

II. THE EFFECT OF CYANIDS AND OF ORGANIC OXIDIZING AGENTS ON THE LIVER INJURY CAUSED BY CHLOROFORM*

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The series of experiments here recorded were undertaken with the hope of throwing some light on the etiology of chloroform liver necrosis. Should this liver injury be caused by a reduction of tissue oxidation, it is conceivable that the cyanids, which apparently act by preventing oxidation, might increase the damage to be expected from chloroform if the two drugs were given simultaneously. On the other hand, iodozo- and iodoxy-benzoic acids and their sodium salts, which readily give up oxygen to the tissues, might lessen the expected injury.¹

We have already reported a few experiments in which potassium cyanid was administered intravenously, either alone or in conjunction with prolonged chloroform anesthesia.² Although large doses of the drug itself produce an extensive fatty alteration in the liver parenchyma, smaller amounts, given during the administration of chloroform, affect the liver changes produced by the anesthetic very little, if at all.

Experimental work by Verworn³ tends to show that the depression caused by narcotics is really an asphyxiation. His hypothetical suggestion is that the oxygen carriers are unable to supply oxygen to the tissues, perhaps because the enzymes are of a lipoidal nature with consequent affinity for the usual anesthetics. Burge⁴ reports that the oxidative enzyme catalase is greatly reduced by anesthetics, most pronouncedly by chloroform. This might be considered to support the views of Verworn. However, both the method⁵ and the conclusions of Burge have frequently been questioned.⁶

* From the George Williams Hooper Foundation for Medical Research, University of California Medical School.

† University of California Fellowship.

1. We are indebted to Professor Loevenhart of the University of Wisconsin for liberal supplies of iodozo- and iodoxy-benzoic acids, also for valuable criticisms and suggestions relative to the experiments.

2. Davis, N. C., and Whipple, G. H.: *Arch. Int. Med.* **23**:636 (May) 1919.

3. Verworn, Max: *Harvey Lectures* **7**: 1911.

4. Burge, W. E.: *Am. J. Physiol.* **43**:545, 1917; *Science* **46**:612, 1917; *J. Pharmacol. & Exper. Therap.* **12**:243, 1918; *ibid.* **14**:121, 1919. Burge, W. E., and Burge, E. L.: *J. Biol. Chem.* **41**:307, 1920. Burge, W. E.; Neill, A. J., and Ashman, R.: *Am. J. Physiol.* **45**:388, 1918.

5. Bodansky, M. J.: *J. Biol. Chem.* **40**:127, 1919.

6. Becht, F. C.: *Am. J. Physiol.* **48**:171, 1919. Reimann, S. P., and Becker, C. A.: *J. Physiol.* **50**:54, 1919. Stehle, R. L.: *J. Biol. Chem.* **39**:403, 1919.

Buckmaster ⁷ has shown that at the point of anesthesia with chloroform, from 64 to 71 per cent. of the drug is held by the red corpuscles; after inhalation of 2 per cent. chloroform for three-quarters of an hour, 98.5 per cent. is found in the erythrocytes. He also demonstrated that in the first three minutes of anesthesia lung ventilation is reduced from 30 to 80 per cent.; he considered, however, that the reduction of oxygen in the blood is not due to the latter cause but to the effect of the chloroform on the corpuscles (oxygen carriers).

Graham ⁸ has stated that chloroform is one of a group of substances whose effect on organs is like that of asphyxiation. He surmised that these drugs dissociate in the body, yielding a molecule with bivalent or unsaturated carbon which eagerly seizes oxygen. However, in a recent article ⁹ he has dismissed this theory. Buckmaster ⁷ has reported that he could not detect the formation of carbon monoxid from chloroform in the body.

Wells ¹⁰ has suggested that chloroform may first unite with the cell lipoids, perhaps physically, then poison the protoplasm by interfering with oxidative or synthetic functions.

The standard textbooks of pharmacology discuss the cyanids quite fully; Evans' ¹¹ article on "Cyanid Anoxemia" is a good recent contribution. Work seems to show that the cyanids prevent oxidations in the tissues themselves, whether by action on protoplasm directly or by inhibition or destruction of intermediary enzymes is not quite clear. The oxygen carrying power of the blood is not impaired.

Loevenhart and his co-workers have for many years been interested in physiologic oxidations. They have been able to produce central necrosis in rabbits' livers by suboxidation, the animals being left in an atmosphere of low oxygen tension for several days at a time.¹² Loevenhart and Grove,¹³ also Arkin ¹⁴ have studied the preparation of iodoxy-

7. Buckmaster, G. A.: *Proc. Roy. Soc. Med., Lond., Sect. Anesth.* **11**:15, 1918.

8. Graham, E. A.: *J. Exper. M.* **15**:307, 1912; *J. A. M. A.* **69**:1666 (Nov. 17) 1917.

9. Graham, E. A.: *Arch. Int. Med.* **25**:575 (June) 1920.

10. Wells, H. G.: *J. A. M. A.* **46**:341 (Jan. 29) 1906; *Arch. Int. Med.* **1**:589 (April) 1908; *Chemical Pathology*, Philadelphia, W. B. Saunders Co., 1918, Ed. 3.

