STUDIES IN LIVER AND KIDNEY FUNCTION IN EXPERIMENTAL PHOSPHORUS AND CHLOROFORM POISONING.

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(Received for publication, May 25, 1915.)

INTRODUCTION.

In the course of a somewhat extended study of the value of various tests of liver function in clinical diseases of the liver,[1] it seemed advisable to determine whether a rough approximate standardization of the functional changes could be obtained through comparison of the findings in clinical conditions with graded experimental liver injuries in animals.

Chloroform and phosphorus poisoning have probably been most used in producing experimental liver lesions. The non-specificity of the injury to the liver is at once apparent in the consideration of this method, for it is well known that pathological changes in the kidneys, heart, suprarenals, and voluntary muscles occur as the results of these poisons. The general opinion that the liver lesions are the predominating pathological features and that they are responsible for certain metabolic changes led us to investigate these conditions. However, it may be stated at once that we feel that deductions concerning liver function drawn from such studies are not free from criticism, and may be applied to findings in clinical material only with reserve.

An acceleration of protein metabolism, as evidenced by increased nitrogen output in the urine, has been shown to occur in phosphorus poisoning by Bauer,[2]

[1] Rowntree, L. G., Marshall, E. K., Jr., and Chesney, A. M., *Tr. Assn. Am. Phys.*, 1914, xxix, 586. Chesney, Marshall, and Rowntree, *Jour. Am. Med. Assn.*, 1914, lxiii, 1533.

[2] Bauer, J., *Ztschr. f. Biol.*, 1871, vii, 63; 1878, xiv, 527.

and in chloroform poisoning by Strassmann.[3] This has been generally confirmed by all subsequent workers.[4] It has been attributed to a decrease in the oxidation processes in the body and to autolysis of tissues.[5] However, Lusk[6] has shown that there is no reduction in the amount of total metabolism in phosphorus poisoning, but there may be rather an increase due to fever and the increased protein metabolism.

In general, the changes occurring in the urine and blood in phosphorus and in chloroform poisoning are similar to those which seem to be characteristic of certain types of liver disease in man.[7] Vidal[8] showed a slight decrease in the urea, and increase in the uric acid, creatinine, and undetermined nitrogen percentages of the total in experimental chloroform poisoning. Paton[9] distinguished between the effects of its inhalation and of its administration by the stomach. In the former he found a normal or increased urea and normal or decreased ammonia percentage; in the latter, a decided decrease in the urea and increase in the ammonia, uric acid, and undetermined nitrogen percentages of the total. He did not think the discrepancy due to size of the dose. Given hypodermically, the same effect, though less pronounced, was noted as when administered by mouth. Howland and Richards,[10] studying delayed chloroform poisoning (anesthesia) in dogs, noted slightly decreased urea and increased ammonia and undetermined nitrogen percentages of the total. Lindsay[11] confirmed these results and found the allantoin, creatine, amino-acids, and polypeptides increased.

The excretion of amino-acids in the urine in phosphorus poisoning has been shown by Abderhalden and Bergell[12] and Wohlgemuth.[13] Fischler and Bardach[14] found in dogs, with and without an Eck fistula, no difference in the nitrogen partition in phosphorus poisoning, low urea and high ammonia percentages, and increase in formol-titratable nitrogen in both. Ishihara[15] has recently studied

[3] Strassmann, F., *Virchows Arch. f. path. Anat.*, 1889, cxv, 1.

[4] Taniguti, K., *Virchows Arch. f. path. Anat.*, 1890, cxx, 121. Savelieff, N., *Virchows Arch. f. path. Anat.*, 1894, cxxxvi, 195. Vidal, E., Influence de l'anesthésie chloroformique sur les phenomènes chimiques de l'organisme, Thèse de Paris, 1897. Paton, D. N., *Proc. Roy. Soc. Edinburgh*, 1908, xxviii, 472. Howland, J., and Richards, A. N., *Jour. Exper. Med.*, 1909, xi, 344. Lindsay, D. E., *Biochem. Jour.*, 1910–11, v, 407.

[5] Jacoby, M., *Ztschr. f. physiol. Chem.*, 1900, xxx, 149. Waldvogel, *Arch. f. klin. Med.*, 1905, lxxxii, 437. Wells, H. G., *Jour. Biol. Chem.*, 1908–09, v, 129.

[6] Lusk, G., *Am. Jour. Physiol.*, 1907, xix, 461. Mandel, A. R., and Lusk, G., *Am. Jour. Physiol.*, 1906, xvi, 129.

