THE INFLUENCE OF DIET UPON NECROSIS CAUSED BY HEPATIC AND RENAL POISONS.

PART II. DIET AND THE NEPHRITIS CAUSED BY POTASSIUM CHROMATE, URANIUM NITRATE, OR CHLOROFORM.*

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Since the experiments which have been recorded demonstrate that lesions of the liver produced by chloroform and phosphorus may be profoundly influenced by diet, experiments have been performed to determine if substances which produce necrosis of the parenchymatous cells of the kidney exhibit similar relations to diet. Potassium chromate and uranium nitrate have been chosen because they have a selective action on the kidney causing necrosis of the renal tubules and leaving the parenchymatous cells of the liver and other organs relatively unaffected. These substances have the further advantage that they have been much used to produce acute nephritis in animals. They cause albuminuria, and in the white rat casts are very numerous within the tubules of the kidney.

THE EFFECT OF DIET UPON THE KIDNEY.

White rats were given during five and eight days (a) oats and sugar, (b) meat alone, or (c) fat alone, and the amount of visible fat within the kidney was determined by staining with Sudan III. In all kidneys examined the stain gives to the tubules of the cortex and of the subcortical zone containing the loops of Henle a pinkish color, but in animals which have received the carbohydrate diet fat droplets are almost wholly absent. Groups of fine, brightly stained fat droplets were occasionally found in a convoluted tubule. In animals which received meat fat was somewhat more abundant. The kidney of one animal which had received meat

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contained a moderate amount of visible fat present in tubules within the medullary striæ, in convoluted tubules situated in the part of the cortex in contact with the medulla, and occasionally in the loops of Henle extending into the medulla. This animal had bronchopneumonia with multiple abscesses in the lungs. The kidneys of animals which had received a diet of fat contained fat in fair abundance. Many of the tubules within the medullary rays of the cortex and many of the loops of Henle in the medulla immediately below the cortex contained conspicuously stained coarse fat droplets so abundant that the affected tubules were sharply outlined. In the convoluted tubules there was little fat save in the lowermost part of the cortex; here groups of tubules contain fat droplets closely crowded together. The distribution of fat indicates that the fatty diet may cause accumulation of fat in the cells of the loops of Henle.

POTASSIUM CHROMATE.

Potassium chromate in 2.5 per cent. aqueous solution has been injected subcutaneously in quantities varying from 0.05 to 0.3 of a cubic centimeter (0.00125 to 0.0075 of a gram). In the following series of experiments animals were given during nineteen days diets consisting of (a) oats and sugar, (b) pig's heart, and (c) beef fat. The special diets were continued after administration of potassium chromate (table I).

In the following series of experiments diets of meat and fat administered during an unusually long period have been accompanied by considerable loss of weight (for meat an average of 22 grams,

TABLE I.

Potassium Chromate.

Diet.	Weight before diet.	Weight after 19 dys.	Dose per 100 gm.	Length of life.	Remarks.
Oats and sugar	125 gm.	143 gm.	0.1 c.c.	*	
	158 gm.	148 gm.	0.15 c.c.	5 dys.	Necrosis $\frac{2}{3}$ convoluted tubules; calcification.
	148 gm.	152 gm.	0.2 c.c.	8 dys.	Necrosis $\frac{1}{2}$ convoluted tubules; calcification.
	154 gm.	155 gm.	0.25 c.c.	1 dy.	Beginning necrosis of convoluted tubules.
	155 gm.	172 gm.	0.3 c.c.	3 dys.	Necrosis $\frac{1}{3}$ convoluted tubules.

Average length of survival with carbohydrate diet.....$4\frac{1}{4}$+dys.

TABLE I.—*Concluded.*

Diet.	Weight before diet.	Weight after 19 dys.	Dose per 100 gm.	Length of Life.	Remarks.
Meat	125 gm.	100 gm.	0.1 c.c.	3 dys.	Casts in kidney.
	155 gm.	128 gm.	0.15 c.c.	3 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	157 gm.	132 gm.	0.2 c.c.	1 dy.	Casts in kidney.
	153 gm.	134 gm.	0.25 c.c.	2 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	160 gm.	142 gm.	0.3 c.c.	1 dy.	Beginning necrosis of convoluted tubules.
	Average length of survival with meat diet.....2 dys.				
Fat	145 gm.	109 gm.	0.1 c.c.	6 dys.	Necrosis $\frac{1}{2}$ convoluted tubules; calcification.
	135 gm.	110 gm.	0.15 c.c.	3 dys.	Necrosis $\frac{3}{4}$ convoluted tubules.
	151 gm.	121 gm.	0.2 c.c.	3 dys.	Necrosis $\frac{2}{3}$ convoluted tubules.
	155 gm.	127 gm.	0.25 c.c.	2 dys.	Beginning necrosis of convoluted tubules.
	160 gm.	133 gm.	0.3 c.c.	1 dy.	No lesion found.
	Average length of survival with fat diet.....3 dys.				

