FIBRINOGEN OF THE BLOOD AS INFLUENCED BY THE LIVER NECROSIS OF CHLOROFORM POISONING.1

BY G. H. WHIPPLE, M.D., AND S. H. HURWITZ.

(From the Hunterian Laboratory of Experimental Pathology, Johns Hopkins Medical School, Baltimore.)

During the course of some experiments on chloroform poisoning in dogs, it was noted that operations upon many of these animals resulted fatally because of uncontrollable hemorrhage. At autopsy the abdominal cavity was frequently full of fluid blood and, in cases of extreme poisoning, even the blood vessels and heart contained fluid blood and no clots. The present series of experiments was undertaken with the hope of clearing up this feature of chloroform poisoning, but, as the work progressed, it seemed to throw some light on interesting problems connected with blood coagulation and liver function. These experiments seem to indicate clearly that fibrinogen is formed by the liver or that its formation is quite dependent upon the functional activity of that organ.

It will be seen that fibrinogen in the blood can be made to decrease or almost to vanish at will through the production of liver necrosis by chloroform anesthesia. Also, the drop of fibrinogen is found to parallel closely the extent of liver necrosis and, in severe poisoning where the liver shows extensive necrosis, the fibrinogen may be practically absent. In this latter condition, which is not unlike hemophilia, the animal bleeds steadily from large or small cuts. Moreover, the fibrinogen reappears in the blood as the liver begins to repair the injury to its lobules and keeps pace with the repair of the liver cells. The repair is very rapid and may be complete in five or six days. Finally, at the end of the reparatory activity of the liver, we may find an excess of fibrinogen in the blood, an over-production corresponding to Weigert's law of tissue injury and repair (Chart I).

1 Received for publication, August 10, 1910.
It has been shown that the liver is quite capable of repairing the injury done to it by chloroform anesthesia (14). Even when the central necrosis of each lobule involves one-half to three-fifths of all the liver cells, the repair begins on the second or third day and is almost complete in six or seven days. The dead liver cells are removed by autolysis and phagocytosis, and the reticulum is filled in by elongation of the cords of liver cells effected by a rapid mitotic division. The end result is a normal liver. If this mechanism of liver necrosis and repair is kept in mind, it is easy to follow the fluctuation in the fibrinogen content of the blood, as the two are parallel.

The two other factors of blood clotting, thrombin and calcium, have been followed in several of the severe cases of poisoning and are normal. This is in harmony with observations on the character and formation of the blood clots in these cases. Blood drawn from animals poisoned by chloroform anesthesia will clot in the usual time (four to seven minutes), but the clot is more or less flabby, depending upon the amount of fibrinogen present. In severe cases, the clot may be a filmy net-work which will shrink away from the walls of the cylinder and settle to the bottom, forming a very soft, purplish jelly-like mass of not more than five to ten per cent. of the volume of blood. A glass rod can be passed through it with ease, and the threads of fibrin are very delicate and inconspicuous. It is obvious that hemorrhage, which may be a marked feature of these cases, is due to an inefficient blood clot and not to the absence of clotting. It is almost impossible to render an animal's blood quite free from fibrinogen by chloroform poisoning, but it is easy to damage the liver and lower the fibrinogen content to such a degree that the animal will bleed for hours from pricks or cuts in any tissue. It may be said, in general, that these animals do not show a tendency to hemorrhage until the fibrinogen has been reduced 10 per cent. The clot forms in the usual time, but lacks body and stiffness. As the latter characteristic is dependent on the presence of fibrinogen, the clots are too weak and flabby to close the ruptured capillaries and vessels.

A discussion here of the various theories concerning coagulation of the blood is not needed, as excellent reviews of this complicated
Liver Necrosis of Chloroform Poisoning.

subject may be found in the recent writings of Howell (6), Rettger (13), and Loeb (8). We wish to review very briefly some of the work of different observers which is or is not in harmony with our observations and experiments described below.

It has been clearly shown by Doyon and Kareff (2) and by Nolf (11), (12) and his pupils, that extirpation of the liver causes profound changes in the blood, particularly a rapid disappearance of fibrinogen, which may be practically absent at the end of two hours. Nolf claims that the thrombogen also disappears from the blood.