[7] For a full discussion of the functional changes occurring in liver diseases, see Rowntree, Marshall, and Chesney, *loc. cit.* Here also will be found a full discussion of the methods employed in our liver functional studies.

[8] Vidal, *loc. cit.*

[9] Paton, *loc. cit.*

[10] Howland and Richards, *loc. cit.*

[11] Lindsay, *loc. cit.*

[12] Abderhalden, E., and Bergell, P., *Ztschr. f. physiol. Chem.*, 1903, xxxix, 464.

[13] Wohlgemuth, J., *Ztschr. f. physiol. Chem.*, 1905, xliv, 74.

[14] Fischler, F., and Bardach, K., *Ztschr. f. physiol. Chem.*, 1912, lxxviii, 435.

[15] Ishihara, H., *Biochem. Ztschr.*, 1912, xli, 315.

subchronic phosphorus poisoning in dogs, and could demonstrate no noticeable changes in the distribution of the nitrogen between the creatine, creatinine, amino-acids, and ammonia.

The most noticeable changes which have been shown in the blood are the marked decrease in fibrinogen and increase in the lipolytic activity. Corin and Ansiaux[16] and Jacoby[17] found the blood incoagulable in phosphorus poisoning, while this decrease in fibrinogen was shown by Doyon and Billet[18] for chloroform poisoning. Whipple[19] and his coworkers have shown the increase in the lipolytic activity of the blood in these conditions, and have further made an extensive study on lipase and fibrinogen in experimental liver injuries (chloroform, phosphorus, hydrazine poisoning, etc.), and in certain clinical diseases of the liver. Ragazzi[20] found that the viscosity and electrical conductivity of the blood were increased in phosphorus poisoning. Frank and Isaac[21] found the blood sugar to be decreased to zero shortly before death from phosphorus poisoning. Opie, Barker, and Dochez[22] showed an increase in the proteolytic enzymes of the blood after chloroform and phosphorus poisonings.

Simultaneous clinical studies by Rowntree, Hurwitz, and Bloomfield,[23] and experimental studies by Whipple, Peightal, and Clark[24] were carried on to determine whether the excretion of phenoltetrachlorphthalein by the bowels in health and disease afforded information relative to liver function. Whipple's studies revealed the facts that in chloroform and phosphorus poisonings marked decrease in the phthalein output resulted, together with the appearance of the drug in the urine, and further that, associated with decrease in the phthalein output, decrease in fibrinogen and increase in blood lipase occurred. In the recent study of lactose tolerance in chloroform and phosphorus poisonings in dogs, Bloomfield and Hurwitz[25] have reviewed the literature on sugar tolerance.

Methods.

Before inducing the chloroform or phosphorus poisoning, each dog was subjected to the series of tests to establish its normals. No

[16] Corin, G., and Ansiaux, G., *Jahresb. f. Thierchem.*, 1894, xxiv, 642.

[17] Jacoby, *loc. cit.*

[18] Doyon, M., and Billet, J., *Compt. rend. Soc. de biol.*, 1905, lvii, 852.

[19] Whipple, G. H., Mason, V. R., and Peightal, T. C., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 207. Whipple, G. H., and Hurwitz, S. H., *Jour. Exper. Med.*, 1911, xiii, 136. Whipple, G. H., *Arch. Int. Med.*, 1912, ix, 365.

[20] Ragazzi, C., *Jahresb. f. Thierchem.*, 1910, xl, 1276.

[21] Frank, E., and Isaac, S., *Arch. f. exper. Path. u. Pharmakol.*, 1911, lxiv, 274.

[22] Opie, E. L., Barker, B. I., and Dochez, A. R., *Jour. Exper. Med.*, 1911, xiii, 162.

[23] Rowntree, L. G., Hurwitz, S. H., and Bloomfield, A. L., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 327.

[24] Whipple, G. H., Peightal, T. C., and Clark, A. H., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 343.

