A CONSIDERATION OF THE RELATIVE TOXICITY OF URANIUM NITRATE FOR ANIMALS OF DIFFERENT AGES. I.*

By WILLIAM deB. MacNIDER, M.D.

(From the Laboratory of Pharmacology of the University of North Carolina, Chapel Hill.)

PLATES 1 AND 2.

(Received for publication, January 31, 1917.)

The various ways in which the age of an organism expresses itself has received little consideration in the interpretation of many reactions that occur naturally and that are induced experimentally. The toxic effect of a substance experimentally introduced into an organism is usually interpreted either morphologically, by certain cell changes, or by some alteration in the functional capacity of an organ or a certain group of organs.

In a preliminary note[1] the observation was recorded that animals of different ages showed a variation in their response to the toxic effect of uranium nitrate when the poison was given subcutaneously in a constant quantity per kilo of body weight. The younger animals not only withstood the toxic effect of uranium for a longer period without developing an albuminuria and a glycosuria, but when these animals finally became both albuminuric and glycosuric, the quantitative output of these substances was much less in the younger than in the older animals.

In a more recent paper[2] the observation referred to has been confirmed in a second series of animals. A further observation has been made, that, judging by the time of appearance and the amount of acetone which occurred in the urine,

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] MacNider, W. deB., On the difference in the response of animals of different ages to a constant quantity of uranium nitrate, *Proc. Soc. Exp. Biol. and Med.*, 1913–14, xi, 159.

[2] MacNider, The inhibition of the toxicity of uranium nitrate by sodium carbonate, and the protection of the kidney acutely nephropathic from uranium from the toxic action of an anesthetic by sodium carbonate, *J. Exp. Med.*, 1916, xxiii, 171.

the older animals gave evidence of developing an acid intoxication much earlier than did the younger ones. In this paper it was also shown that a solution of sodium carbonate given intravenously would protect the kidney of an animal against the toxic effect of uranium, and that it was also possible to protect the kidney of a young animal acutely nephropathic from uranium against the toxic effect of an anesthetic by the use of a solution of sodium carbonate. The ability to furnish such a protection decreased as the age of the animal increased.

It is important to ascertain in a more detailed manner the way in which animals of different ages express their variation to the toxic effect of uranium. The present investigation is therefore primarily concerned with a study of acute uranium intoxications in animals of different ages as indicated by a disturbance in the metabolism of the animals, the severity of which shows a parallel with the age of the animal. During the course of such intoxications the animals become nephropathic. In these animals the kidney has been selected as an organ in which to study the functional and morphological variations which develop during the intoxication. The severity of these changes will be studied in relation to the age of the animal.

EXPERIMENTAL.

Dogs were employed in the experiments. The animals varied in age from pups of 8 months to dogs 8 years of age. The younger animals were raised in the laboratory kennels, while the older animals were obtained from people in the surrounding country who had raised the dogs and could vouch for their age within a few months.

The animals were placed in metabolism cages and given a liberal amount of bread with which was cooked a small amount of meat. The animals received 500 cc. of water daily by stomach tube. After a period of 3 days, which was allowed for normal observations, and during which time animals with a naturally acquired nephropathy or with glucose or acetone bodies in the urine could be excluded, the animals were given subcutaneously on 2 successive days 5 mg. of uranium nitrate per kilo of body weight. In all the animals the uranium intoxication was allowed to persist for 48 hours. At the end of this period the animals were either killed and autopsied, or the nephropathic animals were anesthetized and employed for cer-

tain functional studies which will be reported in Part II of this investigation.

The relative toxicity of uranium for these animals has been investigated by a study of the hydrogen ion content and alkali reserve of the blood, the tension of alveolar air carbon dioxide, and by the time of appearance and quantitative output of acetone and diacetic acid in the urine. The functional capacity of the kidney was ascertained by a study, at different periods during the intoxication, of the time of appearance and total output of phenolsulfonephthalein in the urine and by a study of the urea content of the blood.

The hydrogen ion determinations have been made by the indicator method recently devised by Levy, Rowntree, and Marriott.[3] The alkali reserve of the blood and the determinations of alveolar air carbon dioxide have been made by the methods of Marriott.[4, 5] The quantitative determinations of acetone and diacetic acid in the urine have been made by Folin's[6] method as modified by Hart.[7] The output of diacetic acid is expressed in terms of acetone. The blood urea determinations have been made by the method of Marshall,[8] following the modification suggested by Van Slyke and Cullen.[9] The phenolsulfonephthalein test for kidney function was conducted according to the method outlined by Rowntree and Geraghty.[10]

[3] Levy, R. L., Rowntree, L. G., and Marriott, W. McK., A simplified method for determining variations in the hydrogen ion content of the blood, *Arch. Int. Med.*, 1915, xvi, 389.

