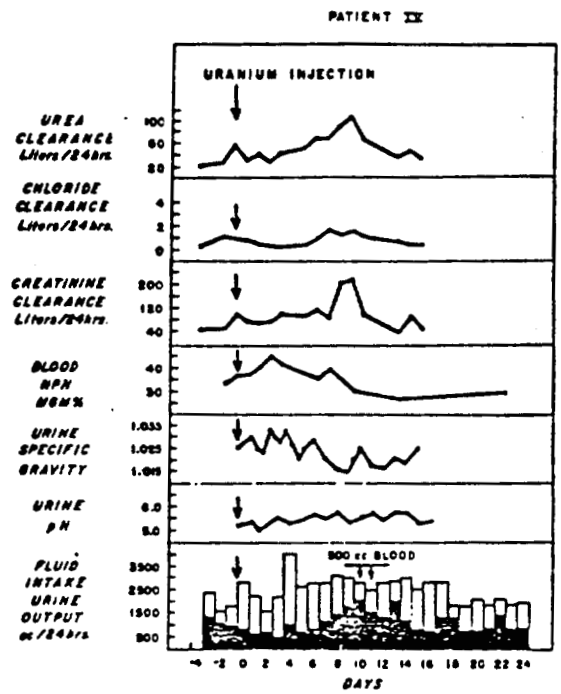

FIG. 9

day in the case of Patient IV. During the third day, this patient received supplemental intravenous fluids increasing the in-

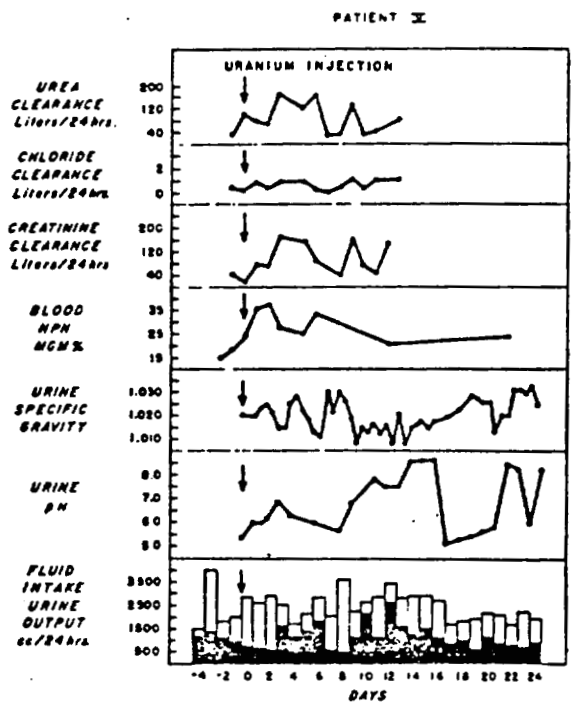

FIG. 10

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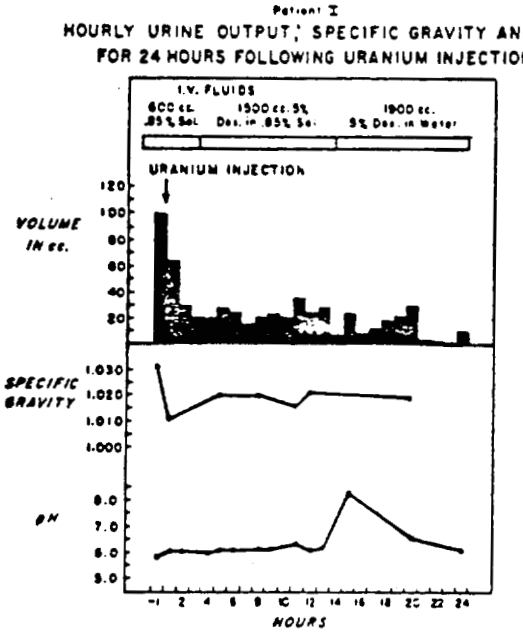

Fig. 11

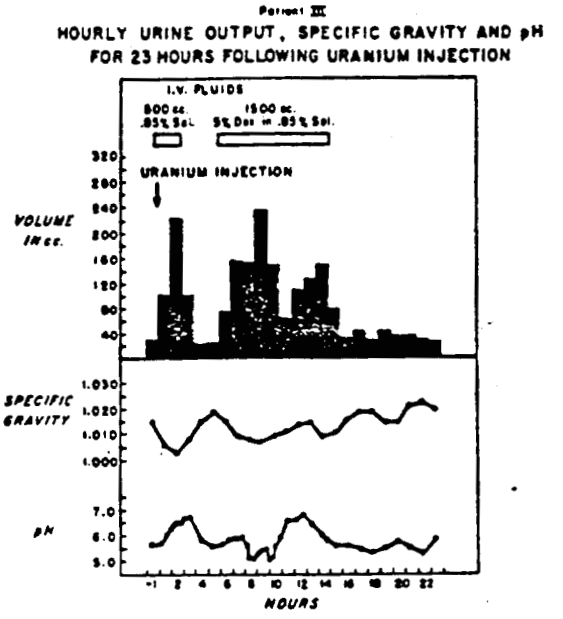

Fig. 13

take to 4,000 cc., but this failed to be reflected in the output.