11. Evans, C. L.: *J. Physiol.* **53**:17, 1919.

12. Martin, G. H.; Bunting, C. H., and Loevenhart, A. S.: *J. Pharmacol. & Exper. Therap.* **8**:112, 1916.

13. Loevenhart, A. S., and Grove, W. E.: *J. Pharmacol. & Exper. Therap.* **1**:289, 1909.

14. Arkin, A.: *J. Infect. Dis.* **13**:408, 1913.

15. Amberg, S., and Knox, J. H. M.: *J. Pharmacol. & Exper. Therap.* **3**:223, 1911. Amberg, S.: *Ztschr. f. d. ges. exper. med.* **2**:19, 1913. Amberg, S.; Loevenhart, A. S., and McClure, W. B.: *J. Pharmacol. & Exper. Therap.* **10**:209, 1917.

16. Loevenhart, A. S., and Grove, W. E.: *J. Pharmacol. & Exper. Therap.* **3**:101, 1911. Grove, W. E., and Loevenhart, A. S.: *J. Pharmacol. & Exper. Therap.* **3**:131, 1911.

benzoic and iodozo-benzoic acids. Amberg,¹⁵ Loevenhart,¹⁶ Arkin,¹⁷ and others have studied the effect of these acids and their sodium salts on inflammatory reactions, and the antagonism to cyanids. It has been found that cyanids intensify and organic oxidizing agents diminish inflammatory processes, such as the mustard oil reaction and the intracutaneous reaction in serum sensitization; these results seem to be due to the effect on bodily oxidations and reductions. Arkin¹⁸ tested the bactericidal property of sodium iodoxy-benzoate and found a definite relationship between this and its oxidizing power. Arkin and Fink¹⁹ also studied the effect of this sodium salt on catalase in the blood and tissues; they found that catalase normally varies immensely and that the values after administration of iodoxy-benzoate fell within normal limits. Loevenhart and associates²⁰ have recommended the therapeutic use of sodium cyanid as a respiratory stimulant. Loevenhart and Eyster²¹ found that iodozo- and iodoxy-benzoates were unable to replace molecular oxygen for the maintenance of activity in the isolated mammalian heart. These oxidizing agents were reduced, however, and caused the heart to develop rigor, probably because of the oxidation of some substance in its musculature. Iodozo- and iodoxy-benzoates apparently transfer their available oxygen directly to the substance to be oxidized, without an intermediate liberation of free oxygen; certain oxidizing enzymes seem to facilitate the transfer; in certain peroxidase reactions the organic oxidizing agents act in a manner comparable to hydrogen peroxid.¹⁶

METHODS

Dogs have been used as experimental animals. Rats were tried, but even the controls differed so much in reaction that the experiments are of little value; these individual variations were probably due, in part, to nutritional differences and the difficulty of imposing a fasting period to reduce the animals to a starvation base line; moreover, the immediate mortality from chloroform injection was very high. Dogs have always been starved three or four days to eliminate the effect of diet on the chloroform injury.

Squibbs' chloroform has been used for both anesthesia and injections. Chloroform anesthesia has been light; the drug was administered by the drop method. When given subcutaneously, chloroform was mixed with twice its volume of liquid petrolatum; syringe plungers

17. Arkin, A.: *J. Infect. Dis.* **16**:349, 1915.

18. Arkin, A.: *J. Pharmacol. & Exper. Therap.* **3**:145, 1911.

19. Arkin, A., and Fink, E. B.: *J. Infect. Dis.* **22**:515, 1918.

20. Loevenhart, A. S.; Lorenz, W. F.; Martin, H. G., and Malone, J. Y.: *Arch. Int. Med.* **21**:109 (Jan.) 1918.

21. Loevenhart, A. S., and Eyster, J. A. E.: *J. Pharmacol. & Exper. Therap.* **5**:21, 1913.

were removed and the substances measured directly into the barrels, first, the oil then the chloroform from a 1 c.c. pipet calibrated to hundredths; the mixtures were injected immediately after measurement. Dogs were under light ether anesthesia during the injection of chloroform and other substances. The amount of chloroform injected has not been of itself sufficient to produce unconsciousness, or even instability of equilibrium in many cases.

A liver injury produced by either method may be repeated accurately under the same experimental conditions if an intervening recovery period of three or four weeks is allowed. This possibility has been discussed in a previous communication²² and subsequent experiments have verified our belief.