[4] Marriott, W. McK., A method for the determination of the alkali reserve of the blood plasma, *Arch. Int. Med.*, 1916, xvii, 840.

[5] Marriott, The determination of alveolar carbon dioxide tension by a simple method, *J. Am. Med. Assn.*, 1916, lxvi, 1594.

[6] Folin, O., On the separate determination of acetone and diacetic acid in diabetic urines, *J. Biol. Chem.*, 1907, iii, 177.

[7] Hart, T. S., On the quantitative determination of acetone in the urine, *J. Biol. Chem.*, 1908, iv, 477.

[8] Marshall, E. K., Jr., A rapid clinical method for the estimation of urea in urine, *J. Biol. Chem.*, 1913, xiv, 283.

[9] Van Slyke, D. D., and Cullen, G. E., A permanent preparation of urease, and its use in the determination of urea, *J. Biol. Chem.*, 1914, xix, 211.

[10] Rowntree, L. G., and Geraghty, J. T., An experimental and clinical study of the functional activity of the kidneys by means of phenolsulphonephthalein, *J. Pharm. and Exp. Therap.*, 1909–10, i, 579.

TABLE I.

Normal Animals.

No. of experiment.	Age.	Weight.	Water in 24 hrs.	Urine in 24 hrs.	Sulfonephthalein.		Blood urea.	P_H.	R. P_H.	Carbon dioxide tension.	Albumin, glucose, acetone, diacetic acid.
					Time of appearance.	Output in 2 hrs.					
	yrs.	*kg.*	*cc.*	*cc.*	*min.*	*per cent*	*per cent*			*mm.*	
1	8 mos.	15.9	500	769	5	68	0.012	7.45	8.0	40	0
2	8 "	10.5	500	509	8	71	0.012	7.4	8.1	40	0
3	1	15.81	500	660			0.015	7.45	8.1	37	0
4	1	19.0	500	960	5	67	0.015	7.4	8.1	43	0
5	2	14.68	500	630			0.015	7.45	8.05	43	0
6	3+	17.01	500	670	5	67	0.015	7.45	8.0	44	0
7	3+	9.8	500	762	4½	67	0.015	7.4	8.0	38	0
8	4+	13.15	500	670			0.015	7.45	8.0	39	0
9	5+	13.3	500	465	4	66	0.012	7.45	8.05	40	0
10	5+	18.2	500	520			0.020	7.45	8.0	40	0
11	8	10.6	500	640	5	73	0.016	7.45	8.0	40	0
12	8+	8.53	500	430			0.015	7.45	8.0	39	0

TABLE II.

Nephropathic Animals after 24 Hours.

No. of experiment.	Age.	Uranium nitrate per kilo.	Urine, 1st 24 hrs.	Sulfonephthalein.		Blood urea.	Acetone per 100 cc.	Diacetic acid per 100 cc.	P_H.	R. P_H.	Carbon dioxide tension.
				Time of appearance.	Output in 2 hrs.						
	yrs.	*mg.*	*cc.*	*min.*	*per cent*	*per cent*	*mg.*	*mg.*			*mm.*
1	8 mos.	5	483	4	64	0.012	0	0	7.45	8.0	38
2	8 "	5	704	5	66	0.012	0	0	7.4	8.1	35
3	1	5	995			0.015	0	0	7.45	8.1	35
4	1	5	745	5	64	0.015	0	0	7.45	8.1	43
5	2	5	459			0.015	0	0	7.45	8.05	35
6	3+	5	1,150	7	54	0.016	0	0	7.35	8.0	40
7	3+	5	435	3½	51	0.015	0	0	7.4	8.0	35
8	4+	5	360			0.015	0	0	7.3	7.95	34
9	5+	5	1,060	8	31	0.015	0	0	7.4	7.95	36
10	5+	5	1,300			0.015	0	0	7.4	7.9	36
11	8	5	645	10	17	0.016	2.6109	3.4812	7.4	7.9	37
12	8+	5	520			0.015	2.6592	2.7559	7.25	7.9	34

TABLE III.

Nephropathic Animals after 48 Hours.