The curves illustrating the twenty-four hour clearances of chloride, urea, and endogenous creatinine failed to reveal any decrease below control values. However, in the face of unaltered clearances, high specific gravity, and low pH of urine, Patients IV and V showed a significant rise in nonprotein nitrogen during the first three days after injection. These elevations coincided with the periods of decreased urinary out-

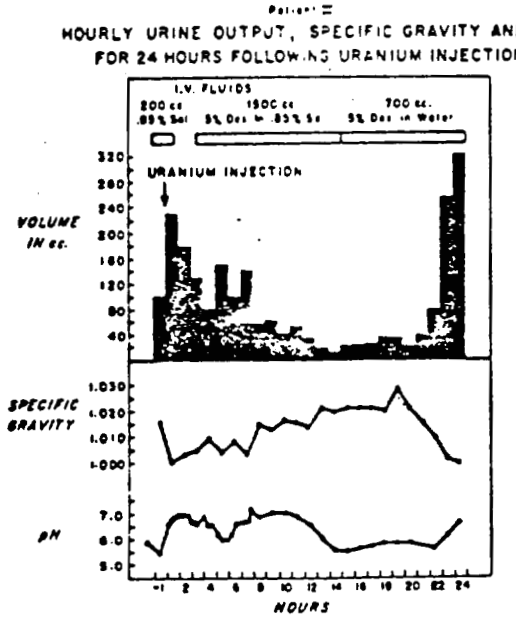

Fig. 12

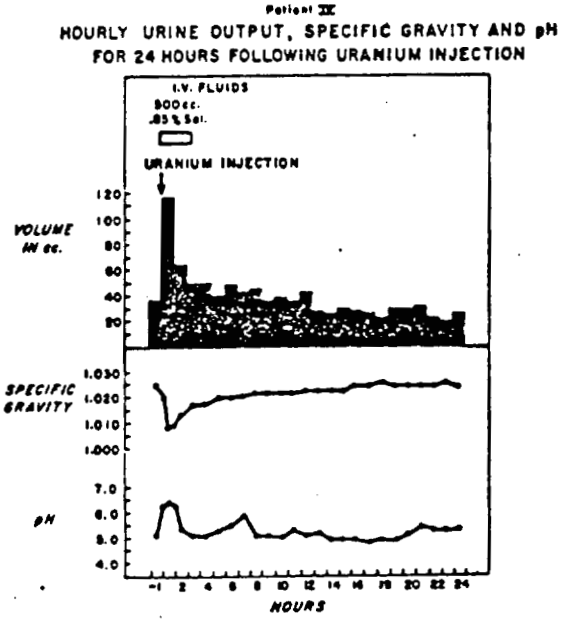

Fig. 14

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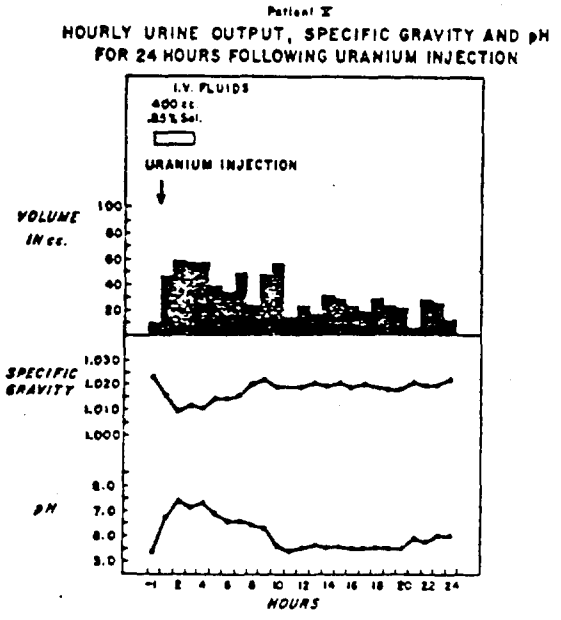

FIG. 15

put and a decline in the clearances of urea and creatinine manifest immediately post-injection.