[25] Bloomfield, A. L., and Hurwitz, S. H., *Bull. Johns Hopkins Hosp.*, 1913, xxiv, 375.

attempt was made to maintain nitrogenous equilibrium except in two animals, although in the others the diet was kept fairly constant throughout the period of observation.[26] The chloroform was given by inhalation, and the phosphorus subcutaneously, in oil. The phenoltetrachlorphthalein test was employed, according to the technique of Rowntree, Hurwitz, and Bloomfield.[27] The fibrinogen determinations were made by the heat coagulation method of Whipple,[28] and the lipolytic activity of the blood plasma by Loevenhart's[29] method. The total non-protein nitrogen, urea nitrogen, and amino-acid nitrogen of the blood serum or whole blood were estimated by the micro-Kjeldahl method of Folin and Denis,[30] the urease method of Marshall,[31] and the nitrous acid method of Van Slyke,[32] respectively. In the urine, total nitrogen was determined by the usual Kjeldahl, the urea by Marshall's,[33] the ammonia by Folin's,[34] and the free amino-acids by Van Slyke's[35] method. The tolerance towards levulose and galactose was determined in the usual way. The hydrogen ion concentration of the blood was determined by the method recently described by Levy, Marriott, and Rowntree.[36]

Liver Function in Phosphorus Poisoning.

In the following protocols of the various animals, the fibrinogen, total non-protein nitrogen, urea nitrogen, and amino nitrogen are expressed in mg. per 100 cc. of blood serum. The urine quantity, unless otherwise stated, is for 24 hours, and the urea nitrogen, ammonia nitrogen, and free amino nitrogen are expressed as percentages of the total nitrogen of the urine. The lipolytic activity of the blood

[26] In the more severe stages of poisoning, the animals refused food for several days.

[27] Rowntree, Hurwitz, and Bloomfield, *loc. cit.*

[28] Whipple, G. H., *Am. Jour. Physiol.*, 1914, xxxiii, 50.

[29] Loevenhart, A. S., *Am. Jour. Physiol.*, 1901–02, vi, 331.

[30] Folin, O., and Denis, W., *Jour. Biol. Chem.*, 1911–12, xi, 527.

[31] Marshall, E. K., Jr., *Jour. Biol. Chem.*, 1913, xv, 487.

[32] Van Slyke, D. D., and Meyer, G. M., *Jour. Biol. Chem.*, 1912, xii, 399.

[33] Marshall, E. K., Jr., *Jour. Biol. Chem.*, 1913, xiv, 283; xv, 495.

[34] Folin, O., *Ztschr. f. physiol. Chem.*, 1902–03, xxxvii, 161.

[35] Van Slyke, D. D., *Jour. Biol. Chem.*, 1913, xvi, 125.

[36] Levy, R. L., Marriott, W. M., and Rowntree, L. G., *Arch. Int. Med.*, 1915, xvi (in press).

plasma is expressed in the number of cc. of N/10 acid produced in 24 hours at 38° C. by a mixture of 1 cc. of plasma, 4 cc. of water, and 0.26 cc. of ethyl butyrate.

Dog I.—Weight 12 kilos. *Normals:* tolerates 0.3 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 32 per cent. *Blood:* fibrinogen, 490 mg.; total non-protein N, 44 mg.; urea N, 20 mg. (46 per cent); amino N, 3.7 mg. *Urine:* 175 cc. containing 4.4 gm. nitrogen; urea N, 83 per cent; ammonia N, 7.4 per cent; amino N, 0.45 per cent.

Mar. 6, 1914. Given 10 mg. phosphorus in oil subcutaneously.

Mar. 8. Given 7.5 mg. phosphorus. 0.2 gm. per kilo of galactose not tolerated. *Urine:* 450 cc. containing 10.3 gm. nitrogen; urea N, 84 per cent; ammonia N, 5.4 per cent; amino N, 0.3 per cent.

Mar. 9. Bled and phthalein injected at noon; died at 3 p. m. *Blood:* lipase, 1.55; fibrinogen, 13 mg.; total non-protein N, 91 mg.; urea N, 33 mg. (36 per cent); amino N, 13.4 mg.

Autopsy.—Liver: light brownish yellow in appearance, very friable, and soft. On section the parenchyma has a mottled appearance, areas of depression alternating with those which project. *Lungs:* pale and edematous.

Microscopic Examination.[37]*—Liver:* diffuse peripheral hemorrhagic necrosis. Necrotic cells are found scattered through all parts of the liver lobule. There are no normal liver cells seen. Those remaining alive show extreme parenchymatous and fatty degeneration.

Dog II.—Weight 23 kilos. *Normals:* tolerates 0.25 gm. but not 0.30 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 31 per cent. *Blood:* lipase, 0.25; fibrinogen, 243 mg.; total non-protein N, 51 mg.; urea N, 20 mg. (39 per cent); amino N, 3.8 mg. *Urine:* 390 cc. containing 12.0 gm. nitrogen; urea N, 85 per cent; ammonia N, 5.3 per cent; amino N, 1.5 per cent.

Mar. 21, 1914. Given 13 mg. phosphorus.