No. of experiment.	Age.	Uranium nitrate per kilo.	Urine, 2nd 24 hrs.	Sulfonephthalein.		Blood urea.	Acetone per 100 cc.	Diacetic acid per 100 cc.	P_H.	R. P_H.	Carbon dioxide tension.
				Time of appearance.	Output in 2 hrs.						
	yrs.	*mg.*	*cc.*	*min.*	*per cent*	*per cent*	*mg.*	*mg.*			*mm.*
1	8 mos.	5	769	5	20	0.012	2.9493	2.2756	7.4	8.0	37
2	8 "	5	509	7	18	0.012	1.3054	4.0614	7.4	8.0	35
3	1	5	660			0.020	3.8680	2.3208	7.4	8.0	35
4	1	5	960	11	21	0.020	2.3691	4.3015	7.35	8.0	39
5	2	5	630			0.020	2.8043	4.1099	7.35	7.9	30
6	3+	5	670	10	24	0.020	1.4010	4.2064	7.35	7.9	35
7	3+	5	762	21	4	0.042	2.3045	4.1092	7.3	7.85	31
8	4+	5	595			0.026	3.7862	4.6416	7.3	7.85	32
9	5+	5	465	20	8	0.022	3.3845	9.6700	7.4	7.9	35
10	5+	5	520			0.022	0.3703	2.2241	7.3	7.85	34
11	8	5	640	20	4	0.030	4.9800	2.2241	7.25	7.85	34
12	8+	5	430			0.030	2.6592	7.3975	7.2	7.8	30

Tables I, II, and III give the normal findings in these animals of different ages for 1 normal day and for the 2 subsequent days of the uranium intoxication. The tables show the results obtained in twelve of the animals which have been selected according to their age as representative of the total number of animals employed in the experiments.

Observations on Normal Animals of Different Ages.

All the animals during the period of 3 days allowed for normal observations were freely diuretic. The total output of urine for the last day of observation varied in the respective animals from a minimum of 430 cc. to a maximum output of 960 cc. The urine was free from albumin, acetone bodies, and glucose, and did not contain casts.

Table I shows that in conducting the sulfonephthalein test for kidney function, the appearance of the dye in the urine varied slightly in the different animals. The earliest appearance was 4 minutes following the injection, while in one animal the appearance of the

dye was delayed for 8 minutes. The total output of the dye in a 2 hour period varied between a minimum of 66 per cent to a maximum output of 73 per cent. These variations have apparently no connection with the age of the animal. The highest output of the dye in this series of animals occurred in an animal 8 years old (Experiment 11, Table I).

The percentage of blood urea has been very constant for animals of all ages. In the normal dog the urea content per 100 cc. of blood has varied between 0.012 to 0.020 per cent.

The hydrogen ion determinations have been made from the oxalated whole blood. In such determinations the hydrogen ion concentration expresses both the volatile and non-volatile acid content of the blood. Recently Marriott[4] has shown that frequently such readings are misleading, and that a more accurate conception of the changes in the hydrogen ion concentration of the blood may be obtained by removing the carbon dioxide from the dialysate, in this way obtaining a reading which represents any change in hydrogen ion concentration which may be due to non-volatile acids. Marriott refers to such a reading as the reserve alkali content of the blood (R. P_H).

In the normal animals of all ages the hydrogen ion concentration of the whole blood (P_H) has varied between 7.3 to 7.45, while the alkali reserve of the blood (R. P_H) has varied between 8.0 to 8.1.

The determinations of the tension of alveolar air carbon dioxide have shown a variation within the limits of normality, 37 to 44 mm. The Marriott method for such determinations was employed in seventy-one dogs and has given remarkably constant results.

From the observations which have been made on the normal animals we may conclude that the animals of different ages show no appreciable difference in their ability to eliminate sulfonephthalein, that the blood urea content in these animals is very constant, and that the animals even though varying much in age show naturally no tendency towards an acid intoxication.

Observations on Animals of Different Ages Intoxicated by Uranium Nitrate.

In the following study of the relative toxicity of uranium, it first became necessary to ascertain whether the weight of the animal, and therefore the total amount of the poison introduced, had any effect in determining the toxicity of the substance. By referring to Tables II and III it will be seen that this factor apparently does not influence the toxic response on the part of the animals. For instance the pup of Experiment 1, with a weight of 15.9 kilos, showed a delayed and slight toxic effect from uranium, while the animal of Experiment 12, 8 years old, and with a weight of only 8.53 kilos, showed clearly the toxic action of uranium during the first 24 hours following the initial injection, and by the end of the second 24 hour period was severely intoxicated. This type of observation has remained constant in all the animals employed in this study.

Following the first injection of uranium all the animals remained freely diuretic. In several of the animals (Experiments 6, 9, and 10) the output of urine was greatly increased.

A study of the sulfonephthalein output by the animals of different ages shows the following variations: There is only a slight change in the time of the appearance of the dye in the urine as compared with the normal animals. The delay in the time of appearance has been most marked in the older animals, while in the youngest animals the time of appearance has either been increased over the normal or remained unchanged.