Serum Electrolytes, Protein, Alkaline Phosphatase, and Amylase. These determinations are shown graphically in Figures 16 to 18. With the exception of Patient V, there was a slight but consistent decrease in serum sodium to below normal values. A

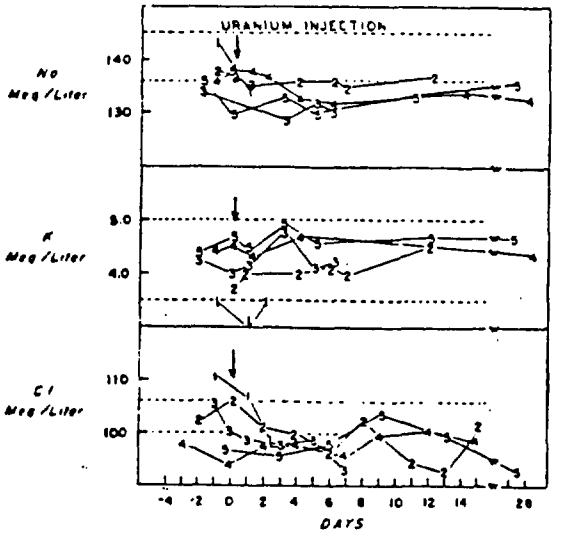

FIG. 16

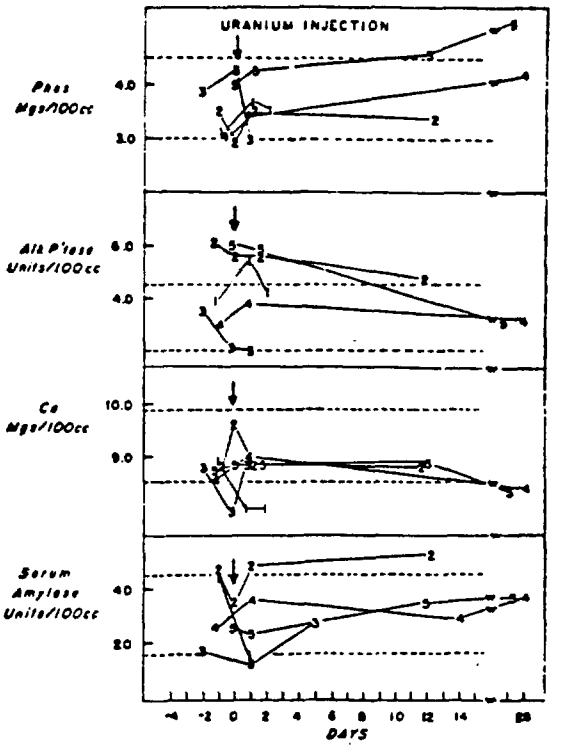

FIG. 17

similar drop in serum chloride was noted for all except Patient IV. A rise in potassium during the first five days was seen in all the patients. Patients IV and V showed a rise in phosphorus and a decline in calcium several weeks after injection. The total protein was low for all the patients except Patient I and remained so for the duration of observation. The serum globulin, pH, and CO_2 showed no consistent changes. The initially high values for alkaline phosphatase in Patients II and V dropped toward normal following injection. The postinjection serum amylase values remained essentially within the normal range with the exception of Patient II, who had an elevation slightly above normal on the first day following injection which was again noted on the twelfth day.

Urine Glucose and Ketone Bodies. Daily testing of the urine of all the patients during the period of observation failed to reveal the presence of glucose or ketone bodies.

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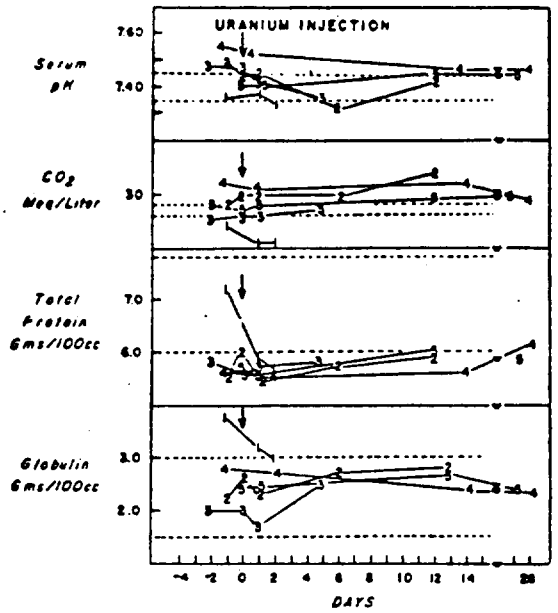

FIG. 18

Pathologic Findings. The final anatomic and microscopic diagnosis on all the patients except Patient IV, for whom autopsy permission was not granted, are given below. Two separate opinions concerning the microscopic examination of the kidneys are listed.*

Patient I

1. Kidney: (A) Congestion; a small venous thrombosis; occasional cast in lower nephron, especially collecting tubules. (B) Albuminous casts present in collecting tubules and a few containing clumps of polymorphonuclear leukocytes; a questionable acute pyelonephritis.
2. Necrotizing bronchopneumonia with septicemia.
3. Phlebothrombosis, left femoral and iliac vessels with extension to the vena cava.
4. Pulmonary emboli, small, with infarction.
5. Glioblastoma multiforme, right frontal lobe.