Mar. 23. Given 5 mg. phosphorus.

Mar. 24. Phthalein 23 per cent positive in urine.

Mar. 26. Given 5 mg. phosphorus. Tolerates 0.20 gm. galactose per kilo. *Urine:* 600 cc. containing 12.1 gm. nitrogen; urea N, 84 per cent; ammonia N, 5.6 per cent; amino N, 0.99 per cent. Bile in urine, but no jaundice; dog seems sick.

Mar. 27. Given 3 mg. phosphorus.

Mar. 28. Tolerates 1.2 gm. levulose per kilo; phthalein 0 per cent; positive in urine. *Blood:* lipase, 0.60; fibrinogen, 10 mg.; total non-protein N, 110 mg.; urea N, 61 mg. (55 per cent); amino N, 11.3 mg. *Urine:* 1,200 cc. containing 18.2 gm. nitrogen; urea N, 77 per cent; ammonia N, 6.0 per cent; amino N, 4.6 per cent.

Mar. 29. Dog very sick; comatose. Died. *Blood:* (just after death) lipase, 1.30; total non-protein N, 225 mg.; urea N, 179 mg. (79 per cent); amino N, 21 mg. *Bladder urine:* 100 cc. containing 1.6 gm. nitrogen; urea N, 82 per cent; ammonia N, 4.7 per cent; amino N, 2.1 per cent.

Dog III.—Weight 22 kilos. *Normals:* tolerates 0.30 gm. galactose and 1.5

[37] For the microscopic reports we are indebted to Dr. G. H. Whipple.

gm. levulose per kilo. Phthalein 30 per cent. *Blood:* lipase, 0.35; fibrinogen, 330 mg.; total non-protein N, 28 mg.; urea N, 8 mg. (29 per cent); amino N, 3.4 mg. *Urine:* 533 cc. containing 10.6 gm. nitrogen; urea N, 81 per cent; ammonia N, 6.7 per cent; amino N, 1.4 per cent.

Mar. 18, 1914. Given 10 mg. phosphorus.

Mar. 19. Tolerates 0.20 gm. galactose per kilo.

Mar. 20. Given 8 mg. phosphorus.

Mar. 21. Tolerates 1.25 gm. levulose per kilo.

Mar. 23. Phthalein 6 per cent; lipase, 0.85; fibrinogen, 200 mg.; total non-protein N, 50 mg.; urea N, 22 mg. (44 per cent); amino N, 3.4 mg.

Mar. 26. *Urine:* 410 cc. containing 7.4 gm. nitrogen; urea N, 83 per cent; ammonia N, 7.4 per cent; amino N, 1.1 per cent.

Mar. 27. Playful. Given 7.5 mg. phosphorus.

Mar. 28. Playful. Does not tolerate 1.2 gm. levulose per kilo. Phthalein 17 per cent positive in urine.

Apr. 2. Does not tolerate 0.20 gm. galactose per kilo.

Apr. 3. Does not tolerate 1.0 gm. levulose per kilo. Given 8 mg. phosphorus.

Apr. 4. Phenolsulphonephthalein 50 per cent for 1¼ hours.

Apr. 5. Does not tolerate 0.18 gm. galactose per kilo. Phthalein 26 per cent. Apparently has been in splendid condition for past week.

Apr. 6. Given in a. m. 11 mg. phosphorus and in p. m. 8 mg. more. *Blood:* lipase, 0.75; fibrinogen, 400 mg.; total non-protein N, 42 mg.; urea N, 18 mg. (43 per cent); amino N, 2.0 mg.

Apr. 7. Vomited. Looks depressed. Wants to lie down. Phenolsulphonephthalein 44 per cent in 1½ hours. *Urine:* 530 cc. containing 8.5 gm. nitrogen; urea N, 82 per cent; ammonia N, 3.2 per cent; amino N, 1.7 per cent. Tolerates 0.8 gm. levulose per kilo.

Apr. 8. Found dead. Weight 17.4 kilos. Has not eaten anything for 2 to 3 days. *Blood:* does not coagulate; total non-protein N, 84 mg.; urea N, 28 mg. (33 per cent); amino N, 11.3 mg.

Autopsy.—Kidney: diffuse epithelial necrosis, involving particularly the convoluted tubules.