In the animals of all ages the total elimination of sulfonephthalein in a 2 hour period is reduced. The reduction is slight in the young animals and marked in the old animals. In Experiment 1, a pup 8 months old, the total output of the dye was only reduced from the normal of 68 to 64 per cent. In Experiment 11, an animal 8 years old, a reduction occurred from the normal of 73 to 17 per cent.

Following the second injection of uranium there is a continuation of the relatively greater toxic effect of this poison for the kidneys of the older animals. The youngest animals which have been recorded in Tables II and III were 8 months old and from the same litter. These animals at the end of the 48 hour period of intoxication by

uranium had a sulfonephthalein output of 20 and 18 per cent respectively. The two oldest animals in which sulfonephthalein determinations were made show an output of the dye of 8 and 4 per cent.

The toxic effect of uranium for the kidney as shown by the reduction in the output of sulfonephthalein increases with the age of the animals.

The determinations of blood urea in the pathological as compared with the normal animals enable observations to be made concerning the degree of urea retention in the animals of different ages and also permit a study of the relation between the output of sulfonephthalein by the kidney and the amount of urea retained.

Following the first injection of uranium the percentage of blood urea remained practically constant in all the animals. There was no evidence of a retention of urea even though the output of sulfonephthalein had been greatly reduced. In Experiment 7, Table II, the sulfonephthalein output was reduced to 51 per cent while the percentage of blood urea remained constant. In Experiment 11, the sulfonephthalein output was reduced following the first injection of uranium from 73 to 17 per cent. The percentage of blood urea was uninfluenced by this degree of kidney injury and remained at the normal reading of 0.016 per cent.

Following the second injection of uranium a retention of blood urea was found to occur in all the animals over 8 months old. The animals which show the greatest reduction in the output of sulfonephthalein also show the highest retention of blood urea. The tables of experiments furthermore show that the decrease in the functional capacity of the kidney as indicated by a retention of blood urea increases with the age of the animal.

From these observations it would appear that as compared with the sulfonephthalein test for kidney function, the retention of blood urea is a much later manifestation of kidney inefficiency. When, however, the kidney shows serious impairment of function as indicated by the sulfonephthalein test, there occurs a retention of blood urea which shows a parallel with this test for renal function.

The degree to which the kidney may be impaired and yet show no evidence of a retention of blood urea is illustrated by the first two experiments of Table III. The output of sulfonephthalein by these

two young animals was 20 and 18 per cent, respectively, and yet the percentage of blood urea showed no variation from the normal.

In a previous paper[2] on uranium intoxications in animals of different age it was shown that the time of the appearance of acetone bodies in the urine and the relative amount of these bodies increased with the age of the animal. This observation was interpreted as indicating the development of an acid intoxication, and furthermore to furnish ground for the belief that such an intoxication was more readily induced by uranium in an old animal than in a young animal. This interpretation was strengthened by a series of experiments in which it was found possible to protect an animal against the toxic effect of uranium by the use of an alkaline solution intravenously. The degree of protection conferred by such injections was largely dependent upon the age of the animal. Young animals were more readily protected than were old animals.

Howland and Marriott[11] have recently called attention to the fact that the presence of acetone bodies in the urine is in itself insufficient evidence of a tissue acidosis. It is furthermore well known that in conditions of tissue acidosis the output of these bodies in the urine may be reduced as a result of a decrease in the functional capacity of the kidney. It has therefore seemed advisable in this series of animals to ascertain whether there was any evidence of an acid intoxication other than that shown by the appearance of acetone bodies in the urine. With this object in view determinations of the hydrogen ion content of the blood, the alkali reserve of the blood, and the carbon dioxide tension of alveolar air have been made to determine first, if by these methods any evidence can be obtained of a tissue acidosis, second, if the degree of acid intoxication shows any parallel with the quantitative output of acetone bodies in the urine, and, finally, if the degree of intoxication as indicated by these different methods shows any variation with the age of the animal.

Reference to Table II shows that following the first injection of uranium none of the animals under 8 years of age had a urine which contained acetone or diacetic acid. However, the two oldest ani-

[11] Howland, J., and Marriott, W. McK., A discussion of acidosis. With special reference to that occurring in diseases of children, *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 63.

mals of the series, Experiments 11 and 12, one 8 years old and the other 8 years and a few months old, showed both acetone and diacetic acid in the urine at this early period of the uranium intoxication. By referring to Table II it will also be observed that the appearance of acetone and diacetic acid in the urine of the two oldest animals coincides with the development of other indications of an acid intoxication. These animals also show an increase in the hydrogen ion concentration of the blood, a reduction in the alkali reserve of the blood, and a decrease in the tension of alveolar air carbon dioxide.