Doyon (3), (4), (5) and his co-workers have reported on several occasions a single experiment with chloroform poisoning. Chloroform was given to the dog by mouth on three consecutive days. The blood obtained one hour before death contained almost no fibrinogen (0.44 per 1,000). Icterus and liver necrosis were present. We have been unable to find any recorded observations, either experimental or clinical, of a very low fibrinogen content of the blood without some accompanying liver injury.

Phosphorus poisoning in animals has added some interesting facts. Corin and Ansiaux (1) have been able to show the absence of fibrinogen in the blood of poisoned animals, and explain the lack of coagulation by this fact. They state that prothrombin and fibrinogen run parallel courses and that both disappear at the same time. Loeb confirmed their findings. They believe that both fibrinogen and prothrombin are formed in the liver. Our observations confirm the findings of Corin and Ansiaux and Loeb in part only, for in our experiments the thrombin remained normal even in the severest cases of chloroform poisoning.

Jacoby (7) states that animals poisoned with phosphorus have no fibrinogen in their blood and that the blood of such animals clots when mixed with normal blood, thus indicating the absence of anticoaguline. We have obtained exactly similar results in chloroform poisoning. Jacoby also states that the injection into a normal animal of the blood of an animal poisoned with phosphorus lowers the coagulability of the latter's blood. In our chloroform experiments we have had a different result, and have shown that, if anything, there is a rise in fibrinogen.

Matthews (9) and, later, Müller (10) found a parallel relationship between the number of leucocytes and the amount of fibrinogen in the blood. Müller injected into animals killed bacterial cultures which produced a rise in fibrinogen in the blood and bone marrow extract, and also a parallel rise in the leucocytes. He concluded that the source of fibrinogen is the leucocyte and that the marrow is a most important factor.

We believe that our experiments exclude the possibility of the marrow's being an important factor and that they show beyond doubt that the white cells are in no way responsible for the fibrinogen of the blood. With the rapid drop in fibrinogen following chloroform poisoning, there is a leucocytosis caused by the liver necrosis and repair. The leucocytosis may be extreme just at the
time when the fibrinogen is lowest. If the liver necrosis is combined with a bronchitis and bronchopneumonia, the white cells may number 320,000 to 330,000 (see table IV, dog c-6), and yet be accompanied by a very low fibrinogen fraction. The bone marrow is never injured by chloroform poisoning and reacts only by a hyperplasia which is the result of the call for blood cells.

**METHOD.**

*Fibrinogen.*—To insure a clear plasma, the dogs were starved for twenty-four hours before the blood was taken from the jugular vein by means of a cannula. To one part of 1 per cent. sodium oxalate in a cylinder were added nine parts of blood. The oxalated blood was thoroughly mixed and then centrifuged for about half an hour. As a rule, the perfectly clear plasma, equal to about one-half the total volume, could be removed with a pipette. The oxalated plasma was then carefully acidified with a few drops of 5 per cent. acetic acid, until at last the very faintest acid reaction to litmus was obtained. Of this slightly acidified plasma, 25 c.c. were taken in a pipette and placed in a centrifuge tube. When the amount of fibrinogen was suspected to be low, 50 c.c. of plasma, if available, were taken instead of 25 c.c. The centrifuge tubes were then placed in a water bath whose temperature was kept carefully at 58° to 60° C., and allowed to remain there for ten to twelve minutes. The tubes were then placed in the centrifuge and flocculent precipitate was thrown down completely by prolonged centrifugalization. It is important that the tubes be centrifuged for a long time at this stage to insure a perfectly clear supernatant fluid. If some of the finer particles are not thrown down, filtration is difficult or impossible, and the procedure becomes very arduous and at times inaccurate. The supernatant fluid is then poured through the weighed filter, and the pure white precipitate is broken up and washed in the centrifuge tubes with an equal volume, 25 to 50 c.c., of cold water.

This mixture was again centrifuged and the supernatant fluid was poured through the filter. The precipitate was then washed several times with hot water. After each washing the mixture was centrifuged, and the supernatant fluid was poured through the weighed filter. This procedure was continued until the supernatant fluid gave no precipitate with silver nitrate. Usually three washings will accomplish this and remove all the chlorides. The precipitate was then washed repeatedly with 95 per cent. alcohol and by means of alcohol was carefully washed into the weighed filter. The precipitate on the weighed filter was washed with ether and allowed to dry in the air. The filter was removed carefully to a weighing tube and suspended in a desiccator over sulphuric acid. The filters were dried in an oven at 110° C. for two hours and replaced in the desiccator for at least two hours. The tubes were weighed subsequently at intervals of one to three days until a constant weight, correct within 0.2 mg., was obtained.