The tests on the three dogs suffering from phosphorus poisoning (I, II, and III) showed the following definite agreement in all: The phthalein excretion in the feces was markedly diminished and the dye appeared in the urine; the lipolytic activity of the blood was greatly increased; the fibrinogen sank to a very low level; the total non-protein nitrogen, urea nitrogen, and amino nitrogen of the blood were all more or less increased. The urines showed no conclusive changes in all, while the galactose and levulose tolerance was rather definitely decreased. The urea nitrogen percentage of the total non-protein nitrogen of the blood showed an increase in one case and decrease in the other two. However, this is not at all striking, and no conclusion concerning it can be drawn. The high

total non-protein nitrogen and urea nitrogen of the blood in Dog II are worthy of note. The highest blood amino nitrogen (21 mg. to 100 cc.) which we have encountered was seen in this case, in which also the partition of the urinary nitrogen was most markedly disturbed. However, with the possible exception of Dog III (March 23), this was the only time when a severe stage of poisoning (liver injury) as indicated by other tests and the general condition of the animals was coincident with a urine analysis. In Dog II we see the gradual drop of the phthalein to zero, and the liver injury, as shown by the test, was probably in this case the most severe. Dog III apparently had a remarkable resistance to phosphorus. The liver repair indicated on April 5 to 6 is worthy of note.

TABLE I.

Dog IV.

Date (1914).	Phosphorus.	Urine.					Total N.			Blood.			Weight.
		Amount.	Total N.	Urea N.	Ammonia N.	Amino N.	Urea N.	Ammonia N.	Amino N.	Total non-protein N.	Urea N.	Amino N.	
										Per 100 cc.			
	mg.	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*mg.*	*mg.*	*mg.*	*kilos*
Nov. 30		112	4.33	3.53	0.18		82	4.2					8.40
Dec. 2													
" 3	20	205	7.53	6.33	0.41	0.044	84	5.5	0.6	28	12	3.7	8.10
" 4	10	154	3.23	2.52	0.11	0.031	78	3.4	1.0				8.20
" 5		200	5.78	4.91	0.33	0.092	85	5.7	1.6				
" 6	20	218	8.74	6.21	0.26	0.055	72	3.0	0.7				
" 7										88	66	7.7	7.45
Dog V.													
Dec. 11		237	4.19	3.60	0.09		86	2.2					8.40
" 15		78	4.52	3.93		0.05	87		1.1				
" 16	20	110	6.60	5.61	0.76	0.03	85	4.0	0.5	29	12	4.3	8.45
" 17	5	170	4.66	3.90	0.16		83	3.6					
" 19										88	49	7.5	
" 20		115	2.00	1.42	0.16	0.14	71	8.0	7.0	140	90	13.2	7.25

Nitrogen Metabolism of Dogs in Phosphorus Poisoning.

It was considered advisable to obtain data on the nitrogen metabolism in phosphorus poisoning, in view of the cumulative phenomena observed in the blood. Information concerning the relation between the amount of the nitrogenous bodies in the blood and their excretion

in the urine is desirable particularly to determine if renal function is involved.

Two dogs (IV and V) were placed on a diet of 200 gm. of ground meat and 150 cc. of water until nitrogenous equilibrium was obtained. Two to three days prior to the administration of phosphorus, the urinary partition (urea N, ammonia N, and amino N) was determined, as well as the total non-protein N, urea N, and amino N of the whole blood. Phosphorus poisoning was induced, and the metabolic studies were continued, the results appearing in Table I.

Dog IV continued to eat until the day of death, whereas Dog V ate nothing for the last two days. Dog IV shows a marked increase in the output of urinary N, with a slight decrease in the urea nitrogen per cent, but no change in the ammonia and amino-acid N per cent, together with an absolute increase in total non-protein N, the urea N and amino N of the blood. Dog V exhibited practically normal urinary N figures until the day of death, when decrease in the total N, an absolute and relative decrease in urea N, and a relative increase in the ammonia and amino N percentages were found. Very marked accumulation of total non-protein N, urea, and amino N occurred in blood.

Renal Function in Phosphorus Poisoning in Dogs.

Since urinary nitrogen changes are utilized in studies of liver function, it is obviously necessary to have some information concerning the effect on renal function of drugs used for the production of these liver injuries. The cumulative phenomena in the blood encountered in our studies on liver function in phosphorus poisoning suggested that the kidney function was affected shortly before death. It was also considered advisable to determine whether an acidosis occurs, as this would affect conclusions drawn from the urinary ammonia findings. The phenolsulphonephthalein output in the urine, and the urea and hydrogen ion concentrations in the blood, were utilized in this connection. The results of studies on four dogs appear in Table II.

In these dogs sufficient phosphorus was given to insure definite functional and anatomical liver injury. Death from the phosphorus resulted in three of them, and jaundice and incoagulable blood were seen in all. These rarely appear except in fatal poisoning.