A study of the animals of Experiments 8, 9, and 10, which have varied in ages between $4\frac{1}{2}$ and 5 years and 2 months, shows that an acid intoxication may exist without the appearance of acetone bodies in the urine. All these animals had a urine which was free from both acetone and diacetic acid and yet all three of the animals gave other evidence of a beginning acid intoxication. It would therefore seem that the appearance of acetone and diacetic acid in the urine may indicate a beginning acid intoxication from uranium. On the other hand, the absence of these bodies from the urine does not exclude a tissue acidosis, for when other tests are employed such a state may be found to exist.

During the second 24 hours of the uranium intoxication the animals of all ages showed the presence of acetone and diacetic acid in the urine. In so far as the development of an acid intoxication can be determined by the presence of these substances in the urine, at this stage of the intoxication the tendency of the animals to develop an acidosis has extended so as to include not only the old animals but the animals of all ages.

The quantitative output of acetone and diacetic acid shows no constant increase with the increasing age of the animal. In general the combined acetone and diacetic acid output is greater in an old animal than in a young animal, but throughout the series of experiments numerous instances have been observed in which there is no true correlation between the age of the animal and the acetone and diacetic acid content of the urine. For example, in Experiment 2, Table III, in a pup 8 months old, the urine, following the second injection of uranium, contained 1.3054 mg. of acetone and 4.0614

mg. of diacetic acid in terms of acetone per 100 cc. of urine. In Experiment 10, in an animal 5 years and 2 months old, the output of acetone was only 0.3703 mg. per 100 cc. of urine, and the output of diacetic acid which was 2.2241 mg. per 100 cc. was but slightly over half the quantity found in the urine of the young animal.

A study of the relation between the quantitative output of acetone bodies in the urine and the changes in blood and alveolar air shows that when these bodies appear in the urine changes also occur in the blood and alveolar air indicative of a beginning acid intoxication.

The experiments also show that at this stage of the intoxication there is no correlation between the total output of acetone bodies and the other indications of the development of a tissue acidosis. For instance, in the animal of Experiment 10, at the end of the uranium intoxication the hydrogen ion content of the blood had been increased to 7.3, the alkali reserve of the blood was reduced to 7.85, and the tension of carbon dioxide in alveolar air gave a reading of 34 mm. The urine, however, showed a remarkably low output of both acetone and diacetic acid, acetone 0.3703 mg. and diacetic acid 2.2241 mg. per 100 cc. of urine.

From this review of the output of acetone and diacetic acid by animals of different ages intoxicated by uranium, the following conclusions may be drawn. The oldest animals show the toxic effect from uranium by the appearance of these bodies in the urine 24 hours before they appear in the urine of the younger animals. When animals of any age show the presence of these substances in the urine, they also show other evidence of a beginning acid intoxication.

The acid intoxication which develops from uranium cannot be solely ascribed to the formation of acetone bodies. These substances may fail to appear in the urine when there is other evidence of a beginning acid intoxication, and when as shown by the sulfonephthalein test the kidney has not become functionally inactive to such a degree as to cause a retention of these bodies. We must therefore conclude that the acid intoxication which develops from uranium is certainly dependent in part upon the formation or retention of acids other than those of the acetone series. Finally, it has been shown that the quantitative output of acetone and diacetic acid bears no constant relation to the age of the animal, and also that there is no

definite correlation between the quantitative output of acetone bodies and the degree of acid intoxication which may be demonstrated by other tests which have been employed for this purpose.

The variation in the toxicity of uranium for animals of different ages is more clearly expressed in changes in the acid-base equilibrium of the blood than by any other response which has been induced during the course of the intoxication.

Reference to Table II shows that following the first injection of uranium the animals under 4 years of age maintain a hydrogen ion content of the blood which varies only slightly from the normal. None of these animals have shown any change in the alkali reserve of the blood. In contrast with this indication of stability on the part of the animals under 4 years of age, all the animals over 4 years old have not only shown an increase in the hydrogen ion content of the blood but the alkali reserve of the blood has constantly shown a depletion. In these older animals in which the alkali reserve has been reduced, the tension of carbon dioxide in alveolar air has varied between 37 to 34 mm. In the younger animals in which the alkali reserve has remained unaffected the tension of carbon dioxide has varied between 43 and 35 mm.

The relative degree of acid intoxication induced in the animals of different ages is even more clearly shown following the second injection of uranium. By this stage of the intoxication, all the animals over 1 year of age not only show an increase in the hydrogen ion content of the blood, but the alkali reserve of the blood has been severely drawn upon. The younger animals, those under 1 year of age, have either shown no change in the hydrogen ion content of the blood, or in the few experiments in which these readings have varied, the hydrogen ion content has not been increased above 7.4, a point which may be considered within the limit of normal variations. In these younger animals the alkali reserve of the blood has either remained unaffected, or it has not been reduced below 8.0.