* = lived.

TABLE II.

Potassium Chromate.

Diet.	Weight before diet.	Weight after 6 dys.	Dose per 100 gm.	Length of life.	Remarks.
Oats and sugar	72 gm.	77 gm.	0.05 c.c.	*	
	67 gm.	77 gm.	0.075 c.c.	4 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	87 gm.	100 gm.	0.1 c.c.	3 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	200 gm.	205 gm.	0.125 c.c.	5 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	200 gm.	205 gm.	0.15 c.c.	5 dys.	Necrosis $\frac{2}{3}$ convoluted tubules.
	Average length of survival with carbohydrate diet.....$4\frac{1}{4}$+dys.				
Meat and oats	67 gm.	80 gm.	0.05 c.c.	*	
	85 gm.	97 gm.	0.075 c.c.	3 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	80 gm.	108 gm.	0.1 c.c.	4 dys.	Necrosis $\frac{2}{3}$ convoluted tubules; calcification.
	198 gm.	102 gm.	0.125 c.c.	3 dys.	Necrosis $\frac{3}{4}$ convoluted tubules.
	262 gm.	270 gm.	0.15 c.c.	3 dys.	Necrosis $\frac{3}{4}$ convoluted tubules.
	Average length of survival with meat diet.....$3\frac{1}{4}$+dys.				
Fat and oats	65 gm.	60 gm.	0.05 c.c.	*	
	70 gm.	75 gm.	0.075 c.c.	3 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	120 gm.	125 gm.	0.1 c.c.	3 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	155 gm.	150 gm.	0.125 c.c.	3 dys.	Necrosis $\frac{2}{3}$ convoluted tubules.
	275 gm.	275 gm.	0.15 c.c.	5 dys.	Necrosis $\frac{2}{3}$ convoluted tubules.
	Average length of survival with fat diet.....$3\frac{1}{2}$+dys.				

* = lived.

for fat, 27.2 grams), whereas animals which received oats and sugar gained in weight (an average of 6 grams). To avoid the disturbance of nutrition produced by prolonged feeding with meat or fat, animals have been fed in the preceding series of experiments (table II) during six days with (a) oats and cane sugar, (b) beefsteak and rolled oats, and (c) fat inseparably mixed with rolled oats. Animals which received oats and sugar and those which received meat and oats gained in weight; those with oats and fat maintained their weight.

TABLE III.

Potassium Chromate.

Diet.	Weight before diet.	Weight after diet.	Dose per 100 gm.	Length of life.	Remarks.
Oats and sugar	71 gm.	67 gm.	0.05 c.c.	*	
	75 gm.	68 gm.	0.1 c.c.	*	
	80 gm.	72 gm.	0.15 c.c.	*	
	88 gm.	82 gm.	0.2 c.c.	*	
	94 gm.	85 gm.	0.25 c.c.	5 dys.	Necrosis $\frac{1}{3}$ convoluted tubules.
	Average length of survival with carbohydrate diet.....5+dys.				
Egg-yolk	82 gm.	82 gm.	0.05 c.c.	*	
	77 gm.	84 gm.	0.1 c.c.	*	
	84 gm.	94 gm.	0.15 c.c.	*	
	93 gm.	97 gm.	0.2 c.c.	6 dys.	Necrosis $\frac{1}{3}$ convoluted tubules; calcification.
	93 gm.	100 gm.	0.25 c.c.	3 dys.	Necrosis $\frac{3}{4}$ convoluted tubules; calcification.
	Average length of survival with egg diet.....$4\frac{1}{2}$+dys.				
Meat	63 gm.	60 gm.	0.05 c.c.	2 dys.	No lesion of kidney found.
	78 gm.	74 gm.	0.1 c.c.	2 dys.	No lesion of kidney found.
	82 gm.	82 gm.	0.15 c.c.	4 dys.	Necrosis $\frac{1}{2}$ convoluted tubules; calcification.
	85 gm.	92 gm.	0.2 c.c.	5 dys.	Necrosis $\frac{1}{2}$ convoluted tubules.
	95 gm.	104 gm.	0.25 c.c.	3 dys.	Necrosis $\frac{2}{3}$ convoluted tubules.
	Average length of survival with meat diet.....$3\frac{1}{5}$ dys.				