Patient II

1. Kidney: (A) Mild nephrosclerosis; hyaline casts in collecting tubules. (B) Epithelium of convoluted tubules somewhat swollen and containing numerous fine hyaline droplets. No other abnormality noted.
2. Alveolar adenocarcinoma of both lungs, with metastases to heart.
3. Fatty metamorphosis of liver.
4. Intussusception of ileum, agonal.
5. Chronic ulcer of esophagus.
6. Cachexia.

Patient III

1. Kidney: (A) No abnormality seen.
2. Recurrent glioblastoma multiforme, left parietal lobe.
3. Bilateral bronchopneumonia.
4. Leiomyoma, 1 cm. in diameter, in wall of first portion of duodenum.
5. Multiple neurofibromatosis with café au lait spots (Von Recklinghausen's neurofibromatosis).

Patient V

1. Kidney: (A) Very mild focal chronic pyelonephritis; question of some edema of the medulla. (B) Changes in convoluted tubules similar to Patient II and also no other abnormalities.
2. Astrocytoma of the left temporoparietal lobe.
3. Bronchopneumonia, bilateral, lower lobes.
4. Encrusted cystitis, chronic.
5. Multiple decubitus ulcers.
6. Atrophy of testicles.
7. Cachexia.

DISCUSSION

Before discussing the significant findings of this study, it is pertinent to review briefly the observations made on these patients concerning the distribution and excretion of hexavalent uranium and the

* We are grateful to Dr. Robert F. Scully of the Department of Pathology of the Massachusetts General Hospital and Dr. Kenneth Mallory of the Mallory Institute of Pathology of the Boston City Hospital for their review of these slides.

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FIG. 19. A gross autoradiogram of a kidney from Patient v, showing greatest concentration of the uranium in the cortical zone.

extensive small mammal studies concerning the mechanism of toxicity.

The distribution and excretion in man[8] was found to be in good agreement with the small mammal data.[11] The injected hexavalent uranium rapidly leaves the blood stream, 99 per cent of the dose being cleared in about twenty hours. In small animals this degree of clearance requires only two to five hours. However, the data pertaining to the fractions of the injected dose initially deposited in the major sites, *i.e.*, the bone, kidney, and urine, coincide more closely. Neuman and Neuman[5] state that the data in rats show 5 per cent to 20 per cent of the dose deposited in the skeletal system, about 20 per cent in the kidney, and 50 per cent to 75 per cent excreted in the urine during the first twenty-four hours following injection. The comparable human data are 6 per cent to 14 per cent deposited in the skeletal system, 16 per cent in the kidneys, and 49 per cent to 84 per cent excreted in the urine.

The specific locus of deposition within bone and kidney is identical in man and the small mammals studied. The uranium first becomes deposited along the luminal surfaces of the proximal convoluted tubules in the outer and midzones of the cortex. Following this, it associates with cellular debris in the lumens of the tubules. The gross autoradiograms of Tannenbaum, Silverstone, and Koziol[10] confirmed the cortical distribution in the kidneys of rats. A gross autoradiogram of a kidney from Patient v is shown in Figure 19. Here, as in the other observations, the uranium is nonuniformly distributed, being more concentrated in the cortical zone. Figure 20 shows a microscopic autoradiogram from Patient v. This indicates that the uranium atoms are associated with cast formation in the lumen of a tubule. This mode of renal deposition was a constant finding, thus confirming the small mammal observations.

The characteristics of bone deposition have been demonstrated by the gross autoradiograms of rat femurs prepared by Tannenbaum, Silverstone, and Koziol,[10] and Neuman and Neuman.[5] A periosteal and endosteal locus is clearly depicted in these, and the scattered spots of activity in cancellous bone suggest a deposition within the haversian system as well. Autoradiograms from a femur of Patient 1 are shown in Figure 21. The characteristics of deposition appear to be identical to those of the rat.

The inference from small mammalian studies[11] is that following injection the uranyl cation is distributed mainly between two complexes, one with the bicarbonate and the other with the protein of the plasma—approximately in equal amounts with each when the CO_2 combining power is normal. The uranyl bicarbonate complexes pass through the renal glomeruli, and as the pH of the glomerular filtrate decreases and the bicarbonate becomes absorbed by the cells of the proximal convoluted tubule, the uranyl cations are liberated to combine with the protein along the tubular wall. That the distal portion of the proximal