A study of Tables II and III not only indicates the differences in the hydrogen ion content and alkali reserve of the blood, which, as has been pointed out, exist between the animals of two age limits, those under 1 year of age and those over 1 year of age, but it also serves to demonstrate that as the animal increases in age there is a pro-

gressive increase in the degree of acidosis. For instance, at the end of the 2nd day of the uranium intoxication the animal of Experiment 6, 3 years and 2 months old, had a hydrogen ion concentration of 7.35 and an alkali reserve of 7.9, while the animal of Experiment 12, 8 years old, had a hydrogen ion content of 7.2 and an alkali reserve of 7.8.

The determinations of carbon dioxide tension in alveolar air of the animals of different ages at the end of the 2nd day of the uranium intoxication show that the older animals that have developed a reduction in the alkali reserve of the blood have an alveolar air carbon dioxide tension which varies between 35 to 30 mm. The younger animals in which no change has taken place in the alkali reserve, or in which the alkali reserve has not been reduced below 8.0, show a tension of carbon dioxide which varies between 39 to 35 mm.

From the observations the conclusion appears clear that one of the constant manifestations of the toxic effect of uranium is the development of an acid intoxication, and that the severity of this intoxication is associated with the age of the animal. The older animals develop a severer intoxication than do the younger animals.

The Pathology of the Kidney in Animals of Different Ages Intoxicated by Uranium.

At the end of the 2nd day of the uranium intoxication, twelve of the animals which have been employed in the experiments were killed and kidney tissue was at once fixed in 10 per cent formalin, Zenker's fluid, and in mercuric chloride-acetic acid to be used in the histological study. The animals from which tissue was obtained varied in age between 8 months, and 7 years and 4 months.

In the foregoing discussion of the relative toxicity of uranium in animals of different ages it has been shown that the toxicity of uranium increases with the age of the animal, and furthermore that the degree of toxicity of this substance is associated with the severity of the acid intoxication which develops in these animals. The following histological study has been undertaken with the object of ascertaining whether there is any pathological difference in the kidneys of animals which have shown very mild grades as contrasted

with severe grades of acid intoxication. The functional tests that have been employed have shown a correlation between the degree of acid intoxication and the ability of the kidney to eliminate sulfonephthalein and urea.

In the histological study it has not been found possible to differentiate between kidneys which were obtained from animals near the same age. In kidneys which were obtained from animals that varied 3 years or more in age there has been found such a difference in the pathological response of the organs as to permit a classification of the kidneys into two groups. The first group is represented by kidneys of animals not over 1 year of age and the second group by kidneys of animals that were over 3 years of age.

The kidneys of the younger animals show no evidence of damage to the vascular tissue. The glomerular capillaries are usually well filled with blood. No exudate has been observed in the subcapsular space or between the tubules. The connective tissue of the kidney has not been edematous. The endothelial nuclei of the glomerular vessels and of the cells lining the capsule of the glomerulus stain normally and show no evidence of degeneration or of proliferation.

The epithelium of the tubules, and especially of the convoluted tubules, shows a definite shrinkage. The lumen of the tubules is prominent and usually free from albuminous material. The nuclei of these cells are large in proportion to the surrounding cytoplasm and stain intensely (Fig. 1).

No stainable fat has been observed in the convoluted tubules. In frozen sections of the kidney stained for fat by Herxheimer's modification of the Scharlach R stain, there has been found in the ascending and descending limb of Henle's loop a very small amount of fat which appears as dust-like particles.

The pathology of the kidney of the older group of animals shows a similarity with the younger group in that no demonstrable changes have taken place in the vascular element of the kidney. The epithelium of the tubules shows the following changes which serve to separate the kidneys of the animals of different ages into the two groups.

The cells of the convoluted tubules show an increase in volume which is variable. In some of the animals the swelling has been marked, while in other animals this change has taken place to a

much less extent. Associated with the swelling of the cells the cytoplasm becomes distinctly granular and occasionally shows vacuolation. The free border of the cells appears ragged. The nuclei are decreased in size and stain less intensely (Fig. 2).

In such cells, unless the animal is very old, no stainable fat has been demonstrated. However, in the loops of Henle the amount of stainable fat has greatly increased over that which has been observed in the younger group of animals. The fat appears as large droplets which frequently coalesce. The difference in the amount of stainable fat in the kidneys of these two groups of animals of different ages is the most constant and striking histological variation.

When these differences in the pathology of the kidney are compared with the variations in the degree of acid intoxication and the alterations in the functional capacity of the kidney that are shown by the two groups of animals, the following differences are found to exist.