It appears that this method is quite accurate and constant under fixed conditions (compare table VI, dog 95). In this instance, two specimens of blood
Liver Necrosis of Chloroform Poisoning.

were taken on the same day from the same animal, examined, and weighed. There was a difference of only 4.4 mg. between the two specimens. In another experiment two fractions from dog 101 were examined on the same day. One specimen of 25 c.c. gave 0.0092 gm. per 100 c.c., and the other specimen of 50 c.c. gave 0.0120 gm. per 100 c.c. It is probable that the latter determination was more nearly correct. When the blood contains such small amounts of fibrinogen, the limits of error are apparently greater than usual.

To test the method further, blood from a normal animal was allowed to clot in a cylinder, the clear serum was rendered very slightly acid to litmus and heated at 60° C. for ten minutes. Prolonged centrifugation gave no precipitate, indicating that this procedure does not throw down any of the proteins of the blood except fibrinogen. In another experiment the clear supernatant fluid obtained after the first precipitation of fibrinogen by heat and centrifugation, was again placed in a tube and heated at 58° C. for ten minutes. Prolonged centrifugation of this specimen gave no precipitate, indicating that the fibrinogen had been totally precipitated by the first heating and completely removed by centrifugizing.

Calcium.—Measured amounts of blood, 50 c.c. unless otherwise stated, were analyzed for calcium by adding a little nitric acid and evaporating slowly in a porcelain dish until the consistency of a thick syrup was reached. Further evaporation and finally combustion to remove all organic matter, were carried out in a platinum dish. The residue was washed out carefully with a little hydrochloric acid and hot water, boiled in a beaker with sufficient hydrochloric acid to clear up any turbidity, and diluted to 300 to 400 c.c. The whole was neutralized with sodium carbonate until a slight opalescence was obtained. This was cleared again by adding a slight excess of hydrochloric acid. Fifteen grams of ammonium acetate were now added and the whole was boiled for one minute and filtered hot to remove the iron acetate. The precipitate was washed several times with a dilute hot solution of ammonium acetate. The filtrate was evaporated to 200 c.c. and to it were added a small amount of ammonium hydroxide to faint alkalinity and then acetic acid to a faint acid reaction to litmus. On adding 25 c.c. of a 10 per cent. solution of ammonium oxalate, a precipitate of calcium oxalate formed on letting the fluid stand for at least eight hours. The precipitate was collected in the cold on a hard, smooth filter paper, and was washed at least three times with boiling water. The precipitate was then washed into a beaker with hot water, 5 c.c. of concentrated sulphuric acid were added, and the mixture was diluted to 100 c.c. The calcium oxalate was dissolved and the oxalic acid set free. The mixture was then heated to 60° to 65° C and titrated against a standard solution of potassium permanganate from which the amount of calcium was readily determined.

Thrombin.—The determination of thrombin (Schmidt's method) is only qualitative. In the three estimations of this substance, one of which (dog 85) was made by Dr. W. H. Howell, the method of Schmidt, described by Howell and Rettger, was employed.
line and shows little variation except a slight rise, as a rule, when bile pigments were present in the serum. Each vertical division represents 0.002 gm. of calcium per 100 c.c. of blood.

EXPERIMENTS.