* = lived.

In the next series of experiments (table III) animals have been given during six days diets consisting of (a) oats and cane sugar, (b) beefsteak, and (c) boiled egg-yolk which contains 35 per cent. of fat including cholesterin and lecithin. After injection of potas-

sium chromate animals on diets of meat and of egg-yolk were allowed to eat freely of rolled oats.

Animals which received oats and sugar lost in weight, the average loss being 6.8 grams, whereas those on meat gained an average of 1.8 grams, and those on egg-yolk, 5.6 grams. The last three series of experiments fully confirm the view that the effect of diet upon the toxicity of the substances which have been tested bears no constant relation to the body-weight and is not the result of partial inanition.

In the three series of experiments with potassium chromate the number of animals which have survived and the average length of life of those which have died uniformly demonstrate the protective influence of a carbohydrate diet. By combining oats with meat and with fat (table II) differences between the three diets have been reduced to a minimum but still persist. The protective action of the carbohydrate diet is referable to the period preceding the production of nephritis by potassium chromate, for this protection is evident even though carbohydrate has been given to all animals after the poison has been administered.

Animals which have received meat have shown the greatest susceptibility to the action of potassium chromate, but there has been little difference between the effect of protein and of fat. Animals which have received egg-yolk which contains cholesterin in abundance have not exhibited remarkable susceptibility to potassium chromate.

The changes produced in the kidney of the white rat by potassium chromate have been studied carefully in order to define their relation to the lesions of the liver produced by chloroform and phosphorus and to compare the severity of the lesions in animals which have been given different diets. Necrosis is limited to the convoluted tubules within the cortex of the kidney; there is no necrosis of the liver. In animals which have died within one day after administration of potassium chromate no changes may be found in the kidney; within one or two days hyaline casts are usually found in the straight tubules and a few of the convoluted tubules may exhibit necrosis; the cells are swollen and occasionally hyaline and the nuclei have disappeared or remain as contracted, deeply stained bodies.

At the end of three days the extent of necrosis is well defined, and with large doses of potassium chromate almost every convoluted tubule of the kidney is a swollen hyaline column without nuclei; the endothelial cells of the capillaries persist, and the glomeruli, the medullary rays of the cortex, and the loops of Henle in the medulla just below the cortex are intact. An attempt has been made to estimate roughly the extent of necrosis in animals which have survived three or more days. When the kidneys of animals which have lived three or more days are compared, necrosis is usually found to be somewhat more extensive in animals which have received meat or fat than in animals on a carbohydrate diet. In table II, in which all animals lived three days, necrosis is most severe in animals with meat, less with fat, and least with carbohydrate. In animals which have lived four or more days calcium is often deposited in the necrotic tubules; there is apparently no relation between diet and the occurrence of calcification.

URANIUM NITRATE.

Uranium nitrate, like potassium chromate, causes necrosis of the renal tubules. The physiological studies of Schlayer and Hedinger,[1] Pearce,[2] and others have shown that reactions referable to the vascular apparatus of the kidney are not impaired by these substances; whereas with the nephritis produced, for example, by cantharidin or arsenic, the volume of the kidney does not diminish under the influence of sensory stimuli or adrenalin; while diuretics, such as caffein or 5 per cent. salt solution, do not dilate the blood vessels and fail to produce diuresis. Uranium nitrate exhibits peculiarities not observed with potassium chromate. At a certain stage of intoxication with uranium nitrate, diuretics, such as 5 per cent. salt solution, urea, dextrose, etc., cause dilatation of the blood vessels of the kidney but no increased flow of urine.[3] Well known observations of Richter[4] have demonstrated that uranium nitrate given to rabbits causes subcutaneous edema and accumulation of fluid in the serous cavities when hydremic plethora is produced by the daily administra-

[1] Schlayer and Hedinger, *Deutsch. Arch. f. klin. Med.*, 1907, xc, 1.

[2] Pearce, R. M., *Arch. Int. Med.*, 1909, iii, 422.

[3] Schlayer, Hedinger, and Takayasu, *Deutsch. Arch. f. klin. Med.*, 1907, xci, 59.

[4] Richter, P. F., *Beitr. z. klin. Med., Festschr. f. Senator*, 1904, 283; *Berl. klin. Wchnschr.*, 1905, xlii, 384.

tion of water by stomach. Edema is not similarly caused by potassium chromate. A further difference between the action of the two substances has been noted by Christian and O'Hare[5] who found that uranium nitrate causes a lesion of the glomeruli characterized by the presence of hyaline droplets in the capillary loops and by other changes.