The kidneys of the younger group of animals in which the epithelium is histologically well preserved except for the appearance of a slight amount of fat in the tubules of Henle show only slight evidence of a beginning tissue acidosis and the functional capacity of the kidney is much less impaired than is the case with the second group of older animals.

The kidneys of the older animals which give histological evidence of a beginning degeneration of the convoluted tubules and which have shown a marked accumulation of fat in the tubules of Henle, show a much severer grade of acid intoxication than the younger animals, and also show that the functional capacity of the kidney has been severely impaired.

In the older group of animals there is an association between the degree of epithelial injury and the amount of fat in the kidney, with the severity of the acid intoxication, and the extent to which the functional capacity of the kidney has been affected.

As a result of this observation the question naturally arises: Does the kidney injury develop primarily, and the tissue acidosis result from a retention of acid bodies which the kidney is unable to remove, or is the kidney injury secondary to, and dependent upon the acid intoxication, resulting from the administration of uranium?

This question has been in a measure answered in the communication[2] previously referred to. If young animals that are being intoxicated by uranium are given a solution of sodium carbonate intravenously they fail to develop an acid intoxication and the renal epithelium either shows no evidence of degeneration, or the degenerative changes are slight when compared with the epithelial damage which occurs in animals unprotected by the use of an alkali.

CONCLUSIONS.

1. The toxic effect of uranium when given in a constant quantity per kilo of body weight is variable. This variation has been constantly associated with differences in the age of the animals. Uranium is more toxic for an old animal than for a young animal. The establishment of this fact, namely, that the age of an animal may modify the toxicity of a substance, should be taken into account in establishing by animal experiment the degree of activity of substances which are to be used for therapeutic purposes.

2. The toxic effect of uranium nitrate is constantly associated with its ability to induce a tissue acidosis. A severer grade of acidosis is induced in an old animal from uranium than is induced in a young animal.

It would appear that in the response of dogs of different ages to uranium the animals represent a reaction system to this substance which shows an increasing susceptibility as the animal advances from youth to senility.

Insufficient experimental data are as yet available to allow a discussion of the mechanism by which such an acid intoxication is produced.

3. The toxic effect of uranium is manifested locally by certain degenerative changes in the kidney. These changes are more marked in the kidney of an old animal than they are in the kidney of a young animal.

Associated with the severer kidney changes which are especially characterized by a beginning swelling of the renal epithelium and by an accumulation of stainable fat in these cells is the development of a severe grade of tissue acidosis.

4. The functional capacity of the kidney shows a parallel with the degree of acid intoxication and with the severity of the histological changes which have developed in the renal epithelium.

EXPLANATION OF PLATES.

PLATE 1.

FIG. 1. Camera lucida drawing, Leitz oc. 1, obj. 6. The figure is from the kidney of the young dog of Experiment 3. The glomerular vessels are well filled with blood. The epithelium of the convoluted tubules, *a*, shows a distinct shrinkage; the nuclei are large and stain intensely. The lumen of the tubules is prominent. At *b* are shown tubules in which the epithelium is beginning to show an early swelling. The animal gave little evidence of an acid intoxication.

PLATE 2.

FIG. 2. Camera lucida drawing, Leitz oc. 1, obj. 6. The figure is from the old animal of Experiment 11. The glomerulus shows no degenerative changes. The epithelium of the convoluted tubules, *a*, appears granular, stains less intensely than the epithelium of the younger animal of Fig. 1, and the nuclei are small and hypochromatic. The free borders of the cells are ragged. At *b* are shown tubules in which the cells are swollen. At *c* the epithelium shows vacuolation. The animal had developed a severe acid intoxication.