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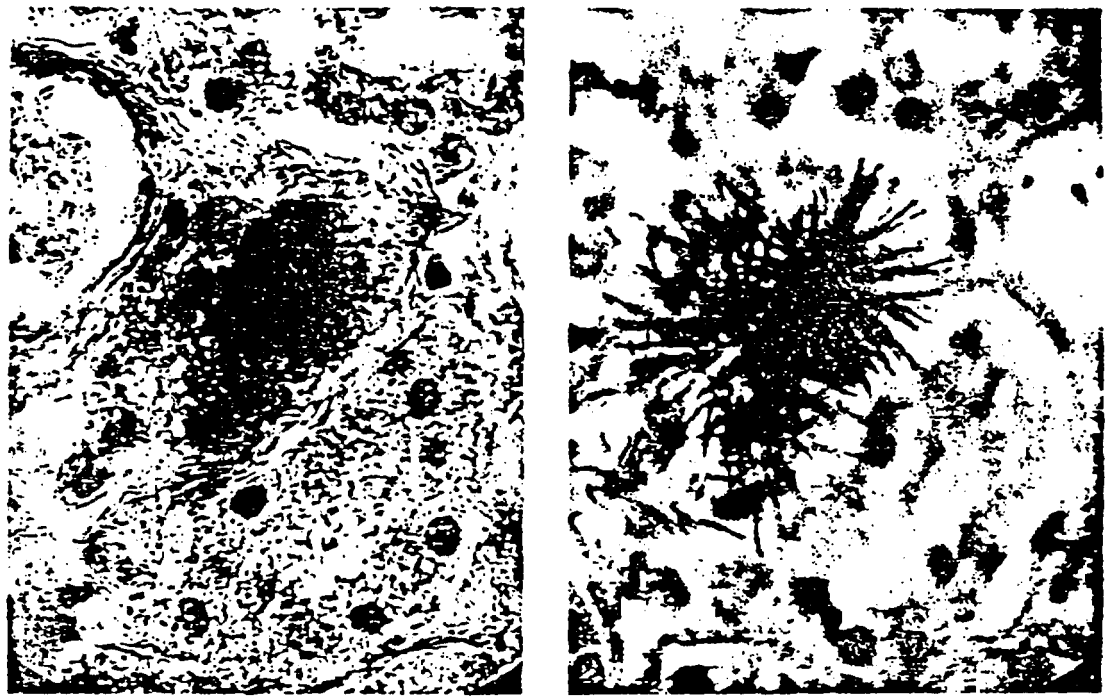

FIG. 20. A microscopic autoradiogram of a kidney section from Patient V, showing a cluster of α tracts associated with debris within the lumen of a renal tubule.

convoluted tubule is the locus of initial damage is confirmed by pathologic examination of the kidneys and clearance data.

The physiologic consequences of the juxtaposition of a high concentration of uranyl cations and the epithelium of the proximal convoluted tubule are apparent immediately. Bicarbonate, chloride, glucose, and amino acids, which are mainly dealt with in the proximal convoluted tubule, appear in the urine in greater abundance. There is an immediate diuresis which, with large doses of uranium, may rapidly decrease to oliguria or anuria. Substances such as urea and NH_4, which are handled by other tubular portions, and creatinine, inulin, and xylose, the excretion of which depends upon glomerular filtration, are unaffected when the damage is not severe.

Numerous studies[11] have shown that minimal effects upon the tubular cells can lead to the loss of protein and enzymes, such as catalase and phosphatase which are abundant in these cells, before the metabolic functioning of these cells is seriously jeopardized. Upon this characteristic depends the increased appearance of these substances in the urine, serving as a sensitive index of tubular damage.

Efforts to elucidate more specifically the mechanisms of toxicity of uranium have led to numerous *in vitro* and *in vivo* metabolic and biochemical studies. *In vitro* studies have demonstrated protein precipitation and the reversible inhibition of certain enzymes, most particularly phosphorylase. The high concentration in the tissue fluids of anions forming complexes made the *in vivo* conditions less suitable for enzyme inhibition studies. However, various metabolic effects unrelated to the renal effects have been described. These include elevated and prolonged glucose tolerance curves and interference with muscle glycogen formation in dogs and rats, elevation of blood inorganic phosphorus in rats, a slight decline in serum protein in rats, changes in the unsaturation of phospholipids of the blood, liver, and kidney