Specimens of iodozo- and iodoxy-benzoic acids were furnished by Dr. Loevenhart. His directions for preparing solutions for intravenous injections were briefly: Dissolve 1.32 gm. iodozo-benzoic acid, or 1.4 gm. iodoxy-benzoic acid, in 20 c.c. water and 10 c.c. normal sodium hydroxid and dilute to 100 c.c. with water; solutions are to be prepared fresh just before using (intravenously). We never had occasion to use 100 c.c. at one time, hence we made up smaller amounts proportionately and by similar steps. To facilitate giving over an extended period of anesthesia these amounts were further diluted with physiologic sodium chlorid solution. When given subcutaneously, iodoxy-benzoic acid was used in relatively large amounts in small volumes of alkaline fluid. Since sodium hydroxid was the solvent, the drug actually employed was sodium iodoxy-benzoate or iodozo-benzoate, as the case might be; however, in the tables and protocols the record gives only the amount of acid corresponding to the volume of solution used. For instance, if 0.2 gm. acid was dissolved in 200 c.c. alkaline fluid, and 150 c.c. of this solution was used, the records show 0.15 gm. acid administered. Iodoxy-benzoate solution given subcutaneously was diluted with 10 per cent. acacia solution; iodozo-benzoic acid was given subcutaneously as a suspension in 10 per cent. acacia solution.

The potassium cyanid employed was a Braun-Knecht-Heimann product, from 98 to 99 per cent. pure; the sodium cyanid was supposed to be chemically pure. The cyanids were dissolved in physiologic sodium chlorid solution immediately before use.

Large amounts of fluid given intravenously have been administered by gravity method, from a funnel through a rubber tube with a glass section near the vein to act as a window, into the external jugular vein through an ordinary large syringe needle; rapidity of flow has been controlled with a screw pinch-cock.

22. Davis, N. C., and Whipple, G. H.: *Arch. Int. Med.* **23**:612 (May) 1919.

In case the dogs survived, it has been our routine to operate two days after the administration of chloroform and remove a small piece of liver, either by ligating and snipping off the tip of a pointed lobe, or by inserting a mattress suture in the edge of a larger lobe and excising a wedge of tissue from the area thus deprived of blood. Operations have been performed always under ether anesthesia, and with aseptic precautions. We consider that the best time to estimate tissue injury is on the second day following the injection of the chloroform, since the necrosis is then usually well demarcated and debris is not yet in process of removal. Specimens are fixed in 10 per cent. formaldehyde solution and divided for (1) frozen sections (fat stains, etc.), and (2) paraffin embedding with subsequent hematoxylin and eosin staining. Estimates of necrosis and fatty degeneration are always based on microscopic examination of the paraffin sections.

The fact that we consider one small specimen of liver tissue as representative of the whole may appear unwarranted. Dowler and Mottram²³ studied the fat content of different parts of the liver after injection of fat into the hepatic artery, glycogen after a heavy meal of cane sugar, and fatty acids during fasting periods, and concluded that the distribution of these substances is irregular and unpredictable; however, they admit that the liver of a fasting dog gives the nearest approach to even distribution. Sérège²⁴ in determining urea and glycogen, and Bartlett, Corper and Long²⁵ in studying the distribution in the liver of fat injected in the portal vein found considerable differences between the lobes as a whole, but intralobular uniformity. Differences of distribution of these various substances have been correlated with differences in blood supply, i.e., the blood from various portal tributaries does not thoroughly mix, hence is distributed differently. On the other hand, Brissaud and Bauer,²⁶ also Gilbert and Villaret²⁷ found an equal distribution of injected gelatin throughout the liver.

When dogs have died of chloroform liver injury we have always taken sections from various parts of the liver, representing several lobes. Ignoring the single layer of liver lobules immediately beneath the capsule (which are usually severely injured), these sections have invariably shown the same picture. Consequently, we have always felt justified in basing our estimations on a biopsy specimen from one lobe only.

23. Dowler, V. B., and Mottram, V. H.: *J. Physiol.* **52**:166, 1918.

24. Sérège: *Compt. rend. Soc. d. Biol.* **54**:200, 1902; **56**:597, 600, 1904; **58**:519, 1905. (Cited by Dowler.)

25. Bartlett, F. K.; Corper, H. J., and Long, E. R.: *Am. J. Physiol.* **35**:36, 1914.

26. Brissaud and Bauer: *J. d. l'Anat.* **45**:1, 1909.

27. Gilbert, A., and Villaret, M.: *Arch. d. méd. exper.* **21**:373, 1909.

EXPERIMENTAL OBSERVATIONS

Table 1 summarizes the essential details of experiments in which organic oxidizing agents were given intravenously during chloroform anesthesia. A representative protocol follows; it is scarcely necessary to add protocols for all the dogs.

EXPERIMENT 2.—*Control. Simple Starvation and Chloroform Anesthesia.*
Dog 19-39, female Airdale.

Oct. 8, 1918: Weight, 26.1 pounds. Isolated before daily feeding; in good condition. Fasting begun.

October 9: Weight, 25.8 pounds; October 10: weight, 24.8 pounds. No food.
October 11: Weight, 24.5 pounds. Active and healthy. Chloroform anesthesia for one and a quarter hours (from 5:50 to 7:05 p. m.).

October 12: Weight, 23.1 pounds. Bright and active.

October 13: Weight, 22.9 pounds. Bright and active.

October 14: Weight, 22.8 pounds. Piece of liver removed at 9:30 a. m. Sections show approximately 60 per cent. necrosis (beginning resolution); fat (scattered). Mixed diet.

Chloroform Anesthesia Plus Sodium Iodoxy-Benzoate Intravenously.—
Dog 19-39, female Airdale.