Uranium nitrate has been administered in 0.5 per cent. aqueous solution by subcutaneous injection. In the following series of experiments (table IV) doses varying from 0.05 to 0.3 of a cubic centimeter (0.00025 to 0.0015 of a gram) have been given to animals which during four days had received (a) oats and cane sugar, (b) beefsteak, or (c) beef fat; the diets were continued six days after injection of uranium nitrate.

TABLE IV.

Uranium Nitrate.

Diet.	Weight before diet.	Weight after 4 dys.	Dose per 100 gm.	Length of life.	Remarks.
Oats and sugar	95 gm.	106 gm.	0.05 c.c.	*	
	119 gm.	110 gm.	0.1 c.c.	6 dys.	Kidney: necrosis ($\frac{1}{2}$).
	138 gm.	140 gm.	0.15 c.c.	7 dys.	Kidney: scant necrosis; calcification.
	150 gm.	142 gm.	0.2 c.c.	9 dys.	Kidney: necrosis ($\frac{1}{2}$).
	132 gm.	149 gm.	0.25 c.c.	*	Kidney: necrosis ($\frac{1}{3}$); calcification.
	165 gm.	172 gm.	0.3 c.c.	10 dys.	
	Average length of survival with carbohydrate diet.....8+dys.				
Meat	85 gm.	88 gm.	0.05 c.c.	*	
	95 gm.	93 gm.	0.1 c.c.	*	
	127 gm.	127 gm.	0.15 c.c.	7 dys.	Kidney: scant necrosis.
	127 gm.	132 gm.	0.2 c.c.	4 dys.	Kidney: necrosis ($\frac{1}{4}$).
	145 gm.	135 gm.	0.25 c.c.	4 dys.	Kidney: necrosis ($\frac{1}{4}$); calcification.
	280 gm.	267 gm.	0.3 c.c.	8 dys.	Kidney: necrosis ($\frac{1}{5}$); calcification.
	Average length of survival with meat diet.....$5\frac{3}{4}$ dys.				
Fat	85 gm.	78 gm.	0.05 c.c.	10 dys.	Kidney: necrosis ($\frac{1}{5}$); calcification.
	96 gm.	96 gm.	0.1 c.c.	8 dys.	Kidney: scant necrosis; calcification.
	128 gm.	114 gm.	0.15 c.c.	6 dys.	Kidney: necrosis ($\frac{2}{3}$); calcification.
	140 gm.	130 gm.	0.2 c.c.	7 dys.	Kidney: necrosis ($\frac{2}{3}$); calcification.
	145 gm.	135 gm.	0.25 c.c.	6 dys.	Kidney: necrosis ($\frac{2}{3}$).
	230 gm.	225 gm.	0.3 c.c.	9 dys.	Kidney: necrosis ($\frac{2}{3}$); calcification.
	Average length of survival with fat diet.....$7\frac{2}{3}$ dys.				

* = lived.

[5] Christian, H. A., and O'Hare, J. P., *Jour. Med. Research*, 1913, xxviii, 227.

In this series of experiments animals upon a diet of fat have shown greatest susceptibility to uranium nitrate. The number of animals which survived has been the same on diets of meat and of carbohydrate, but the average duration of life has been considerably longer after a carbohydrate diet. These differences have been almost obliterated when oats have been given with meat and fat. In the following series of experiments (table V) oats were combined with meat and fat during three days; during the next three days animals received meat alone and fat alone and after administration of uranium nitrate all animals received oats. Since animals were allowed to eat oats freely, it is probable that the special diets were effective only during three days.

TABLE V.

Uranium Nitrate.