In Dogs VI and VII, acutely poisoned, no decrease in the phenolsulphonephthalein occurred. The blood urea increased only as a terminal event. Acidosis is indicated in each on the day before death.

Dog VIII, which recovered, showed at one time a phenolsulphonephthalein output considerably decreased,—28 per cent. However, the phthalein rapidly returned to normal. No marked increase in blood urea and no acidosis accompanied this change.

TABLE II.

Dog VI.

Date (1915).	Weight.	Phosphorus.	Sulphonephthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.
	kilos	*mg.*	*per cent*	*mg.*	
Mar. 30			78		
" 31	8.0			24	7.70
Apr. 3			68		
" 7	8.4	12.5	70	32	7.45
" 8	8.55		55	35	7.65
" 9	8.40	5.0	80	40	
" 10			60	68	7.25
" 11				176	

Dog VII.

Date (1915).	Weight.	Phosphorus.	Sulphonephthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.	Hydrogen ion concentration of serum.
	kilos	*mg.*	*per cent*	*mg.*		
Mar. 31	12.22		60	30	7.55	7.55
Apr. 3			60			
" 7	13.00	20		36	7.50	7.70
" 8	13.50		70	37	7.55	7.65
" 9	12.60	7.5	56	35		
" 10			75	35		
" 11			65	46	7.25	
				127 at death		

Dog VIII.

Date (1915).	Weight.	Phosphorus.	Sulphonephthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.
	kilos	*mg.*	*per cent*	*mg.*	
Apr. 13			62	20	7.60
" 14	7.60	5			
" 16		5	67		7.45
" 17			75	40	
" 19		5		30	7.40
" 20				30	
" 21	6.35		58		
" 22			28	40	
" 23			45		7.45
" 26		5		36	7.60
" 27			63		
" 28		5			
" 29	6.30		75	51	7.60
" 30		5		36	
May 3	5.85		60	22	7.65
" 7			61	17	

TABLE II.—*Concluded.*

Dog IX.

Date (1915).	Weight.	Phosphorus.	Sulphone-phthalein, 2 hrs.	Blood urea per 100 cc.	Hydrogen ion concentration of blood.
	kilos	*mg.*	*per cent*	*mg.*	
Apr. 13			64	23	7.60
" 14	5.80	5			
" 16		5	75	30	7.45
" 17			80	27	
" 19				43	7.30
" 20				31	
" 21	5.35	5	73		
" 22			58		
" 23			50		7.35
" 26	5.25	5	65		
" 28		5			
" 29	5.15		53	55	7.55
" 30		5	45	32	7.60
May 3			20	79	7.35
" 4	4.75			112	

Dog IX exhibited a progressively decreasing renal function, as indicated by sulphonephthalein for the week previous to death, a normal increase in the blood urea, and an acidosis early in the poisoning, which reappeared before death.

That considerable injury to renal function does occur occasionally during the course of the intoxication, but that it is usually a terminal event, is evident from the study of these dogs. The renal injury, however, is exceedingly slight, and is late in comparison with the functional injury to the liver. The functional renal injury cannot be absolutely ignored, but it plays only a minor part in the interpretation of the functional changes of the liver resulting from the administration of phosphorus.

Liver Function in Chloroform Poisoning.

In the following protocols the results of liver studies similar to those carried out on the dogs poisoned with phosphorus are reported. No special renal functional studies, however, were made in chloroform poisoning.

Dog X.—Weight 24 kilos. *Normals:* tolerates 0.30 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 30 per cent. *Blood:* lipase, 0.25; total non-protein N, 58 mg.; urea N, 30 mg. (52 per cent); amino N, 3.4 mg. *Urine:* 1,140 cc. containing 15.1 gm. nitrogen; urea N, 91 per cent; ammonia N, 5.8 per cent; amino N, 1.4 per cent.

Mar. 6, 1914. Chloroform anesthesia for 2 hours.

Mar. 8. Phthalein 22 per cent.

Mar. 10. Does not tolerate 0.20 gm. galactose per kilo.

Mar. 13. Tolerates 1.0 gm. levulose per kilo.

Mar. 14. *Blood:* lipase, 1.20; fibrinogen, 217 mg.; total non-protein N, 56 mg.; urea N, 26 mg. (46 per cent); amino N, 3.0 mg. *Urine:* 250 cc. containing 5.7 gm. nitrogen; urea N, 84 per cent; ammonia N, 6 per cent; amino N, 6.9 per cent.