March 5, 1919: Weight, 33.8 pounds. Isolated before daily feeding; fasting begun; has completely recovered from previous experiments; in excellent condition.

March 6 and 7: No food.

March 8: Weight, 31.5 pounds. Active and healthy. Chloroform anesthesia for one and a quarter hours (from 2:45 to 4 p. m.). Intermittently during this period 0.21 gm. iodoxy-benzoic acid (freshly prepared in 15 c.c. alkaline solution, then diluted to 200 c.c. with physiologic sodium chlorid solution) was given intravenously. No ill effects of moment were observed either during or following the injection.

March 9: Active. Mixed food.

March 10: Active. Piece of liver removed at 1:30 p. m. Considerable bleeding occurred. Milk and mixed diet. Sections show approximately 60 per cent. necrosis with a scattering of fat in the dead tissue.

These experiments seem to indicate that iodoxy- and iodozo-benzoic acids have little, if any, protective value against liver necrosis when given intravenously. This may be due to the small amounts of the drugs so given. The intravenous solutions were administered slowly and fairly uniformly throughout anesthesia, intermittently (as in Experiment 2), or steadily during the first forty-five or sixty minutes of anesthesia and none the last few minutes. In Experiment 1 some difficulty was encountered due to apnea during anesthesia, but the dog roused somewhat afterward; death, probably from the iodoxy-benzoate, occurred too early to determine accurately the extent of liver injury; there seemed to be some central disintegration, however. In Experiment 4 there appeared to be a definite lessening of necrosis after injection of iodozo-benzoate; in this case intravenous injection was continued steadily during the first hour of anesthesia; the liver showed one-quarter to one-third necrosis with extensive vacuolization and fat up to one-half; the uninjured lobule peripheries showed considerable

TABLE 1.—CHLOROFORM INHALATION PLUS ORGANIC OXIDIZING AGENTS INTRAVENOUSLY

Exptl- ment Number	Dog Number	Date, 1913	Weight, Lbs.	Fasting Period, Days	Chloro- form Anes- thesia, Hours	Organic Oxidizing Agent	Liver Injury		Remarks
							Oxidizer Plus Chloro- form	Control Chloro- form	
1	19-30	Feb. 12	18.6	4	1¼	0.21 gm. iodoxy- benzoic acid	?	60% necrosis	Found dead next morning; liver shows some central disintegra- tion, necrosis not determin- able; viscera congested
2	19-39	Mar. 8	31.5	4	1¼	0.21 gm. iodoxy- benzoic acid	60% necrosis	60% necrosis	
3	19-74 (a)	Feb. 25	13.6	4	1¼	0.14 gm. iodoxy- benzoic acid	50% necrosis	50% necrosis	
4	19-74 (b)	Apr. 16	13.8	4	1¼	0.25 gm. iodozo- benzoic acid	25% necrosis	50% necrosis	Glycogen appears in liver lobule peripheries, suggesting dog fed by mistake during starvation period
5	19-28	May 6	19.1	4	1¼	0.24 gm. iodozo- benzoic acid	50% necrosis	50% necrosis	
6	19-135	May 24	46.5	4	1¼	0.35 gm. iodozo- benzoic acid	60% necrosis	Operation two days following chloroform; found dead on sixth day following operation; intense jaundice; hemorrhages in viscera; slight removal of liver debris

glycogen. This glycogen content leads to the question whether or not the dog received food by mistake during the preliminary period of supposed fasting. This storage might have occurred after chloroforming and before operation, but more likely it took place before the administration of chloroform. Glycogen per se may have no protective value against chloroform, but its presence is certainly somewhat of a gage of carbohydrate assimilation, which for some reason is protective. A smaller amount of iodoxy-benzoate had no protective value for this same dog (Experiment 3). Unfortunately, there is no control on the same animal in Experiment 6, but judging from the injuries sustained by the other dogs recorded in the same table, there appears to have been no protection from the amount of iodozo-benzoic acid given.

To summarize Table 1, there seems to have been no protection against chloroform injury from intravenous administration of iodozo- and iodoxy-benzoates in five of six experiments; there seems to have been protection in one case from iodozo-benzoate, but this protection may very well have been from food given by mistake, and not from the drug.

Table 2 summarizes briefly the experiments in which chloroform was given subcutaneously with or without organic oxidizing agents and more ample summaries of certain experiments follow.

EXPERIMENT 13.—Dog 20-17, young, black mongrel, female.

Sept. 26, 1919: Weight, 18.9 pounds. Isolated before daily feeding; healthy and active; fasting begun.

September 27 and 28: No food.

September 29: Weight, 17.2 pounds (7.8 kilos). Active. Gave subcutaneously 1.17 c.c. chloroform (0.15 c.c. per kilo) plus 2.4 c.c. liquid petrolatum; temporary ether anesthesia.

September 30: Bright and active.

October 1: Weight, 16.5 pounds. Removed piece of liver at 2 p. m. Sections show about 35 per cent. necrosis with severe vacuolation and injury up to one-half; fat moderate. Recovery on mixed diet.

EXPERIMENT 21.—Dog 20-17, young black mongrel, female.