Diet.	Weight before diet.	Weight after 6 dys.	Dose per 100 gm.	Length of life.	Remarks.
Oats and sugar	120 gm.	110 gm.	0.05 c.c.	10 dys.	Kidney: necrosis ($\frac{1}{2}$); calcification.
	132 gm.	133 gm.	0.1 c.c.	9 dys.	Kidney: necrosis ($\frac{1}{3}$); calcification.
	145 gm.	143 gm.	0.15 c.c.	9 dys.	Kidney: necrosis ($\frac{1}{3}$).
	145 gm.	143 gm.	0.2 c.c.	7 dys.	Kidney: necrosis ($\frac{1}{3}$); calcification.
	160 gm.	170 gm.	0.25 c.c.	6 dys.	Kidney: scant necrosis; calcification.
	Average length of survival with carbohydrate diet ..... $8\frac{1}{5}$ dys.				
Meat	117 gm.	122 gm.	0.05 c.c.	*	
	145 gm.	127 gm.	0.1 c.c.	6 dys.	Kidney: necrosis ($\frac{1}{5}$).
	150 gm.	133 gm.	0.15 c.c.	7 dys.	Kidney: necrosis ($\frac{1}{2}$); calcification.
	140 gm.	160 gm.	0.2 c.c.	10 dys.	Kidney: necrosis ($\frac{1}{4}$); calcification.
	177 gm.	162 gm.	0.25 c.c.	6 dys.	Kidney: scant necrosis; calcification.
	Average length of survival with meat diet ..... $7\frac{1}{4}$+dys.				
Fat	125 gm.	115 gm.	0.05 c.c.	8 dys.	Kidney: necrosis ($\frac{2}{3}$); calcification.
	135 gm.	128 gm.	0.1 c.c.	8 dys.	Kidney: necrosis ($\frac{2}{3}$); calcification.
	145 gm.	148 gm.	0.15 c.c.	7 dys.	Kidney: necrosis ($\frac{1}{2}$).
	156 gm.	159 gm.	0.2 c.c.	8 dys.	Kidney: necrosis ($\frac{1}{2}$); calcification.
	157 gm.	160 gm.	0.25 c.c.	7 dys.	Kidney: necrosis ($\frac{3}{4}$); calcification.
	Average length of survival with fat diet ..... $7\frac{3}{5}$ dys.				

* = lived.

In the foregoing series of experiments the diets which have been employed have been associated with no noteworthy differences in

the number of animals which died or in the average duration of life. A study of changes in the kidneys has shown that the more advanced necrosis occurs in animals which have received a diet of fat.

Since nearly all animals in the two last series of experiments have died, it has seemed probable that differences might be more accurately tested by smaller doses. In the next series of experiments (table VI) special diets have been administered during five days; from 0.02 to 0.1 of a cubic centimeter of a 0.5 per cent. solution (0.0001 to 0.0005 of a gram) of uranium nitrate has been injected subcutaneously and after its administration oats have been given to all animals.

TABLE VI.

Uranium Nitrate.

Diet.	Weight before diet.	Weight after 5 dys.	Dose per 100 gm.	Length of life.	Remarks.
Oats and sugar	138 gm.	137 gm.	0.02 c.c.	*	
	165 gm.	140 gm.	0.04 c.c.	*	
	155 gm.	154 gm.	0.06 c.c.	7 dys.	Kidney not examined.
	168 gm.	159 gm.	0.08 c.c.	*	
	260 gm.	267 gm.	0.1 c.c.	*	
	Average duration of life with carbohydrate diet.....7+dys.				
Meat	142 gm.	130 gm.	0.02 c.c.	9 dys.	Kidney: scant necrosis.
	138 gm.	132 gm.	0.04 c.c.	*	
	150 gm.	137 gm.	0.06 c.c.	7 dys.	Kidney: scant necrosis.
	164 gm.	162 gm.	0.08 c.c.	10 dys.	Kidney: not examined.
	185 gm.	172 gm.	0.1 cc..	11 dys.	Kidney: scant necrosis.
	Average duration of life with meat diet.....$9\frac{1}{4}$+dys.				
Fat	115 gm.	99 gm.	0.02 c.c.	7 dys.	Kidney: necrosis ($\frac{1}{4}$).
	130 gm.	115 gm.	0.04 c.c.	*	
	142 gm.	127 gm.	0.06 c.c.	9 dys.	Kidney: necrosis ($\frac{1}{4}$).
	155 gm.	140 gm.	0.08 c.c.	11 dys.	Kidney: necrosis ($\frac{1}{4}$).
	290 gm.	280 gm.	0.1 c.c.	7 dys.	Kidney: necrosis ($\frac{1}{3}$).
	Average duration of life with fat diet.....$8\frac{1}{2}$+dys.				

* = lived.

The experiments with uranium nitrate demonstrate that carbohydrate exerts a protective action. A survey of the three last tables shows that only one of six animals on carbohydrate diet receiving

less than 0.1 of a cubic centimeter died, only one of four on meat diet receiving less than 0.06 of a cubic centimeter died, and only one of two animals on a fat diet receiving less than 0.05 of a cubic centimeter died, a fatal result being almost constant after the respective diets with doses above those named. Susceptibility to uranium nitrate after a diet of meat and after a diet of fat is almost the same; susceptibility is somewhat greater after fat.