The following experiment (dog 104) is summarized, since it is fairly complete and quite typical of all the other experiments. The animal was young, which may explain its great susceptibility to chloroform and the almost fatal result following a single anesthesia of two hours. The poisoning was indicated by a drowsy condition, loss of appetite, repeated vomiting, and especially by the capillary hemorrhages from all cuts. For three days following the chloroform anesthesia, this animal had hemophilia. Blood examination showed no abnormality in its formed elements (Dr. Duke), and the calcium content was normal or above normal. Study of the blood in bulk showed that clotting took place in the normal time, but the clot, instead of being a solid elastic mass, was filmy and not tough and bulky enough to control even capillary hemorrhage. From this evidence, and from that of many other experiments, we are sure that the liver of the dog at this time showed an extensive central necrosis involving a large part of each lobule. Judging from other experiments, we feel sure that liver repair began on the second day and was effective on the fifth day (April 26), for at this time the bleeding stopped and the animal began to improve. Four days later, on the ninth day following the chloroform, operation revealed a liver which had almost completed its regeneration (microscopical section). Blood examination showed a fibrinogen content almost three times as great as before the liver injury, and it is likely that this overproduction resulted from the activity of the regenerating liver cells. Blood examination, after an interval of seventeen days, showed a still higher fibrinogen content, but after a month the fibrinogen had returned to normal.

A second chloroform anesthesia produced in the same dog only a moderate drop in fibrinogen and did not cause the severe symptoms of poisoning observed after the first exhibition of the drug. It is evident that, for some unknown reason, the animal was more resistant to the drug and that the liver was not so gravely injured. It
Liver Necrosis of Chloroform Poisoning.

is possible that the newly formed liver cells are more resistant to the harmful action of chloroform (see also dog c-9), but this increased resistance is not a constant feature.

This experiment in connection with others makes it clear that the fibrinogen of the blood is dependent on liver activity, and that any sudden injury to the liver causes a sudden drop in the fibrinogen. The fibrinogen reappears in the blood in normal or increased amount following the regeneration of the liver cells, and the symptoms of poisoning disappear.

Dog 104.

April 16. Young mongrel hound, female; weight 28½ lbs. Bled under ether anesthesia from jugular vein. The fibrinogen was 0.2192 gm. per 100 c.c., and the calcium was 0.0117 gm. per 100 c.c.

April 17 to 20. Dog recovered well and the neck wound healed rapidly.

April 21, 3 p.m. Chloroform anesthesia for two hours; 1 oz. given. Anesthetic well taken.

April 22. Dog was quite sick and rather drowsy, and vomited repeatedly. Dr. Duke followed the bleeding time of this animal and found it to be greatly delayed. A small ear prick bled for twenty minutes.

April 23. Dog was very quiet and refused to eat. At 11 a.m. it was bled, under ether anesthesia, from jugular vein. The fibrinogen was 0.009 gm. per 100 c.c., and the calcium was 0.0133 gm. per 100 c.c. The blood clot was very flabby and, on standing, settled at the bottom of the graduate. Its volume was about 10 per cent. of the blood in the graduate, and offered scarcely any resistance to a glass rod. The plasma obtained after centrifugation was a clear amber. The animal bled, drop by drop, from the operative wound in the neck during the course of the entire day. A bandage was tied tightly about the neck, but did not suffice to check the flow. The cloth became wet with blood, but there was no clotting.

April 24. Animal was very sick and weak. Bleeding continued and the blood did not clot. Animal given one-fourth grain of morphia subcutaneously.

April 25. Dog was very weak and anemic. The pulse was irregular and the bandage was completely blood-soaked. On removing the bandage the wound continued to ooze blood, drop by drop. There were no clots in the wound or on the bandage.

April 26. In the morning some fluid blood was found under the cage, but the neck wound had stopped bleeding and was distended with a very soft, purple, loosely adherent blood clot. Hemoglobin was 60 per cent. which is about half the normal quantity for a dog.

April 27 and 28. Dog was improving slowly and began to eat.

April 29 and 30. Animal was gaining in weight and eating vigorously. Weight 23 lbs. The blood showed normal coagulation and bleeding time, but signs of active regeneration. There was a leucocytosis, and nucleated red blood cells were numerous in the circulation.
Operation.—At 10 a. m. one-third grain of morphia was given subcutaneously. Ether anesthesia. Incision was made through the right rectus, and the liver was exposed with a little difficulty. The organ was very pale, but the lobulation was quite regular and apparently normal. The centers of the lobules were rather conspicuous and appeared as rusty specks, a condition usually found in the last stages of liver regeneration following the central necrosis of chloroform poisoning. The blood clots perfectly and effectually in the liver wound, and there are no operative difficulties. At the end of the operation the animal was bled and the fibrinogen was found to be 0.6130 gm. per 100 c.c.

