significantly decreased the synthesis of acetylcholine *in vitro* while the other extracts had no demonstrable effect on this process. 3. Less acetylcholine was synthesized in the presence of serum from animals injected with the extract of thymus and pancreas than in the presence of serum from non-injected animals or animals injected with extracts of lungs. 4. The extracts of the thymus and pancreas contained neither nucleoproteins nor their decomposition products in an amount demonstrable with ultraviolet spectrography.

14702

Studies on Heavy Metal Poisoning: I. The Use of Natural Radioactivity for Tracer Studies on Uranium.*

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Introduction. Since uranium was introduced as an experimental nephropathic agent by Leconte[1] several attempts have been made to develop a method to measure small quantities of uranium such as are encountered in biological materials in animals poisoned with this heavy metal, but none of these methods has utilized the natural radioactivity of uranium as the basis. Recent developments in the measurement of emanations from radioactive substances have made this possible and with the aid of Dr. A. E. Ruark in the Physics Department a reasonably satisfactory method has been developed.

Our immediate interest in the problem arose from studies by Donnelly and Holman[2] in which it was shown that the intravenous administration of sodium citrate protected dogs against a lethal dose of uranium nitrate. If a method could be perfected for determining small quantities of uranium, it might be possible to determine whether sodium citrate leads to increased elimination of the heavy metal by altering the solubilities of the complex uranium salts, or otherwise. Before these studies could be completed they were interrupted by one of us (W.A.D.) being called away to participate in the war effort. The results to date have indicated: (1) that the method has merit, (2) that uranium nitrate disappears rapidly from the blood stream after intravenous injection, and (3) that approximately ⅔ of the uranium can be recovered in the urine in the first 24 hours after injection.

Methods. Four adult dogs fed the regular kennel diet and allowed free access to water were used. Two of these dogs received daily intravenous injections of sodium citrate (0.33 cc of a saturated aqueous solution [approximately 230 mg] per kg) for 5 days before the injection of uranyl nitrate and for 5 additional days following the injection of the heavy metal. Each of the 4 dogs received an intravenous injection of 5.0 mg/kg of uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$, Merck in 0.5% aqueous solution) accurately transferred by washing out the syringe with blood. Immediately after the injection of uranium nitrate the dogs were catheterized and placed in clean metabolism cages. All urine passed during the next 24 hours was collected. In each case the 24-hour urine output was diluted up to 1500 cc with cage washings. Blood samples were taken immediately before and at 4, 19, 34, 64, 124, and 244 minutes after injecting the heavy metal—also at the end of 24 hours. The blood samples consisted of 12 cc of venous blood (jugular vein) mixed with 2.0 cc of 1.4% sodium oxalate in a 15 cc graduated centrifuge tube and centrifuged at 3,000 r.p.m. for 35 minutes.

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1 Leconte, *Gas. Med. Paris*, 1854, IX, 488, (cited by MacNider, W. deB.), *Physiol. Rev.*, 1924, **4**, 595.

2 Donnelly, G. L., and Holman, R. L., *J. Pharm. and Exp. Therap.*, 1942, **75**, 11.

Corrections were made for this oxalate dilution in the figures recorded. Similar oxalated plasma obtained from normal dogs was used in preparing the standard dilutions from which the calibration curves were made.

Standard calibration curves were prepared by making standard dilutions of uranium nitrate covering the approximate range of dilution determined by previous trial, viz., 1:10, 1:100, 1:200, 1:400, and 1:800. The alpha-particle activity of these standard dilutions of uranium in blood plasma and in urine-blood-plasma (see below) was determined, and the uranium content of the unknown samples was determined from these calibration curves.

The sample of each specimen of standard and unknown blood plasma was 5.0 cc. In the case of the standard and unknown urine analyses, 5 cc of urine were mixed with 5 cc of oxalated blood plasma and these 10 cc of oxalated blood plasma-urine were analyzed. All of the samples were evaporated to dryness at about 110°C for 2-4 hours in flat porcelain dishes, ashed at 650-750°C for 3 hours, cooled, immersed in a liter beaker containing 700 cc of distilled water for 24 hours, the water in the beaker drawn off with a siphon, and the water in the crucible cover evaporated to dryness at 110°C. It was found that this washing for 24 hours removed some of the soluble salts which screened the activity of the uranium complex. Several analyses of the concentrated wash water showed that it never contained as much as 2% of the material washed, and most of the analyses were entirely negative.