Since the object of these experiments upon the kidney has been to determine if diet influences the incidence of necrosis, a careful study of the kidney has been made. The liver and other organs show no change comparable to that which occurs in the kidney. It may be assumed that animals which survive have suffered less profound renal changes than those which die. In the two series recorded in tables IV and V, relatively large doses having been employed, nearly all animals died after an interval which does not vary very greatly. These series offer an opportunity to determine if the extent of the lesion is influenced by diet.

Although uranium nitrate causes necrosis of the renal tubules, the lesion is not identical with that of potassium chromate. Whereas potassium chromate causes death within from one to five days uranium nitrate has rarely caused death before the end of six days. With small doses of uranium nitrate the convoluted tubules are almost wholly unaffected; in the lowermost part of the cortex in immediate contact with the medulla small groups of convoluted tubules have lost their nuclei and become hyaline. In the medullary striæ of the cortex necrosis has usually occurred in many tubules. In the medulla just below the level of the glomeruli tubules which have undergone coagulative necrosis are abundant and have the characters of the loops of Henle, which here dip into the medulla. With more advanced lesions the greater part of all tubules within both the cortical striæ and subcortical zone may be necrotic, whereas the convoluted tubules are little changed. With increasingly severe lesions, such as occur after a fat diet, the convoluted tubules are implicated and a considerable proportion of the cortex undergoes necrosis. Calcium salts are quickly deposited in the dead tissue and, stained with hematoxylin, define very sharply the chief localization

of the lesion in the medullary striæ and the zone just beneath the cortex.

The extent of necrosis is considerably greater in animals which have received fat than in those on other diets, but no noteworthy difference has been found between the kidneys of those with meat and those with carbohydrate. In tables IV, V, and VI a rough estimate has been made of the relation of the necrotic tissue to the total area (cortex plus subcortical zone) in which necrosis occurs. In the tables this estimate is expressed by a fraction in parenthesis. After a diet of fat from two thirds to three fourths of this area may be implicated (tables IV and V), whereas with carbohydrate or meat the necrotic part is usually not more than from one fifth to one third when the corresponding doses of uranium nitrate are employed.

It is noteworthy that necrosis of the renal tubules with uranium nitrate is most severe after a diet of fat and occurs in those situations in which fat has been found in the normal kidney of animals fed upon fat, namely, in the medullary striæ, in the lowermost part of the cortex, and in the medulla immediately below the cortex. The loops of Henle of a rat fed upon fat become unusually susceptible to the action of uranium nitrate.

CHLOROFORM.

The occurrence of necrosis caused by uranium nitrate in parts of the kidney in which fatty infiltration may occur has suggested the possibility that a substance such as chloroform, which is soluble in fat, may produce necrosis in the same situations, for it has been shown that animals which have received fat are especially susceptible to the toxic action of chloroform. Furthermore, fat is deposited in the center of the hepatic lobule of white rats which have received a fat diet and the necrosis of chloroform occurs in this part of the lobule. Renal lesions following the administration of chloroform have been noted in some of the experiments[6] which have been described. When chloroform produces a fatal lesion of the liver death occurs within three or four days. Animals which sur-

[6] Opie, E. L., and Alford, L. B., *Jour. Exper. Med.*, 1915, xxi, 1, tables I, II, and VI.

vive this period may die after from six to eleven days with evident lesions of the kidney. The organ is enlarged and bright yellow. There is fatty degeneration or both necrosis and fatty degeneration of the renal tubules, and casts are abundant (page 6). In animals which have died with hepatic lesions within three or four days, well defined lesions of the kidney are often present.

In table VII changes found in the kidneys of animals which died after various periods following the administration of chloroform are described. These animals are some of those concerning which data are recorded in table VI of the preceding paper.[7]

TABLE VII.

Chloroform.

Diet.	Dose per 100 gm.	Length of life.	Kidney.
Fat	0.025 c.c.	8 dys.	Necrosis ($\frac{1}{2}$) of medullary striæ, of convoluted tubules in lowermost half of cortex, and of loops of Henle in adjacent medulla; slight calcification.
Fat	0.075 c.c.	3 dys.	Casts in tubules.
Fat	0.125 c.c.	6 dys.	Necrosis ($\frac{1}{2}$) in situations named above.
Fat	0.225 c.c.	4 dys.	Fatty degeneration diffusely distributed; albumin in glomerular capsules; casts.
Brain	0.025 c.c.	2 dys.	No lesion found.
Brain	0.075 c.c.	2 dys.	Fatty degeneration diffusely distributed; albumin in glomerular capsules; casts.
Brain	0.125 c.c.	2 dys.	Fatty degeneration most advanced in medullary striæ and subcortical zone.
Brain	0.225 c.c.	4 dys.	Necrosis almost wholly limited to medullary striæ with beginning calcification; fatty degeneration.