October 21: Weight, 20 pounds. In good condition and active; isolated after daily feeding; fasting begun.

October 22, 23 and 24: No food.

October 25: Weight, 18 pounds (8.18 kilos). Active. Gave subcutaneously 1.22 c.c. chloroform (0.15 c.c. per kilo) plus twice its volume liquid petrolatum, followed immediately by 1 gm. iodoxy-benzoic acid dissolved in 7 c.c. tenth-normal sodium hydroxid plus 10 c.c. of a 10 per cent. acacia solution subcutaneously. Temporary ether anesthesia.

October 26: Bright and active.

October 27: Active. Piece of liver removed at 1 p. m. Hard swelling in left lumbar quadrant at point of injection of iodoxy-benzoate. Sections show from 10 to 15 per cent. necrosis; very slight amount of fat over central half of lobules. Recovery on mixed diet. Slough at point of injection.

As will be noted from Table 2, massive doses of the organic oxidizing agents given subcutaneously apparently have protective value against chloroform injury; in some cases this is quite striking. Iodoxy-benzoic

TABLE 2.—COMPARATIVE TABLE—SUBCUTANEOUS INJECTIONS

Chloroform Alone						Chloroform and Oxidizing Agent						
Experiment Number	Dog Number	Fasting Period, Days	Chloroform C.c. per Kilo (subcutaneously)	Liver Injury	Remarks	Experiment Number	Dog Number	Fasting Period, Days	Chloroform C.c. per Kilo (subcutaneously)	Organic Oxidizing Agent (subcutaneously)	Liver Injury	Remarks
7	20-7	4	1.00	75% necrosis	Found dead on second day	19	20-38	4	0.20	Iodoxy-benzoic acid 0.6 gm.	50-60% necrosis	Found dead on seventh day; apparently from toxemia; injection slough; liver repairing well
8	20-10	4	0.50	80% necrosis	Died on second day	20	20-65	4	0.22	Iodoxy-benzoic acid 0.5 gm.	60% (+) necrosis	Operation on second day; found dead on fourth day; debris in liver largely removed; dog developed distemper during experiment
9	20-12	4	0.20	80% necrosis	Found dead on third day							
10	20-13	3	0.10	No necrosis; trace of fat	Minimal injury							
11	20-13	3	0.20	75% necrosis	Found dead on third day	21	20-17	4	0.15	Iodoxy-benzoic acid 1 gm.	10-15% necrosis; slightest possible trace fat	Recovered
12	20-18	4	0.16	70% necrosis	Found dead on next day							
13	20-17	4	0.15	33% necrosis; severe injury to $\frac{1}{2}$	Recovered; see experiment 21 on same dog							
14	20-75	3	0.15	50% (+) necrosis	Operation on second day; found dead on fourth day	22	20-57	3	0.21	Iodoxy-benzoic acid 0.8 gm.	No necrosis; no fat	Liver sections from operation show glycogen; died on seventeenth day from pneumonia; injection slough
15	20-76	3	0.21	60% necrosis	Recovered; see experiment 23 on same dog; second control on same dog shows only about 50% necrosis	23	20-76	3	0.21	Iodoxy-benzoic acid 0.8 gm.	45% necrosis; very severely injured up to $\frac{1}{2}$	Recovered; very slight injury from injection
16	20-31	4	0.18	55% necrosis	Found dead on second day; see experiment 24 on same dog	24	20-31	4	0.18	Iodoxy-benzoic acid 0.5 gm.	Slightest possible trace necrosis; trace fat over $\frac{1}{2}$	Recovered; injection slough
17	20-30	4	0.14	60% necrosis	Recovered; same result obtained on two occasions, once before and once after experiment 25	25	20-30	4	0.14	Iodoxy-benzoic acid 0.5 gm.	45% necrosis	Recovered; injection slough
18	20-106	4	0.14	85% necrosis	Died on second day							

acid was dissolved in tenth normal sodium hydroxid about 0.1 gm. to 1 c.c., and the volume increased by addition of 10 per cent. acacia solution; no doubt the ugly sloughs obtained in such instances were due to the alkalinity of the solution (unfortunately much stronger than necessary), not to the drugs used; controls where alkaline solutions alone were used showed the same sloughs. Iodozo-benzoic acid was given suspended in 10 per cent. acacia solution; these injections did not cause such severe reactions, but the drug seemed to be absorbed only partially. With the exception of the sloughs already mentioned there have been no ill effects noted from injections of either drugs. On one occasion a large dose of iodozo-benzoic acid suspension was given to a dog intraperitoneally; this caused an acute reaction and contributed largely to the dog's death within a few hours (chloroform was given also, subcutaneously). Intraperitoneal injections of from 10 to 20 mg. iodozo-benzoic acid in rats sometimes seem very toxic, in other cases apparently cause no reaction.

It is interesting to note the very small doses of chloroform which may prove fatal when given subcutaneously to dogs after a preliminary fast. Experiments 10 and 11 indicate the narrow zone of safety between a dose producing no injury and that causing death.