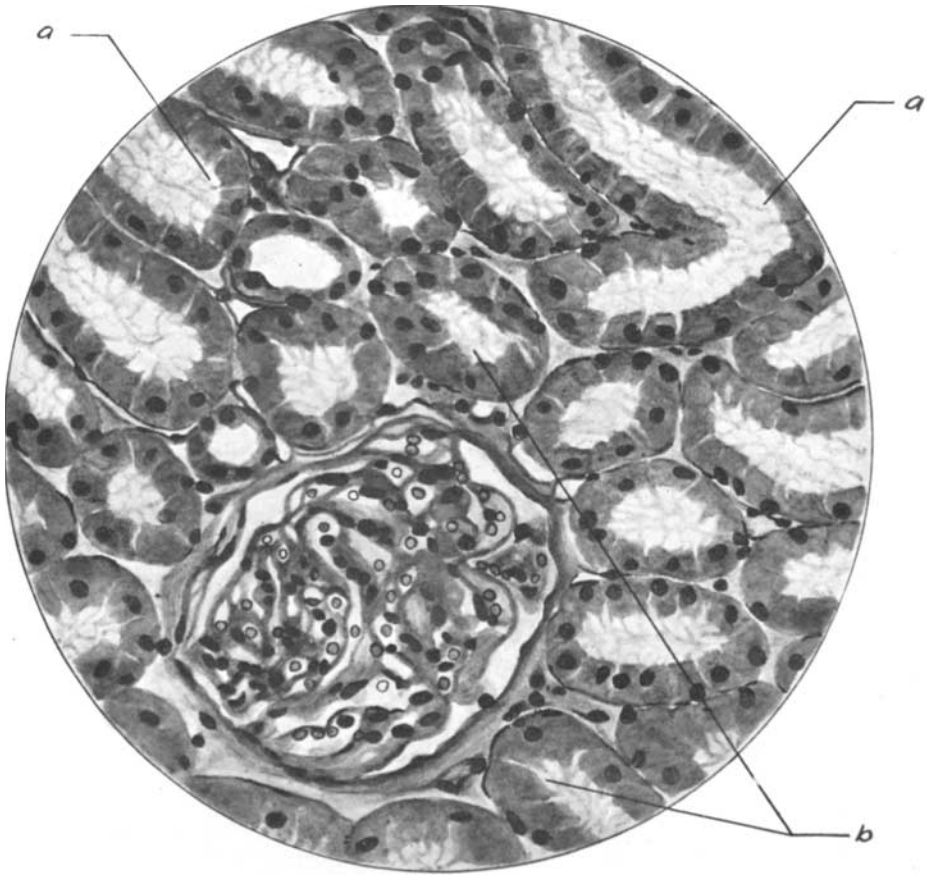

FIG. 1.

(MacNider: Toxicity of uranium. I.)

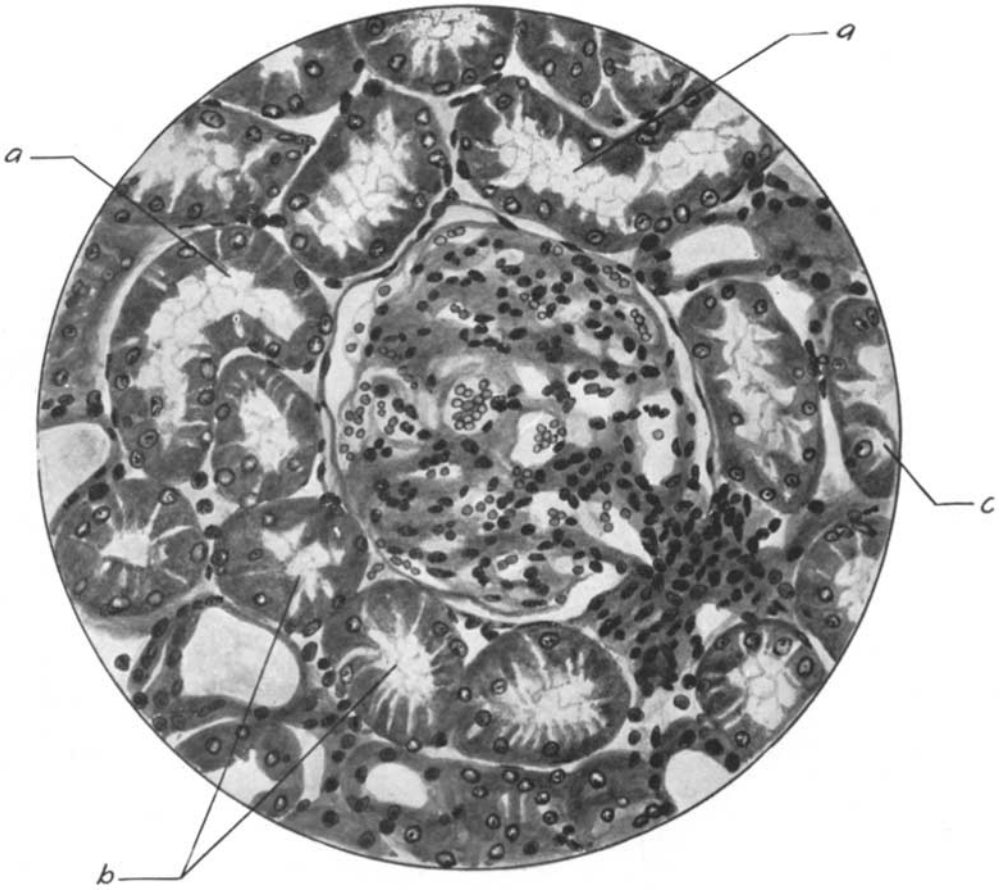

FIG. 2.