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

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

TABLE I.
Effect of Sodium Citrate Injections on Survival in Dogs Given a Lethal Dose of Uranium Nitrate.

Group	First citrate inj. given	Citrate inj. continued for days	No. of dogs	No. surviving	% survival
I	Simultaneously	10	5	5	100
II	(a) After 17 hr	10	5	5	100
	(b) " 17 "	5	6	5	83
III	" 67 "	5	4	4	100
	Total		20	19	95

for PSP, protein, and microscopic analyses were collected on the 3rd, 7th, and 12th day after the injection of the heavy metal.

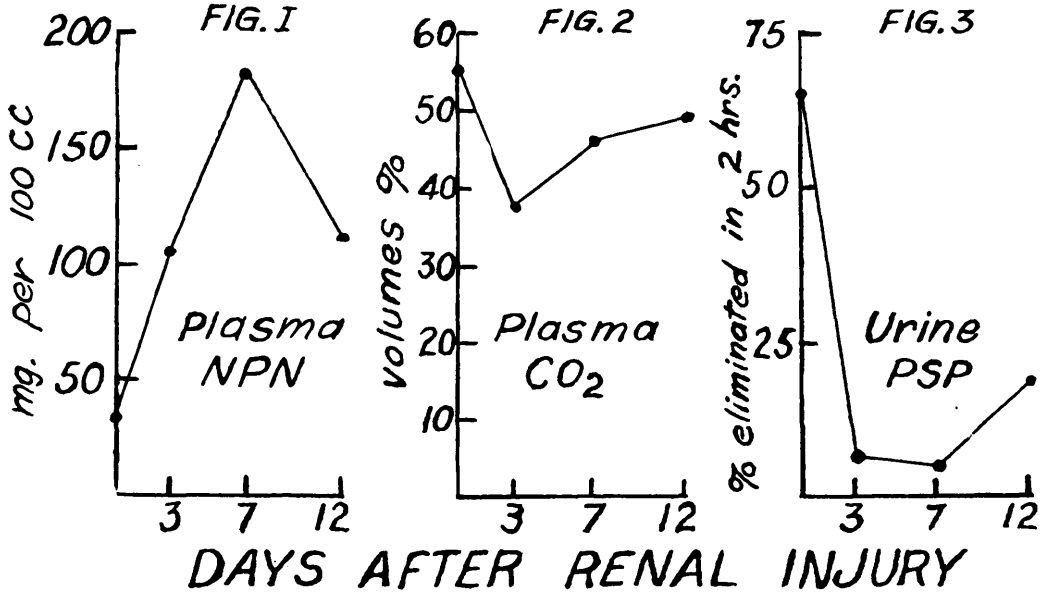
Experimental Observations. Table I gives the survival figures for the group of 20 dogs. Sodium citrate injections started as late as 67 hours after the administration of uranium and continued for only 5 days were as efficacious as 10 daily injections started immediately after the injection of the heavy metal. And the survival figure for the entire group, 95%, is the equivalent of that obtained in the previous series (with citrate injections before and after the uranium injections) in which 13 of 14 dogs (92%) survived.

In Fig. 1, 2, and 3 are presented the changes in NPN, CO_2, and PSP following the heavy metal injury. The points plotted represent the average for the entire group as obtained on the 3rd, 7th, and 12th days after the uranium injection and are practically identical with those obtained in the previous study except that the CO_2 combining power of the plasma did not fall quite as low and started returning toward normal sooner than in the previous study.

The clinical condition of the dogs remained good throughout. All reactions were averted by giving the citrate injections very slowly. No abnormalities in the clotting of blood were noted at any time. The single fatality (Group IIb) occurred on the 12th day.

Anatomical studies in this series of dogs were limited to biopsy and necropsy material

Effect of Sodium Citrate Injections on Blood Chemical and Urinary Excretion Studies in Dogs Given a Lethal Dose of Uranium Nitrate.



obtained from 3 weeks to 9 months after the renal injury. The kidneys of the dog that died on the 12th day showed considerable evidence of regeneration. By the 3rd week approximately ⅔ of the damaged tubules were lined by newly formed flattened epithelium. Anatomical material at later periods showed a progressive decrease in the amount of this "flattened epithelium," and most of the kidneys examined 9 months after the injection of uranium showed normal broad columnar epithelium lining the proximal convoluted tubules.

Discussion. The practical implications of these studies on acute uranium poisoning are obvious. We have not made any studies on chronic poisoning by uranium. The only report that we know of in which sodium citrate has been used to counteract heavy metal poisoning is that by Kety and Letonoff[2] in which sodium citrate gave beneficial results in human cases of lead poisoning. It is possible that some of the success usually attributed to plasma and indirect whole blood transfusions might be due to sodium citrate.

The theoretical considerations of these studies involve not only the method whereby sodium citrate protects against uranium ions but also the pharmacological action of the heavy metal itself.

There are several points that militate against the currently accepted view that sodium citrate exerts its protective action by preventing or combating the acidosis associated with uranium nitrate injury:

1. The alkali reserve as expressed by the CO_2 combining power of the plasma is not maintained by sodium citrate [Fig. 2 and (1, Fig. 2)].