Experiment 22 indicates superficially a very marked protection, but since the liver showed glycogen at operation, it may be a food protection, either because of an inadequate starvation period, or because of feeding by mistake on fasting days.

Table 3 merely amplifies experiments recorded in Table 2 and adds Experiments 26 and 27 to complete the record of Dog 20-30. Experiment 26 will be taken up later in discussing the use of cyanids. Experiment 27 is merely a repetition of 17, to act as double control. This double check was attempted on Dog 20-76 (Table 2), but the second trial with chloroform alone showed only about 50 per cent. necrosis, compared with the original 60 per cent.; this is the only time that two controls on the same animal have failed to check, and this is not a very wide fluctuation considering that estimations are merely relative.

It will be noted that Experiment 17 was intended as a control not only for Experiment 25 on the same animal, but for Experiment 16 on Dog 20-31; while Experiment 24 controls Experiment 16 on the same animal. A point worth noting is that the weights of these dogs in control experiments were less than in the others; in such cases the animals actually received less chloroform; but in the cases with greater weights the surplus fat may have been more than sufficient as a block to chloroform to compensate for the greater injection, ignoring any possible effect from organic oxidizing agents. However, no such

TABLE 3.—EFFECT OF IODOXY-BENZOATE

	Experi- ment Num- ber	Date of Injection	Fast- ing Period, Days	Weight at Time of Injec- tion, Kilos	Chloro- form, C.c. per Kilo (subcuta- neously)	Potas- sium Cyanid (subcuta- neously), Mg.	Iodoxy- benzoic Acid (subcuta- neously), Gm.	Amount of Liver Injury	Remarks
Dog 20-30 Small male terrier	17	11/10/19	4	5.80	0.14	60% necrosis; slightest pos- sible trace fat	Questionable distemper; during convales- cence; wound healed slowly
	25	12/18/19	4	6.42	0.14	..	0.5	45% necrosis; slight trace fat	Iodoxy-benzoic acid dissolved in alkaline acacia solution; injection slough
	26	2/ 4/20	4	6.30	0.14	25	...	45-50% necrosis; slight trace fat	Potassium cyanid dissolved in 12 c.c. physi- ologic sodium chlorid solution
	27	3/22/20	4	6.02	0.14	60% necrosis; slight trace fat	Experiment performed as second control
Dog 26-31 Young female collie	16	11/10/19	4	6.60	0.18	..	0.5	Slightest possi- ble necrosis; trace of fat over % of each lobule	Iodoxy-benzoic acid dissolved in alkaline acacia solution; questionable distemper during convalescence; injection slough
	24	12/18/19	4	5.68	0.18	55% necrosis; slightest pos- sible trace fat	Alkaline acacia solution injected; found dead on second day; necrosis at point of injection; edema and congestion in lungs

explanation will hold in the case of Dog 20-76, for here the weights in control experiments were practically the same, and both slightly greater than the weights in experiments when other substances were injected, in which latter cases the injuries sustained were less.

The tables and protocols which follow present a few experiments in which cyanid were used in conjunction with chloroform.

EXPERIMENT 28.—*Control; Chloroform Anesthesia.* Dog 19-74, young black and white male terrier.

Dec. 7, 1918: Weight, 11.8 pounds. In good condition; fourth day of fasting; chloroform anesthesia for one and a quarter hours.

December 9: Weight, 11.1 pounds. Active. Piece of liver removed in afternoon; bled freely. Sections show necrosis involving one-half of each liver lobule, and a moderate degree of fatty degeneration in zone surrounding necrosis.

Chloroform Anesthesia; Cyanid Subcutaneously. Dog 19-74, a black and white male terrier.

Oct. 18, 1919: Weight, 15 pounds. In good condition; isolated before daily feeding; fasting begun. Since the control experiment (given above) was performed, this animal has been subjected to two experiments in which organic oxidizing agents were injected intravenously (Table 1), also a second control with result the same as in the first.

October 19 and 20: No food.

October 21: Weight, 13.9 pounds. Active. Chloroform anesthesia for one and a quarter hours. Gave 15 mg. potassium cyanid in 30 c.c. physiologic sodium chlorid solution subcutaneously in divided doses at intervals during anesthesia.

October 23: Weight, 13.3 pounds. Active. Piece of liver removed at 1:30 p. m.; many adhesions; considerable bleeding; some subcutaneous edema in area of injections (due to alkalinity of potassium cyanid). Sections show approximately 60 per cent. necrosis.

EXPERIMENT 15.—*Control; Chloroform Subcutaneously.* Dog 20-76, a young, black mongrel, female.

Jan. 24, 1920: Weight, 14.2 pounds. Active and in good condition. Isolated before daily feeding; fasting begun.

January 25: No food.

January 26: (Third day of fasting.) Weight, 12.9 pounds (5.85 kilos). At 5 p. m. gave 1.23 c.c. chloroform (0.21 c.c. per kilo) plus 2.5 c.c. liquid petrolatum subcutaneously; temporary ether anesthesia.

January 27: Weight, 12.4 pounds. Quite active.

January 28: Weight, 12.3 pounds. Dog in fair condition. Piece of liver removed at 3:30 p. m. Sections show 60 per cent. necrosis; fat plus.