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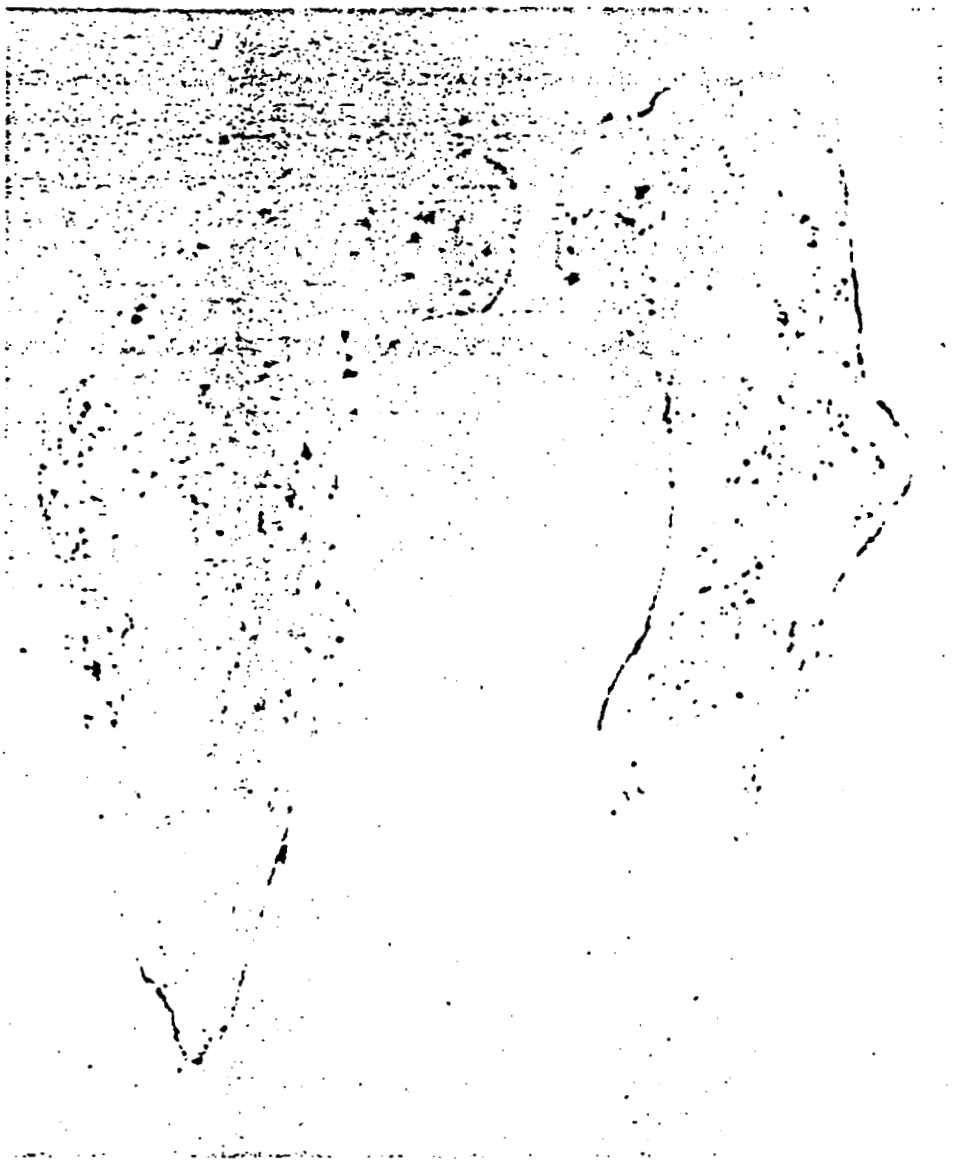

FIG. 21. A gross autoradiogram of the proximal end of a femur from Patient 1, showing periosteal and endosteal concentration and scattered foci of concentration in the cancellous bone consistent with deposition in the haversian system.

in rats, and an increase in blood cholesterol in dogs and rats. Other alterations more related to hepatic function include: decreased efficiency to remove bromsulphalein from the blood, increased excretion of ketone bodies and free phenol, decreased plasma levels of fibrinogen and prothrombin, failure in the conversion of injected lactate into liver glycogen, and some evidence for an increase in total liver lipids.

These findings indicate that it is unlikely that enzyme poisoning, aside from that in the kidney, participates to a significant degree in acute toxicity. This is consistent with the over-all picture of the distribution, excretion, and chemical properties of the uranyl cation. Surface proteins of the cells are only briefly and minimally exposed to uranyl cations after intravenous injection because of the abundance of complexing agents in the tissue fluids and the rapidity with which the cation is cleared

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from the soft tissues. The lumen of the renal tubule affords a unique situation where the uranyl cation is present in comparatively enormous concentration for a longer period and with a relative decrease in complexing agents.

To offset this general scheme of the mechanism of toxicity of the uranyl cation, a particular species would have to possess an essential enzymatic system considerably more sensitive than the enzymatic systems in the renal tubules. This is extremely unlikely, especially in view of the evidence that the action of the uranyl cation on the cell surfaces of the tubules is quite general, being more related to concentration than to a specific enzymatic poisoning.[11]

In numerous small mammal experiments[11] it was demonstrated that urinary catalase has a marked consistent rise following uranium intoxication. The comparative ease of quantitative manometric determination has made the test of great value in animal studies. Studies of the catalase activity of normal human subjects[11] showed a mean value of 4.12 cc. O_2/0.2 cc. urine with the huge standard deviation of 9.37. Because the normal subjects occasionally showed values as high as 50 cc. O_2/0.2 cc. urine, such sporadic elevations were not considered as definitely abnormal. Patients with various types of chronic renal disease showed values above normal in several cases, but the findings were not striking and there was no correlation with proteinuria. Therapeutic doses of mercuhydrin, salyrgan, and mapharsen failed to produce catalasuria or proteinuria in humans but caused catalasuria in proportionally larger doses in animals. Exercise sufficient to cause a significant proteinuria in humans failed to produce catalasuria.