The thin crystalline film in the bottom of the crucible cover had a hard glazed appearance and measured about 25 micra in thickness. Since this is greater than the range of the alpha particles, these films were considered as "thick" sources. The alpha particle activity was counted by an ionization chamber operating a linear amplifier and mechanical counter. The distance from the film to the entrance screen of the ionization chamber was 0.55 cm and the effective entrance area of the opening was for the alpha particles proceeding vertically 3.40 sq cm. All counts were carried on for at least one hour, and in the case of "weak sources" counting was continued for 4-5 hours.

The background or natural counting rate of 20 per hour was subtracted from the count of each specimen measured.

The root-mean-square error in the amount of uranium associated with statistical fluctuations alone is about $100/\sqrt{N}$ if expressed in percent. Here N is the total number of counts. This expression does not make allowance for fluctuations of background but is sufficiently accurate for present purposes. In actual practice this narrowed down to a probable error of about 10% and a maximum error of about 25%.

Experimental Observations. The standard calibration curves for plasma and urine are shown in Fig. 1 and 2 respectively. The points plotted are the average of duplicate determinations, and all of them fall along a straight line within the range tested. It was necessary to add protein to the urine in order to obtain these maximal reproducible results. We do not know the reason for this. Crude chemical and physical tests showed that the straight ashing of standard aqueous and urine dilutions changed the uranium to U_3O_8. Possibly the crystals that formed without the added plasma protein were too large and resulted in some screening, for the counts were much lower with this simple direct technic. Wet ashing with nitric acid did not improve the technic, but we have not exhaustively studied wet ashing methods.

Regardless of the absolute value of the method, when the same arbitrary technic was applied to standard dilutions and to unknown samples of oxalated dog plasma at varying

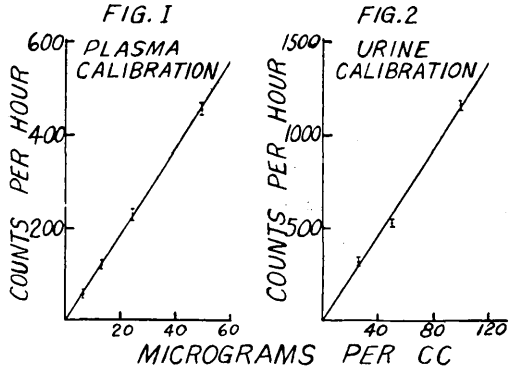
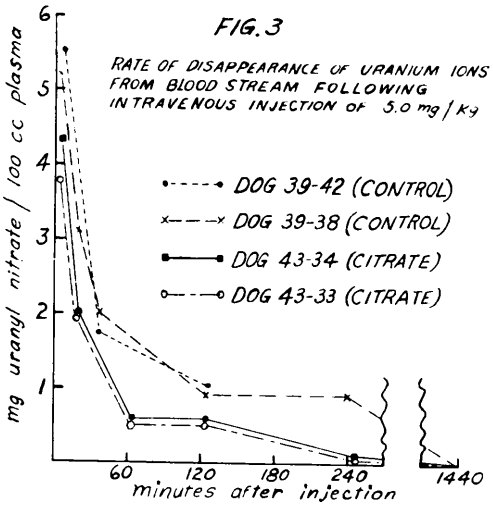


TABLE I.
Recovery of Uranyl Nitrate in Urine in 24 Hours after Intravenous Injection of 5.0 mg/kg.

Dog No.	Sex	Estimated age (yrs)	Body wt (kilos)	Total uranyl nitrate inj. (mg)	Total uranyl nitrate recovered in urine (mg)	% recovery in urine in 24 hr
39-38	M	6	18.6	93.0	72.0	77
39-42	M	5	24.3	121.5	37.5	31
43-33	M	3	18.4	92.0	81.0	88



intervals after the intravenous injection of a minimal lethal dose of uranium nitrate, it was found that the uranium disappeared rapidly from the blood stream. At the end of 4 hours radioactivity in the blood stream was minimal and by the end of 24 hours the count had returned to background level in all 4 dogs (Fig. 3).

It was somewhat of a surprise to us to recover so much of the uranium in the urine that was passed in the first 24 hours after the injection (Table I). This varied from 31% to 88% of the total amount injected and averaged 65% for the 3 dogs tested. Even if the maximal error of 25% occurred in the dog with the highest percentage recovery (dog 43-33) there would still be a recovery of 66% of the amount injected. Unfortunately these studies were not carried on beyond the first 24 hours and no analyses of tissues or bile have been attempted, hence we have no quantitative checks on the total amount injected.