The renal lesion which is produced by chloroform is inconspicuous in animals which have lived less than four days. Necrosis of renal tubules occurs, and although it may affect tubules in any part of the cortex it is most severe in the medullary striæ, in the lowermost part of the cortex, and in the immediately adjacent subcortical part of the medulla. Fat infiltration after a diet rich in fat has been found in the same situations and is much less advanced in other parts of the cortex. Chloroform which is readily soluble in fat produces necrosis in those parts of both liver and kidney in which visible fat accumulates.

[7] Opie, E. L., and Alford, L. B., *loc. cit.*

DISCUSSION.

The effect of diet upon the toxicity of two substances, namely, chloroform and phosphorus, capable of causing necrosis of the liver, has been studied, and a similar investigation of two substances which cause necrosis of renal tubules, namely, potassium chromate and uranium nitrate, has been made. All of these substances are more toxic for animals which have received a diet of meat than for those which have received a diet consisting in great part of carbohydrate (oats and cane sugar). Upon these diets white rats maintain their weight and remain in good health. Study of the histological changes in animals which have died shows that susceptibility to these poisons is dependent upon the varying extent of necrosis in the liver or kidney induced by diet.

The protective action of carbohydrates, preventing destruction of hepatic or renal cells, is in accord with observations which have shown that a carbohydrate diet prevents the disintegration of body protein. Voit, Rubner, and others[8] have shown that carbohydrate given to a healthy individual diminishes the amount of protein needed to maintain nitrogen equilibrium and in starvation reduces the elimination of nitrogen. It is well known that the fever of acute infections is accompanied by increased excretion of nitrogen in the urine due to augmented activity of protein catabolism. Fritz Voit[9] and Linser and Schmidt[10] found that elevation of temperature caused by immersion in warm water was accompanied by increased elimination of nitrogen; they found that it was possible to retard this increased protein catabolism by adding carbohydrate to the food. Shaffer and Coleman[11] have shown that the loss of body protein which occurs with typhoid fever may be retarded by the use of diets of high caloric value especially rich in carbohydrate, and, if the supply of carbohydrate is sufficient, the loss of nitrogen derived from body protein may be prevented. Coincident diminution of the excretion of creatin and of total sulphur indicates that febrile de-

[8] For literature on the subject see Lusk, G., The Elements of the Science of Nutrition, Philadelphia and London, 1909.

[9] Voit, F., *Sitzungsber. d. Gesellsch. f. Morphol. u. Physiol. in München,* 1895, xi, 120; cited by Shaffer, P. A., and Coleman, W., *Arch. Int. Med.,* 1909, iv, 538.

[10] Linser, P., and Schmidt, J., *Deutsch. Arch. f. klin. Med.,* 1904, lxxix, 514.

[11] Shaffer, P. A., and Coleman, W., *loc. cit.*

struction of body protein is prevented. What relation disintegration of body protein bears to parenchymatous degeneration of the organs, focal necrosis of the liver and spleen, and necrosis affecting the specific lesions of typhoid fever cannot be defined with our present knowledge of the subject.

The urine furnishes similar evidence of protein disintegration after administration of chloroform. Strassmann[12] and other observers have shown that anesthesia produced by inhalation of chloroform is followed by increased elimination of urinary nitrogen, sulphur, and phosphoric acid. The experimental studies of Howland and Richards[13] have demonstrated that the increased elimination of nitrogen and sulphur which accompanies intense disintegration of body protein occurs in association with necrosis of the liver, which is the characteristic lesion of delayed chloroform poisoning in all mammals. A similar increase of protein catabolism occurs as the result of phosphorus poisoning (Ray, McDermott, and Lusk[14]).

Necrosis produced by chloroform, phosphorus, and similar substances is perhaps the anatomical expression of advanced disintegration of body protein. Carbohydrate may tend to limit this necrosis by protecting body protein. Protein diet, by virtue of the specific dynamic action of protein diet demonstrated by Rubner,[15] increases the activity of metabolism, indicated by increased heat formation.

It is noteworthy that the quantity of carbohydrate needed to maintain nitrogen equilibrium may be increased by disease of the liver. Tallqvist[16] found that increased quantity of carbohydrate is required with catarrhal jaundice, cholelithiasis with jaundice, cirrhosis, and certain other hepatic diseases. He believes that the change occurs because the liver is no longer able to store glycogen effectively.

The toxicity of two substances, namely, chloroform and uranium nitrate, has been greater after a diet of fat than after diets of meat or of carbohydrate. Necrosis caused by chloroform occurs in the

[12] Strassman, F., *Virchows Arch. f. path. Anat.*, 1889, cxv, 1.