That the source of urinary catalase is the tubular cells rather than increased clearance from the plasma was quite conclusively demonstrated by experiments in which it was found that variations in catalase clearance were not correlated with variations in inulin clearance, and increasing the plasma catalase in uranium poisoned animals did not increase the urinary catalase.[11] The urinary protein, which was found to accompany the catalasuria in animal studies, was found by electrophoretic testing to be derived from plasma protein to a large extent. Since pathologic studies failed to reveal evidence of glomerular damage, the method by which the protein gets into the urine can only be hypothesized.

Following moderate doses of uranium in rabbits and dogs, the catalase elevation reached an initial peak within two to three hours and a maximum within three to six days and then a return to normal in several days.[11] As an indication of the sensitivity of the catalase test, it was found that intravenous doses as small as 0.005 and 0.01 mg. U/kg. in dogs and rabbits can evoke increased catalase excretion within a period of two to four days. In the case of rabbits, this was one-tenth to one-twentieth of the lethal dose. Also with rabbits it was shown that small doses can evoke catalasuria without proteinuria, and with larger doses the catalasuria appears before proteinuria and elevation of non-protein nitrogen. Hence, catalasuria is a more sensitive indicator of tubular damage in the rabbit (and probably in the cat, but only in some of the dogs tested). Also, the catalase test was more specific, reproducible, and had a much greater relative change in all the animals.

The elevation in urinary catalase on the first postinjection day noted in Patient III did not exceed a control value, and the presence of normal values for the subsequent seven days suggested that the tubular effect was insufficient to produce the release of catalase. Also there was no evidence that the injection provoked an albuminuria. In Patients II, IV, and V, who received the largest doses, there was an elevation of both albumin and catalase, with the peaks generally coinciding in Patient II but slightly preceding the catalase peaks in Patients IV and V. With increasing dosage, the magnitude of the albumin peaks became greater. The maximum possible manometric reading using

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undiluted urine was approached by all 3 patients, this being about 160 cc. O_2. In Patients IV and V, in whom diluted samples of urine were tested, the catalase peaks were higher in Patient V, who received the larger dose. It has been shown that urine inhibits catalase by approximately 50 per cent when compared to distilled water, and this is the most likely explanation for the higher values obtained with diluted samples.[11] Although catalasuria and albuminuria both appeared in these 3 patients, the catalase peaks represented a far greater relative increase.

As an explanation for the double peaking of catalase values observed in animals, it was suggested that the first peak, occurring about two hours after injection, resulted from an increase in the membrane permeability of the tubular cells, and the second peak, occurring in two to five days, from actual breakdown of the cell structure. There was a definite tendency for double peaking in these patients, being most obvious in Patient V who received the largest dose. However, although the appearance of the maximum peaks corresponded with the animal studies, the initial peak was delayed for one and a half to three days. An explanation for this may be the slower rate of clearance noted in the human studies, or that these patients received relatively smaller doses than the animals studied.

Other evidence of tubular damage is the appearance of casts in Patients IV and V during the period of albuminuria and catalasuria. Patient V had many cellular and coarsely granular casts on the second day, indicating that actual cellular destruction was taking place. This corresponds to the initial catalase peak. At the time of maximum catalasuria on the sixth day, only finely granular casts were noted. This is a fragment of evidence against the concept that the initial peak results from increased permeability of the tubular cell membrane and the maximum peak from cellular destruction. More conclusive data on this point could be obtained by more frequent and quantitative cast determinations.

The extent to which this tubular damage, as manifested by catalasuria, albuminuria, and casts, is reflected in renal function is not entirely clearcut. During the first twenty-four hours following injection, there did not appear to be any tendency for diuresis beyond that expected from the effects of the intravenous fluids.[7] Also the increased urinary outputs of Patients II and III during the first two days are consistent with the excess of intravenous saline received on the day of injection. Patient IV had a definite decrease in output for six days following injection, but during this time the specific gravities ranged from 1.020 to 1.035, making it doubtful that this represented oliguria secondary to renal damage.

All the patients showed a tendency for a more alkaline urine following injection. That this could be due to loss of base with the uranyl cation excretion is not likely because the tendency is not apparent during the initial twenty-four hours when approximately 99 per cent of the dose is excreted. There was a decline in serum pH in all the patients except Patient V, suggesting some interference with the renal capacity for excreting acid, but the serum CO_2 showed no significant change.

During the time of maximal renal effect, as evidenced by albuminuria and catalasuria, there was an elevation of serum potassium, a drop in serum sodium for all except Patient V, and a drop in serum chloride for all except Patient IV. That these changes are not related to the uranium is suggested by the failure of the degree of change to be correlated with the magnitude of the dose and the participation in these changes of Patient III, who failed to show a significant catalasuria or albuminuria. Also, patients with brain tumors occasionally show electrolyte abnormalities. On the other hand, these changes tend to return to control levels as the apparent renal effect wears off. Therefore, they stand as suggestive evidence of an interference with the renal capacity for sodium and chloride reabsorptions and potassium secretion. It is not as likely that the potassium elevation

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reflects loss from damaged body cells, because this would be expected at a time when the concentration of the uranyl cation is highest in the body fluids, *i.e.*, for a few hours after injection.