2. The acidosis is the result of and not the cause of the renal injury.[3]

3. Sodium citrate does not prevent the curious coagulative necrosis of the proximal convoluted tubules of the kidney which classically follows uranium nitrate injury (1, Fig. 3-8).

4. If the results in the preceding paper in this series[4] are confirmed—namely, that approximately ⅔ of the uranium is eliminated in the urine in the first 24 hours—it is difficult to see how citrate administered 67 hours later can counteract the effects already produced by uranium.

Rather our data indicate that sodium citrate maintains one or more vital equilibria during the critical period (5th-8th day) when regeneration is taking place. This would indicate "replacement" or "supplemental" therapy which is directed at something more fundamental than the acid-base balance.

Possibly uranium damages a specific substance which is formed or concentrated in the cortex of the kidney. Indirect evidence pointing to a selective action by uranium is not lacking: (1) there is little or no reaction about the injection site in the subcutaneum, the obvious site of maximum concentration of uranium ions; (2) within 12 hours after subcutaneous injection there is functional evidence of renal injury (proteinuria) and at the end of 24 hours anatomical evidence of selective damage in the proximal convoluted tubules is demonstrable. Thus the lack of correlation between the concentration of uranium ions and the site of anatomic injury and between the time of maximal concentration at the site of damage (if ⅔ of the total amount injected is lost from the body in the first 24 hours) and the time of effective therapy (as late as 67 hours after the injection) could be used to support the selective affinity hypothesis.

This selective "toxic action" of uranium might logically occur by competition between uranium ions or electrons and those of another cation (perhaps a trace element) in an enzyme system with consequent inhibition of that system. The fact that uranium is hexa-valent makes this hypothesis attractive; and some of the other findings associated with uranium injury (glycosuria and increased susceptibility of pregnant animals[5]) could be put on a more logical basis if this or a similar hypothesis could be established.

[2] Kety, S. S., and Letonoff, T. V., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 476.

[3] Karsner, H. T., Reimann, S. P., and Brooks, S. C., *J. Med. Res.*, 1918, **39**, 177.

[4] Holman, R. L., and Douglas, W. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 72.

[5] MacNider, W. deB., *J. Phar. and Exp. Therap.*, 1929, **35**, 31.

Whether or not this hypothesis holds true, we believe that our data on sodium citrate protection against uranium injury point to the maintenance of one or more vital equilibria (possibly the "citric acid cycle" of carbohydrate metabolism[6]) while the normal processes of repair are taking place, for we have no evidence that repair is accelerated or altered in any way.

Summary. Nineteen of 20 adult dogs survived a lethal dose of uranium nitrate (5.0 mg/kg) when large doses of sodium citrate (0.23 g/kg/day for 5-10 days) were injected intravenously. The citrate injections were as effective when started as late as 67 hours after the heavy metal as when started immediately after the injection of uranium nitrate.

Objections are raised to the currently accepted view that maintenance of the alkali reserve is the mechanism whereby protection is afforded, and the suggestion is offered that sodium citrate supplies ions or molecules that keep the vital "citric acid cycle" of carbohydrate metabolism going while recovery takes place.

[6] Krebs, H. A., and Johnson, W. A., *Biochem. J.*, 1937, **31**, 645.

14704

Maternal Inheritance of Protoporphyrins of the Harderian Glands in Mice.

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Data indicating a parallelism between the amount of protoporphyrins in the Harderian glands of mice and intrinsic susceptibility to cancer of the mammary gland have been published.[1,2] Mice belonging to inbred strains with a high incidence of spontaneous mammary tumors have a very high concentration of protoporphyrins (deep red fluorescence under ultraviolet light); mice of strains with intermediate degrees of tumor susceptibility show an intermediate "pink" fluorescence; and mice of cancer-resistant strains exhibit very little or none of the fluorescence characteristic of protoporphyrins. Moreover, female mice of a cancer-susceptible strain (C_3H) have a higher concentration of protoporphyrins in their Harderian glands than males of the same strain and age.[3] *Only* female mice of a cancer-susceptible strain develop breast cancer spontaneously.

The problem of immediate interest is, can further studies on the determination of protoporphyrin concentration throw additional light (or reveal further parallelisms) on their level and the phenomena of susceptibility and resistance to spontaneous cancer? Among the most interesting (and perhaps the most important) observations in the analysis of the genetic influence on cancer susceptibility and resistance in mice is the finding that susceptibility to cancer of the breast in the F_1 individual is determined by the female parent (maternal inheritance).[4]

Chart I presents the data obtained on the red fluorescence (hence protoporphyrin concentration) in mice of the (a) C_3H strain (high or maximal protoporphyrin in the Harderian gland and high cancer susceptibility), (b) JK strain (minimal or low protoporphyrin and low cancer susceptibility), and (c) the 2 F_1 classes obtained by crossing mice of the parental stocks in both directions. The first F_1 generation was obtained by crossing a

* This experiment has been made possible by grants from The Anna Fuller Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

[1] Strong, L. C., and Figge, F. H. J., *Science*, 1941, **94**, 331.

[2] Figge, F. H. J., Strong, L. C., Strong, L. C., Jr., and Shanbrom, A., *Cancer Research*, 1942, **2**, 335.

[3] Strong, L. C., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 123.

[4] Little, C. C., and Staff Roscoe B. Jackson Memorial Laboratory, *Science*, 1933, **78**, 465.