EXPERIMENT 30.—*Chloroform Subcutaneously; Cyanid Subcutaneously.* Dog 20-76, a young, black mongrel female.

March 19: Weight, 14.8 pounds. In excellent condition. Isolated after daily feeding; fasting begun.

March 22: (Third day of fasting.) Weight, 12.9 pounds (5.85 kilos). Gave 1.23 c.c. chloroform (0.21 c.c. per kilo) plus 2.5 c.c. liquid petrolatum subcutaneously; followed immediately by 35 mg. potassium cyanid in 35 c.c. physiologic sodium chlorid solution subcutaneously; temporary ether anesthesia.

March 23: Active. Full diet.

March 24: Active. Piece of liver removed at 3:15 p. m. Sections show from 35 to 40 per cent. necrosis. Point of injection of potassium cyanid later showed a little local necrosis (due to alkalinity of potassium cyanid).

TABLE 4.—POTASSIUM CYANID AS INFLUENCING CHLOROFORM LIVER INJURY

Experi- ment Number	Dog Number	Weight, Kilos	Fasting Period, Days	Chloroform	Potassium Cyanid (subcuta- neously), Mg.	Liver Injury	Control Liver Injury (Same Dog)	Organic Oxidizing Agent Injection (Same Dog)	Remarks
28	19-74	6.3	4	1½ hours anesthesia	15	60% necrosis	50% necrosis (chloroform anesthesia, no drugs—2 controls with same result)	50% necrosis (chloro- form anesthesia; 0.14 gm. iodoxy-benzoic acid intravenously) 25% necrosis (chloro- form anesthesia; 0.25 gm. iodoxy-benzoic acid intravenously with question of feeding during starva- tion period)	Greater injury caused by potassium cyanid
29	20-17	8.6	4	0.15 c.c. per kilo subcuta- neously	20	35-40% necrosis	35% necrosis (very severe injury up to ½)	10-15% necrosis (1 gm. iodoxy-benzoic acid subcutaneous)	A shade more necrosis in potassium cyanid experiment, but not so much surrounding in- jury
26	20-20	6.3	4	0.14 c.c. per kilo subcuta- neously	25	45-50% necrosis	60% necrosis (2 controls with same result)	45% necrosis (0.5 gm. iodoxy-benzoic acid subcutaneous)	Less necrosis in potas- sium cyanid experi- ment than in controls; about the same as with organic oxidizing agent injection
30	20-76	5.9	3	0.21 c.c. per kilo subcuta- neously	35	35-40% necrosis	(1) 60% ne- crosis (2) 50% ne- crosis	45% necrosis (0.8 gm. iodoxy-benzoic acid subcutaneous)	Less necrosis in potas- sium cyanid experi- ment than in control or when using iodoxy- benzoic acid

Between Experiments 15 and 30 came Experiment 23 in which the dog was given iodozo-benzoic acid subcutaneously (Table 2). In May this dog was used for another control similar to Experiment 15, and showed approximately 50 per cent. necrosis. In a final experiment in June the dog was given 26 mg. sodium cyanid (molecular equivalent of the 35 mg. potassium cyanid in Experiment 30), but 12 mg. of this were injected intraperitoneally and death occurred from respiratory failure in about one hour.

EXPERIMENT 35.—*Chloroform Anesthesia; Sodium Cyanid Intravenously.* Dog 21-18, a brindle mongrel, male; right eye shows keratitis and staphyloma, suggesting previous distemper infection.

Sept. 14, 1920: Weight, 25.5 pounds. In good condition and active. Isolated after daily feeding; fasting begun.

September 15, 16, 17. No food.

September 18: Weight, 23.8 pounds. Active. Chloroform anesthesia for forty-five minutes (3 to 3:45 p. m.). During thirty-five minutes of anesthesia 10 mg. sodium cyanid in 100 c.c. physiologic sodium chlorid solution were given intravenously (0.01 per cent. solution = approximately 1/500 N). Considerable chloroform was administered, but some respiratory difficulty was encountered because of sodium cyanid. More than the 100 c.c. would have been given except for shallow breathing, tendency to apnea, and a rapid, weak pulse.

September 19: Weight, 23.3 pounds. A little dull. Casein feeding.

September 20: Weight, 23.5 pounds. Somewhat dull. Casein feeding. Operation at 3 p. m. Piece of liver removed; liver is grossly injured. Sections show about 35 per cent. necrosis and a trace of fat.

EXPERIMENT 36.—*Chloroform Anesthesia; Physiologic Sodium Chlorid Solution Intravenously.* Dog 21-18. A brindle, mongrel male.

Oct. 19, 1920. Weight, 30.7 pounds. Bright and active. Wounds of former operations healed. Isolated after daily feeding; fasting begun.

October 20, 21 and 22: No food.

October 23: Weight, 27.5 pounds. Active and in good condition. Chloroform anesthesia for forty-five minutes (3:40 to 4:25 p. m.). Gave 100 c.c. physiologic sodium chlorid solution intravenously during anesthesia.

October 24: Weight, 27 pounds. Quite active. Gelatin feeding.