Mar. 15. Died during chloroform anesthesia.

Dog XI.—Weight 16 kilos. *Normals:* phthalein 35 per cent. *Urine:* 425 cc. containing 7.9 gm. nitrogen; urea N, 82 per cent; ammonia N, 10 per cent; amino N, 1.1 per cent.

Mar. 3, 1914. Chloroform anesthesia for 2 hours.

Mar. 5. Found dead in cage. Body still very warm. *Bladder urine:* 100 cc., containing 2.2 gm. nitrogen; urea N, 40 per cent; ammonia N, 9.7 per cent; amino N, 3.0 per cent.

Dog XII.—Weight 8 kilos. *Normals:* tolerates 1.5 gm. levulose per kilo. Phthalein 34 per cent, positive in urine. *Blood:* lipase, 0.39; total non-protein N, 33 mg.; urea N, 11 mg. (33 per cent); amino N, 4.3 mg. *Urine:* 198 cc. containing 7.8 gm. nitrogen; urea N, 84 per cent; ammonia N, 6.4 per cent; amino N, 1.0 per cent.

Feb. 14. Chloroform anesthesia for 1½ hours.

Feb. 15. Phthalein 21 per cent, positive in urine. *Blood:* lipase, 0.93; fibrinogen, 395 mg.; total non-protein N, 52 mg.; urea N, 24 mg. (46 per cent); amino N, 4.9 mg.

Feb. 16. *Urine:* 265 cc., containing 13.4 gm. nitrogen; urea N, 84 per cent; ammonia N, 5.3 per cent; amino N, 1.5 per cent.

Died in subsequent chloroform anesthetization.

Dog XIII.—Weight 12 kilos. *Normals:* tolerates 0.22 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 32 per cent. *Blood:* lipase, 0.38; fibrinogen, 485 mg.; total non-protein N, 39 mg.; urea N, 11 mg. (39 per cent); amino N, 4.8 mg. *Urine:* 360 cc. containing 9.1 gm. nitrogen; urea N, 83 per cent; ammonia N, 6.0 per cent; amino N, 1.2 per cent.

Mar. 19, 1914. Chloroform anesthesia for 2 hours. Very light. All subsequent ones light.

Mar. 23. Phthalein 25 per cent.

Mar. 25. Chloroform anesthesia for ¾ hour. Phthalein 27 per cent.

Mar. 26. Tolerates 0.16 gm. galactose per kilo.

Mar. 27. Chloroform anesthesia for 1 hour.

Mar. 28. Does not tolerate 1.2 gm. levulose per kilo.

Mar. 29. Phthalein 30 per cent.

Apr. 1. Chloroform anesthesia for 2 hours.

Apr. 2. Tolerates 0.16 gm. galactose per kilo.

Apr. 3. Does not tolerate 1 gm. levulose per kilo.

Apr. 4. Phthalein 9 per cent, positive in urine. Does not tolerate 0.16 gm. galactose per kilo.

Apr. 6. *Blood:* lipase, 0.85; fibrinogen, 14 mg.; total non-protein N, 25 mg.; urea N, 13 mg. (52 per cent); amino N, 4.0 mg.

Apr. 7. Tolerates 0.8 gm. levulose per kilo. Phthalein 2 per cent, positive in urine.

Apr. 8. *Blood:* lipase, 1.61; fibrinogen, 44 mg.; total non-protein N, 29 mg.; urea N, 10 mg. (34 per cent); amino N, 4.3 mg. *Urine:* 465 cc. containing 6.1 gm. nitrogen; urea N, 70 per cent; ammonia N, 14.4 per cent; amino N, 1.4 per cent.

Killed by bleeding from carotid.

Autopsy.—Liver: normal size, very pale, and friable. Lobules show very plainly. All tissues stained with bile. Kidney looks normal.

Microscopic Examination.—Kidney: cloudy swelling, moderate grade. Cells of convoluted tubules are swollen and granular. The lumina contain pink staining granules. *Liver:* shows central atrophy; extreme fatty degeneration, involving the entire lobule except a thin zone about the portal area; and numerous scattered necrotic liver cells, especially about the central portions of the lobules.

In order to gain some idea of the variations in normal dogs dependent on diet, Dogs XIV and XV were studied with uncontrolled diet (lungs, corn cake, etc.), and then on April 17 placed on a diet of 400 grams of meat per day. The total nitrogen and urea (whole blood) were taken at various times. Table III shows the variations encountered. The urea figures are expressed as mg. of nitrogen per 100 cc. of blood.