Liver.—Microscopical sections. The central fifth of each liver lobule showed a few fat droplets of varying size situated in the liver cells. No necrosis was seen, as all the necrotic debris had been dissolved and removed. The repair was almost complete, but there was an empty reticulum in the very center of each lobule. In this reticulum were a few polymorphonuclear cells and quite a number of large mononuclear phagocytes which contained finely granular, yellow pigment. Mitotic figures were not found, indicating that the active multiplication of the liver cells was completed. Many of the bile canaliculi were dilated with small, yellowish-green hyaline casts.

May 17. Animal was pretty active. It was bled from jugular vein under ether anesthesia. The fibrinogen was 0.7800 gm. per 100 c.c.

May 21. Animal did not eat well and had signs of distemper.

June 11. Animal bled, under ether anesthesia, from jugular vein. The fibrinogen was 0.2848 gm. per 100 c.c.
Liver Necrosis of Chloroform Poisoning.

### Table I. Dog 104.

<table>
<thead>
<tr>
<th>Date</th>
<th>Chloroform</th>
<th>Fibrinogen in 100 c.c.</th>
<th>Calcium in 100 c.c.</th>
<th>Platelets</th>
<th>White blood corpuscles</th>
<th>Jaundice</th>
<th>Blood ting.</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 16</td>
<td>0.2192 gm.</td>
<td>0.0117 gm.</td>
<td>459,000</td>
<td>23,400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 21 2 hrs.</td>
<td>-</td>
<td>-</td>
<td>236,000</td>
<td>5,270</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 22</td>
<td>-</td>
<td>-</td>
<td>321,000</td>
<td>11,000</td>
<td>?</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>April 23</td>
<td>-</td>
<td>0.090 gm.</td>
<td>128,000</td>
<td>8,900</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>April 27</td>
<td>-</td>
<td>0.6730 gm.</td>
<td>279,000</td>
<td>50,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 16</td>
<td>0.7600 gm.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 22 3 hrs.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 23</td>
<td>-</td>
<td>0.3072 gm.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 24</td>
<td>-</td>
<td>0.100 gm.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**June 24.** Autopsy: liver necrosis involved over two-fifths of each lobule.

June 22. Dog 104 was quite well; weight 24 lbs. At 9:25 a.m. chloroform anesthesia for three hours; ½ oz. given. The anesthesia was very light and well taken; recovery rapid.

June 23, 11 a.m. Animal seemed quite well and was apparently not poisoned as by the previous anesthesia. It was bled, under ether anesthesia, from jugular vein. Fibrinogen was 0.3072 gm. per 100 c.c. The plasma, after centrifugation, had a clear pale lemon color. The calcium was 0.009 gm. per 100 c.c.

June 24. Animal would not eat, but seemed pretty well. There was only a little oozing from the neck wound and no vomiting. Bled to death at 4 p.m. under ether anesthesia from carotid artery. Calcium and fibrinogen determined as usual. Serum had a clear amber color and contained bile pigments in considerable amounts. The clots which formed in the graduate were decidedly soft and flabby.

**Autopsy.**—Performed at once. The serous cavities were all normal except for a few old adhesions about the site of operation in the liver. Heart, lungs, spleen, pancreas, stomach, duodenum, and kidneys were normal, gross and microscopically.

**Liver.**—Decidedly pale. The lobulation was distinct, and in the centers of the lobules were seen collapsed pink areas, while the portal tissue was quite opaque and yellow, the usual picture in chloroform poisoning. The organ was large, rather tense, and friable. Microscopical sections showed a moderate grade of hyaline cell necrosis involving the central two-fifths of each lobule. The necrotic areas were invaded by a good many wandering cells and were surrounded by a thin zone of fatty degeneration, in which all of the liver cells contained large and small fat droplets. About half of the liver cells were uninjured and appeared practically normal. Numerous mitotic figures were present in the boundary zone. The very centers of the lobules showed a few large, pigmented mononuclear phagocytes—all that remained to indicate the first chloroform poisoning, from which recovery had been complete. The bile ducts were all normal.

**Dog 81.**

May 5. Large black and white female; weight 44½ lbs. Bled, under ether anesthesia, from jugular vein. Fibrinogen was 0.2972 gm. per 100 c.c., and calcium was 0.009 gm. per 100 c.c.
May 10. Dog was quite well.