[13] Howland, J., and Richards, A. N., *Jour. Exper. Med.*, 1909, xi, 344.

[14] Ray, W. E., McDermott, T. S., and Lusk, G., *Am. Jour. Physiol.*, 1899, iii, 139.

[15] Rubner, M., Die Gesetze des Energieverbrauchs, Leipzig, 1902.

[16] Tallqvist, T. W., *Arch. f. Hyg.*, 1908, lxv, 39.

centers of the hepatic lobules and in the loops of Henle. Infiltration with fat occurs in these situations when white rats are fed with fat. The ready solubility of chloroform in fat suggests the probability that necrosis occurs because chloroform is fixed by the fat present in the cell. The susceptibility of the cells of the kidney and of the liver to necrosis has a part in the localization of the lesion, for all cells of the body which contain fat do not undergo necrosis. Meyer and Overton maintain that narcosis occurs because a substance soluble in lipoids accumulates in the cells of the central nervous system. They believe that the efficiency of an anesthetic is dependent upon the readiness with which it passes from an aqueous solution into lipoid and may be conveniently expressed by its coefficient of distribution in oil and water. It is probable that the occurrence of necrosis caused by chloroform is dependent upon two factors: (1) the coefficient of distribution in fat and water causing the fixation of chloroform, and (2) the injurious action of chloroform upon the protoplasm of the cell, measurable in part by increased activity of protein catabolism.

Of the two substances which cause necrosis of renal tubules, uranium nitrate exhibits maximum toxicity in animals which have received fat, whereas with potassium chromate the susceptibility of animals which have received meat is somewhat greater than the susceptibility of those which have received fat. It is noteworthy that the lesion of uranium nitrate is severest in those tubules (loops of Henle) which accumulate visible fat when the animal is fed upon fat.

There is a close analogy between certain human lesions and those produced by the substances which have been employed. Toxemia of pregnancy, acute yellow atrophy of the liver, and certain bacterial infections[17] are characterized by necrosis of parenchymatous cells of the liver and not infrequently of the kidney, resembling very closely the changes produced by chloroform. In eclampsia the distribution of hepatic necrosis is similar to that which has been caused by phosphorus. Focal necrosis of parenchymatous cells occurs in the liver, spleen, and other organs during typhoid fever, malaria,

[17] Mallory, F. B., *Jour. Med. Research,* 1901, vi, 264. Opie, E. L., *idem,* 1904, xii, 147.

diphtheria, and many other infections. Study of pathological tissues and experimental studies with chloroform and other substances indicate that necrosis of hepatic cells has an important part in the pathogenesis of cirrhosis of the liver. Our experiments performed with four substances which produce localized necrosis indicate that diet may exert an important influence upon the incidence and progress of similar lesions. Increased susceptibility caused by a protein diet and in some instances by fat may have a part in the production of conditions such as toxemia of pregnancy or eclampsia. The experiments suggest that carbohydrate diet may prevent their occurrence or retard their progress.

CONCLUSIONS.

Diet exerts a profound influence upon the toxicity (tested in the omnivorous white rat) of certain substances, namely, chloroform, phosphorus, potassium chromate, and uranium nitrate, which cause necrosis of the parenchymatous cells of the liver or of the kidney. Susceptibility to intoxication with all of these substances is less after a diet rich in carbohydrate than after a diet consisting of meat.

Carbohydrates protect the parenchymatous cells of the liver or of the kidneys from necrosis caused by any one of these substances.

Chloroform is much more toxic to animals which have received a diet consisting in great part of fat than to those which have received meat. When fat is fed to the white rat fatty infiltration occurs in the centers of the hepatic lobules and in the loops of Henle of the kidney. The necrosis caused by chloroform has the same location. The solubility of chloroform in fat determines the increased susceptibility of animals which have received fat and stored it in the parenchymatous cells of the liver and kidney.

Susceptibility to intoxication with phosphorus which causes fatty degeneration and necrosis of the liver is not increased by a diet of fat. Necrosis caused by phosphorus occurs in the periphery of the hepatic lobule and exhibits maximum intensity in animals which have received meat.

Susceptibility to intoxication with potassium chromate which causes nephritis with necrosis of the convoluted tubules of the kidney is not greater after a diet of fat than after a diet of meat.

Susceptibility to intoxication with uranium nitrate which causes nephritis with advanced necrosis of renal tubules is increased by a diet of fat. The loops of Henle, in which fat is abundant after a diet of fat, are the chief site of necrosis.