Discussion. It is difficult to compare the results obtained by our method with those obtained by other methods, for different amounts of different preparations were administered by different routes to different species of animals. Karsner and Reimann[3] using a chemical method with potassium permanganate titration found about 20% of 50.0 mg/kg in the kidneys of cats after an unstated interval. In another series of experiments they recovered 11% and 14% of the uranium injected in dogs (0.5 mg/kg) in the gall-bladder bile 8 and 11 days later. Jones and Goslin[4] using a magneto-optic method on the blood, urine, liver, spleen, and kidney of a group of rabbits sacrificed at hourly intervals from 1-24 hours following the subcutaneous injection of 0.5 mg/kg of uranyl nitrate found no uranium in the bile, but an average of 20% in the liver and 23% in the kidney. About ⅓ of the uranium was eliminated in the urine in the first 24 hours. These workers noted a rapid disappearance from the blood in the first 6 hours even though the heavy metal was administered subcutaneously. Our results compare more favorably with those of Jones and Goslin, but indicate that in dogs receiving 10 times as much uranium intravenously as did the rabbits by the subcutaneous route, the heavy metal disappears more rapidly from the blood and about twice as much of the uranium is recovered in the urine in the first 24 hours.

We believe that our method possesses certain advantages over either of the 2 methods cited above. The method might be adopted

[3] Karsner, H. T., and Reimann, S. P., *J. Med. Res.*, 1918, **39**, 157.

[4] Jones, H. D., and Goslin, R., *Am. J. Physiol.*, 1933, **105**, 693.

by certain industrial concerns for periodically testing the urine of those exposed to radioactive compounds.

Even though these studies are incomplete a word or two about the immediate problem that attracted our interest to this method, viz., the protective influence of sodium citrate against uranium injury[2] might not be out of place. The 2 dogs which did not receive citrate (39-38 and 39-42) died on the 9th and 8th days respectively while the 2 dogs which did receive citrate injections (42-33 and 43-34) are both alive 12 months after the uranium injection. Both the rate of disappearance from the blood stream and the highest percentage recovery in the urine (88%) were in the dogs that received citrate (dogs 43-33 and 43-34). This might indicate that citrate brings about a more rapid elimination of the heavy metal, but if ⅔ of the heavy metal is eliminated in the first 24 hours without citrate it is difficult to see how citrate administered as late as 67 hours after uranium can be equally effective in protecting against the heavy metal (see next paper).

Summary. 1. A method is described whereby the alpha-particle radioactivity of uranium can be utilized to follow its course in the body. 2. Four hours after the intravenous injection of a minimal lethal dose of uranium nitrate (5.0 mg/kg) practically none of the injected heavy metal can be detected in the blood plasma. 3. Within the first 24 hours after the intravenous injection, approximately ⅔ of the uranium is eliminated in the urine. 4. Similar tracer studies have been applied to dogs protected by sodium citrate injections, but no satisfactory data have been obtained to explain the manner in which sodium citrate protects against a lethal dose of uranium nitrate.

14703

Studies on Heavy Metal Poisoning: II. Sodium Citrate Therapy Effective 67 Hours After Uranium Injury.*

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Last year we reported the survival in 13 of 14 dogs (92%) that had received intravenous injections of sodium citrate for 5 days before and 5 days after a lethal dose of uranium nitrate. Twelve of 13 control dogs (without citrate) succumbed to the same dose of heavy metal (5.0 mg/kg). One week after the injection of uranium the degree of azotemia, of acidosis, of alterations in the urine, and of histological change in the kidney in the two groups of dogs was similar.[1] Apparently something happened between the 5th and 8th day that determined the fate of the dogs.

The timing of the citrate injections in these experiments, *i.e.*, before and after the administration of uranium, was related to the fact that these studies grew out of other studies in which sodium citrate was used as an anticoagulant. Because of theoretical and practical considerations we have repeated these experiments but have given the citrate injections only *after* the uranium poisoning.

Methods. The methods were the same as those employed in the previous study[1] except that the daily intravenous citrate injections (0.33 cc/kg—approximately 0.23 g—of a saturated aqueous solution of tri-sodium citrate) were started at varying intervals *after* the subcutaneous injection of uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$, 0.5 mg/kg in 0.5% aqueous solution). Blood samples for NPN and CO_2 determinations and urine samples

* This investigation was aided by a grant from the John and Mary R. Markle Foundation.

1 Donnelly, G. L., and Holman, R. L., *J. Pharm. and Exp. Therap.*, 1942, **75**, 11.