May 11. Chloroform anesthesia for two hours; 1 oz. given.

May 12. Dog vomited repeatedly and drank much water. It was thought best not to give more chloroform, as the animal appeared to be too sick.

May 13. Chloroform anesthesia for two hours; 1½ oz. given. As the animal was coming out of the anesthesia, she vomited about 75 c.c. of blood and blood clots, but did not seem dangerously ill. At 6 p.m. the dog seemed very sick.

May 14. At 10 a.m., dog was bled from jugular vein under ether anesthesia. The serum had a clear amber color, the fibrinogen was 0.002 gm. per 100 c.c., and the calcium was 0.0097 gm. per 100 c.c. At 3 p.m., the dog was bleeding drop by drop from the neck incision, and about 50 c.c. had collected in the container under the cage. The dog seemed very sick.

May 15. About 100 c.c. of blood had escaped during the night, due to a steady oozing from the small neck wound. Bleeding stopped for a time during the forenoon, but the animal was still very sick.

May 16. The wound bled again during the night, for about 50 c.c. of unclotted blood was found in the container.

May 17. Dog began to eat and was evidently improving.

May 21. Animal was practically normal and weighed 41½ lbs.

June 16. Dog was bled from jugular vein; the fibrinogen was 0.0084 gm. per 100 c.c. and the calcium was 0.010 gm. per 100 c.c.

June 30. Weight 42 lbs. Animal killed with ether; autopsy performed at

Table II. Dog 81.

<table>
<thead>
<tr>
<th>Time</th>
<th>Chloroform</th>
<th>Fibrinogen in 100 c.c.</th>
<th>Calcium in 100 c.c.</th>
<th>Platelets</th>
<th>Jaundice</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 5</td>
<td>—</td>
<td>0.2972 gm.</td>
<td>0.009 gm.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>May 11</td>
<td>2 hrs.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>May 13</td>
<td>2 hrs.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>May 14</td>
<td>—</td>
<td>0.0020 gm.</td>
<td>0.0097 gm.</td>
<td>179,000</td>
<td>?</td>
<td>++</td>
</tr>
<tr>
<td>May 15</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>May 16</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>May 17</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 16</td>
<td>—</td>
<td>0.2984 gm.</td>
<td>0.010 gm.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 30</td>
<td>—</td>
<td>Autopsy: liver normal.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>
Liver Necrosis of Chloroform Poisoning.

Once. There was an extreme grade of chronic pancreatitis, but the other organs were normal.

Liver.—Microscopical sections showed that the liver repair was complete. In the centers of some of the lobules were seen a few pigmented phagocytes. The architecture was perfectly normal.

Dog 85.
January 13. Black mongrel female pup; weight 20 lbs. Chloroform anesthesia for two hours; $ oz. given. The anesthetic was well taken.
January 14. Dog was quite active and appeared well. Urine contained bile pigment. Chloroform anesthesia for one and one-half hours; $ oz. given.
January 15. Dog seemed a little dull. No vomiting. Urine contained a large amount of bile pigment. 11 a.m., operation under ether anesthesia. Incision was made through the right rectus. The liver was exposed and a small wedge-shaped piece was removed. The lobulation was conspicuous and there was evidently a good deal of necrosis. At the site of operation there was almost no clotting of the blood.

Thrombin.—The dog was bled to death at once from the carotid for the determination of thrombin. In a graduate 100 c.c. of blood clotted in the normal time. After one hour the clot retracted and settled at the bottom of the graduate. Its volume was 5 c.c. The clot was almost semifluid and offered no resistance to the passage of a glass rod through it. After centrifugalization the serum had a perfectly clear lemon color and contained a large amount of bile pigment. Fifty cubic centimeters of the clear serum were precipitated with twenty volumes of 95 per cent. alcohol and were examined after a period of six weeks by Dr. W. H. Howell, who demonstrated that thrombin, in normal amount, was present.

Autopsy.—The autopsy was performed at once. The serous cavities were normal; the heart and lungs showed nothing of interest; the thymus was full of small ecchymoses; and the pancreas, stomach, duodenum, and kidneys were all normal.