October 25: Weight, 25.5 pounds. Active. No vomiting. Operation at 2:30 p. m. Piece of liver removed; liver appears injured in gross. Sections show approximately 55 per cent. necrosis and a moderate amount of fat.

The experiments in which potassium cyanid was given subcutaneously appear very irregular and contradictory. On account of the immediate toxicity, the drug was given cautiously in rather small dosage; however, this accounts in no way for the fact that some cases show even less injury than the control experiments on the same animals; it may be coincidence that the larger doses of potassium cyanid (25 and 35 mg.) seem to have been somewhat protective. It will be recalled that we found no effect on chloroform liver injury from small intravenous injections of potassium cyanid.

Since in one case a small area of necrosis appeared at the point of injection of potassium cyanid, and in other cases moderate tissue edema

TABLE 5.—SODIUM CYANID INTRAVENOUSLY AS INFLUENCING CHLOROFORM LIVER INJURY

Experiment Number	Dog Number	Weight, Pounds	Fasting Period, Days	Chloroform Anesthesia, Min.	Sodium Cyanid Intravenously, Mg.	Liver Injury	Remarks
31	20-108	17.5	4	45	..	60% necrosis	Control. Recovery; operation on second day following anesthesia
32	20-112	14.8	4	45	..	70% necrosis	Control. Found dead on second day following anesthesia
33	20-119	28.6	4	45	18	60% (+) necrosis; fat very extensive almost to lobule peripheries	Sodium cyanid in physiologic sodium chlorid solution, 1 mg. to 10 c.c.; intervals of accelerated breathing during anesthesia; periods of apnea the last few minutes; recovery; operation on second day following chloroform
34	20-119	27.4	4	45	..	75% (+) necrosis; fat moderate	Control (see Experiment 33). Found dead on second day following anesthesia
35	21-18	23.8	4	45	10	35% necrosis; trace of fat	Sodium cyanid in physiologic sodium chlorid solution, 1 mg. to 10 c.c.; tendency to apnea and rapid, weak pulse, especially toward the last; recovery; operation on second day
36	21-18	27.5	4	45	..	55% necrosis; fat moderate	Control (see Experiment 25). 100 c.c. physiologic sodium chlorid solution intravenously during anesthesia; recovery; operation on second day

was manifested, we must conclude that a considerable local reaction took place (due in part to alkalinity of the drug), and it seems likely that not only the tissue suffered but that a certain part of the potassium cyanid may have been destroyed locally.

Table 5 gives the results of sodium cyanid injections in two dogs; these same animals were later used as controls; two other control experiments on different animals are also included. These experiments indicate that the cyanid may have some protective value against chloroform injury. The attempt was made to give the anesthetic at the same rate in all cases, but on account of respiratory difficulties caused by the cyanid it is possible that the animals receiving such injections were given somewhat less chloroform. This is suggested as a possibility only, but if so might account for the lessened liver necrosis in such cases. For comparative purposes we consider that controls on the same animals are by far the most valuable.

DISCUSSION

Although these experiments were undertaken to throw some light on the rôle of oxidations in chloroform liver injury, the results are far from conclusive. There appears to have been a definite amount of protection in the cases when organic oxidizing agents were injected in large amounts subcutaneously. Should this have been because an increased supply of oxygen was furnished to a liver hampered by its lack, we should have expected cyanids to have acted in an opposite manner, as found by Loevenhart, Amberg and their associates in various conditions; as a matter of fact, relatively large doses of cyanids seem to have been themselves rather protective in certain instances.

Administration of cyanids intravenously during chloroform anesthesia has been difficult because of respiratory irregularities; this trouble has been much more marked than in control experiments (without the cyanid). Loevenhart has had less difficulty in giving a solution of sodium cyanid five times as concentrated as that used by us. There are two factors in our experiments which may account, in part, for this high immediate cyanid toxicity: (1) preceding fasting period, and (2) simultaneous chloroform anesthesia.

We are safe in saying that the experiments do not prove that liver necrosis following administration of chloroform has any connection with a disturbance of normal tissue oxidation.

At the present state of our knowledge it is hardly justifiable to assume that chloroform narcosis and chloroform liver necrosis are manifestations of the same pharmacologic property of the drug; this point is often left rather indefinite in the literature on the subject. It is relatively simple to obtain one without the other. Since our observa-

tions relate entirely to the phenomenon of liver injury, we do not extend our deductions to bear at all upon the existing theories pertaining to the anesthetic action of chloroform.

SUMMARY

Very small amounts of chloroform given subcutaneously are sufficient to cause an extensive liver injury in fasting animals.

The intravenous injection of small amounts of organic oxidizing agents (iodoxy- and iodozo-benzoic acids and their sodium salts) seems to have no effect on chloroform liver necrosis. Large amounts of these substances subcutaneously appear to afford the liver some protection against chloroform injury.

The administration of cyanids (potassium and sodium) does not effect chloroform liver injury in any constant manner; it seems that large doses may exert some protective action.

These experiments offer no evidence that chloroform liver injury is a result of disturbance in tissue oxidations.