The apparent renal damage was of insufficient magnitude to alter urea and creatinine clearances. It is known that disappearance of several hundred thousand of the circa 2,500,000 nephrons normally present cannot be detected by these tests. As demonstrated by more recent uranium toxicity studies, the tubular capacity to deal with such substances as glucose, vitamine C, and amino acids affords a much more sensitive index of primary tubular damage. However, none of the patients showed glucosuria. Although there were no instances where the urea and creatinine clearances fell below the control values, both Patients IV and V had elevations of blood nonprotein nitrogen with peaks exactly coinciding with the peaks of catalasuria and declines in the magnitudes of the urea and creatinine clearances. However, all these changes also follow the fluctuations in urine output, which are known to influence these factors greatly. The chloride clearances showed no tendency to increase, indicating preservation of the normal capacity to reabsorb this ion. This finding, however, is in conflict with the finding of declines in the level of serum chloride.

Other changes unrelated to renal function which are worthy of note are the development of extrasystoles by Patient IV, a slight elevation of serum amylase in Patient II, elevation of cephalin flocculation above normal on the first postinjection day in Patients II and III, and elevation of serum phosphorus and decline of serum calcium in Patients IV and V several weeks after injection. These changes are not consistent, nor do they deviate from what might be expected in patients in this clinical condition. Therefore, it is not likely that they are ascribable to the uranium injection.

All the patients with the exception of Patient I expired after the time in which the acute tubular effects would be apparent pathologically. The findings described are not beyond those often seen in terminal patients. There was no evidence of atypical tubular cells as described by Oliver[6] in dogs. Since Patient I was showing hyaline casts prior to injection, the casts observed pathologically cannot be attributed to the uranium effect. The tubular cells in this patient failed to show evidence of toxicity.

The alveolar adenocarcinoma found in Patient II was obviously of longer duration than the sixty days that elapsed following the uranium injection, and, therefore, was unrelated. The diagnosis of multiple neurofibromatosis had been made on Patient III prior to injection.

CONCLUSIONS

The toxicity of hexavalent uranium injected intravenously in humans in the dosages employed is readily apparent by a catalasuria and albuminuria which show an initial peak during the first one and a half to four days and a second one at the sixth to eighth day. The minimal dose necessary to produce this effect is of the order of 0.1 mg. of uranium/kg., and with increasing dosage the effect becomes greater. The relative increase in catalase is far greater than that of albumin. Further evidence of tubular damage is indicated by the appearance of cellular casts which can appear as early as the second day. Concomitant with this tubular damage there is suggestive evidence of interference with the renal capacity for reabsorption of Na and Cl and the secretion of potassium. There is no good evidence of a diuretic effect at this dosage. There is a tendency for a more alkaline urine. As evidenced by declines in the twenty-four hour clearances of urea and creatinine, it is suggested that a sufficient number of nephrons were incapacitated to cause a transient rise in serum nonprotein nitrogen with the doses of 0.17 and 0.28 mg. uranium/kg. Impairment of tubular function was of insufficient degree to allow glucosuria at normal blood glucose levels. Evidence for toxic manifestations in other systems or unrelated to

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renal abnormality was lacking. The tubular changes were of insufficient degree to be pathologically detectable after forty-two days, and as early as 2.4 days when the dosage was 0.097 mg. uranium/kg.

The findings permit an estimation of man's relative position with the rabbit, guinea pig, rat, and mouse in regard to approximate lethal dosage. He is certainly more tolerant than the rabbit and guinea pig, which usually succumb with doses of 0.1 and 0.3 mg. uranium/kg. In the rabbit, catalasuria commences at approximately one-tenth the lethal dose. If this is carried over to man, it indicates a lethal dose of about 1 mg. uranium/kg., which is the range of the rat. The findings are against human toleration as high as 10 mg. uranium/kg., which is the lethal dose of the mouse.

SUMMARY

Five terminal patients with brain tumors were given intravenous injection of uranyl nitrate in doses of 0.097, 0.12, 0.07, 0.17, and 0.28 mg. uranium/kg. The 3 patients receiving the largest doses showed an elevation of urinary catalase and albumin. Other evidences of renal toxicity were the appearance of casts in the urine and elevation of nonprotein nitrogen. There was suggestive evidence of the loss of renal capacity for sodium and chloride reabsorption and potassium secretion. Electrocardiograms, liver function studies, hematologic tests, and glucose tolerance tests failed to reveal other evidences of toxicity. Four of the patients who came to autopsy did not reveal any unusual findings. Of the common laboratory animals, man appears to correspond most closely to the rat in regard to intravenous tolerance to uranium.

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