TABLE III.

Dog XIV.

Apr., 1914.	4	5	8	15	16	17	18	19	20	21	22	23	24	25	26	27
Total N, urine.....		4.4	9.0		11.2	10.0	5.8	20.0		13.6		15.2		10.1		
Urea N, blood.....	10		14	9	13			21	19				14			14

Dog XV.

Total N, urine.....		5.1	3.7		7.6	4.7	6.9	18.1		17.0		14.4		6.7		
Urea N, blood.....	11		14	9	15			20	25				18			18

Dog XIV.—Weight 11.9 kilos. *Normals:* phthalein 29 per cent. *Blood:* lipase, 0.35; fibrinogen, 470 mg.; total non-protein N, 34 mg.; urea N, 15 mg. (44 per cent); amino N, 3.7 mg.

May 1, 1914. Deep chloroform anesthesia for 2 hours.

May 2. Phenolsulphonephthalein between 60 and 70 per cent for 2 hours. *Blood:* lipase, 2.75; fibrinogen, 90 mg.; total non-protein N, 32 mg.; urea N, 13 mg. (40 per cent); amino N, 4.2 mg. *Urine:* single specimen; urea N, 44 per cent; ammonia N, 8.3 per cent; amino N, 3.1 per cent. Phthalein, only trace in feces.

Dog XV.—Weight 10.2 kilos. *Normals:* tolerates 0.3 gm. galactose and 1.5 gm. levulose per kilo. Phthalein 37 per cent.

May 5, 1914. Deep chloroform anesthesia for 1½ hours.

May 6. *Blood:* lipase, 2.70; fibrinogen, 30 mg.; total non-protein N, 120 mg.; urea N, 80 mg. (67 per cent); amino N, 6.6 mg. *Urine:* 24 hour specimen lost.

May 7. Dead. *Bladder urine:* urea N, 54 per cent; ammonia N, 10.6 per cent; amino N, 3.6 per cent. Phthalein 3 per cent.

In chloroform poisoning in dogs the following changes are found: The tetrachlorphthalein is decreased, and it appears in the urine; lipase is increased; fibrinogen is decreased; the total non-protein nitrogen, urea nitrogen, and amino nitrogen of the blood suffer practically no change. In the urine a more or less pronounced disturbance of the nitrogen partition is seen, while the galactose and levulose tolerance is definitely decreased.

Dog XV was the only one with chloroform poisoning which showed increase in nitrogenous products in the blood. Here, we have a condition somewhat resembling that in phosphorus dogs. The other tests show a very severe type of poisoning in this dog. This brings out the possibility of cumulative phenomena appearing in the course of chloroform poisoning. High amino-acid content of the urine is noted in Dog X (6.9 per cent); Dog XI (3.0 per cent); Dog XIV (3.1 per cent); and Dog XV (3.6 per cent). Dogs XI, XIV, and XV received poisonings which were rapidly fatal, dying within forty-eight hours. In them, a markedly disturbed nitrogen partition occurs in the urine, *i. e.,* a much decreased urea nitrogen, without a corresponding increase in the free amino-acids or ammonia nitrogen.

In Dog XIV, at a time when the liver function was reduced to a minimum, the sulphonephthalein was absolutely normal. It is very unlikely that renal functional changes are greater in chloroform than in phosphorus poisoning.

CONCLUSIONS.

1. We have confirmed the results of previous workers on tetrachlorphthalein, fibrinogen, and lipase.

2. The total non-protein nitrogen, urea, and amino-acids of the blood serum show a definite and sometimes marked increase in phosphorus poisoning. These changes are not so evident in chloroform poisoning, although they sometimes occur. They are usually terminal phenomena.

3. The urinary nitrogen partition between the urea, ammonia, and amino-acids is not always disturbed. The most important changes which occur are an increased amino nitrogen in chloroform and phosphorus poisoning, and a very low urea nitrogen percentage in severe fatal chloroform poisoning.

4. Sugar tolerance towards galactose and levulose is in general markedly decreased in both types of poisoning.

5. In phosphorus poisoning liver functional changes can and do occur without concomitant renal changes. Renal insufficiency usually arises as a terminal event.

6. Increased nitrogenous products in the blood (total non-protein nitrogen, urea, and amino nitrogen) are associated with an increase of these bodies in the urine. Consequently, an increased protein catabolism, as well as renal insufficiency, is necessary to explain this accumulation.

7. A terminal acidosis, as evidenced by increased hydrogen ion concentration in the blood, usually occurs.