Liver.—The liver showed the characteristics seen in severe chloroform poisoning. It was swollen, tense, and very friable. The lobulation was conspicuous. The centers of the lobules appeared as bright red dots. The liver tissue elsewhere was opaque and yellow. Microscopical sections showed that nearly all the cells had undergone hyaline necrosis. One or two rows of relatively normal liver cells remained about the portal structures.

If we judge from other experiments, it is very unlikely that this animal would have recovered from such an extreme degree of liver necrosis. Unfortunately no accurate determination of fibrinogen was made in this case, but from other experiments we may be sure that it was reduced to a minimum.

At this point, we may refer again to the papers of Nolf, Loeb, and Corin and Ansiaux, who are convinced that the thrombin or
prothrombin has its origin in the liver or, at least, is dependent upon liver activity for its presence in the blood. The last experiment and the two that follow speak strongly against this view, for in these we have extreme chloroform poisoning with liver necrosis involving almost all the cells in each lobule and with the merest trace of fibrinogen in the blood, yet the thrombin was present.

Dog 88.
January 28. Large pregnant female; weight 47 lbs. Chloroform anesthesia for two hours; 1 oz. given. During the anesthesia, incision through the median line, exposure of the pregnant uterus, and removal of a part of the left uterine horn, in which a good-sized fetus was contained.


January 30. Condition remained the same. Urine contained a large amount of bile pigment.

January 31. Animal was quite well. Chloroform anesthesia for two hours; 1½ oz. given. The anesthetic was not well taken and muscular tremors were marked. During the anesthesia, an operative incision was made through the right rectus with exposure of the right uterine horn. A large part of this was resected, two fetuses being removed. Due to placental separation, there had been some intra-uterine hemorrhage. At the time of operation there was much bleeding because of inefficient clotting.

February 1. Animal looked weak. At 2 p. m. animal vomited mucus and water and appeared quite drowsy. Under anesthesia the dog was bled from the carotids and a large amount of blood was collected for the study of thrombin. After the blood clotted, the serum was centrifugalized and 50 c.c. of the clear amber-colored fluid was removed and precipitated with 950 c.c. of 95 per cent alcohol. After standing for five months the precipitate was collected, dried, tested as described above, and thrombin was demonstrated to be present. The blood obtained from this dog clotted in the usual time, but the clots were small and very flabby, breaking up when simply poured out of the cylinder.

Autopsy.—Heart, lungs, spleen, stomach, and intestines were normal. The uterus contained six fetuses. Of these one was alive when removed from the uterus, but three were partly macerated, due to placental hemorrhage and separation. Placental necrosis, hemorrhage, and separation are the rule in pregnant dogs after chloroform anesthesia.

Liver.—In gross, the liver presented the usual appearance. On microscopical section, all the lobules showed central necrosis involving about one-fourth to two-fifths of the liver cells. The remaining liver cells showed an extreme grade of fatty change and were packed with large and small fat droplets. Mitotic figures were not very numerous.

Dog 101.
March 19. Old mongrel hound, male; weight 41½ lbs. Animal was bled, under ether anesthesia, from jugular vein. The fibrinogen was 0.3316 gm per 100 c.c. Serum was clear as water. Chloroform anesthesia for one and one-
Liver Necrosis of Chloroform Poisoning.

half hours; 1½ oz. was given. In the afternoon the dog seemed badly poisoned.

March 20. Animal was very quiet and would not eat. Chloroform anesthesia for one hour; 1 oz. was given. The anesthetic was well taken.

March 21. Chloroform for one and three-fourth hours; 1½ oz. was given.

March 22. Dog seemed pretty well. Under ether anesthesia the dog was bled from carotid. Part of the blood was obtained in oxalate for the determination of fibrinogen. Two different fractions were weighed in order to test the method. One fraction of 50 c.c. gave a fibrinogen content of 0.0120 gm. per 100 c.c. The other fraction of 25 c.c. gave a fibrinogen content of 0.0093 gm. per 100 c.c. The serum was colored a perfectly clear amber. Fifty cubic centimeters of blood were analyzed for calcium and gave a normal amount (0.0108 gm. per 100 c.c.). Thirty cubic centimeters of clear amber-colored serum were analyzed for calcium and gave 0.0083 gm. per 100 c.c. A large amount of blood was received into cylinders and clotted in the usual time. The blood clots were very tenuous and soft. They settled on standing into soft purple masses, occupying 10 to 20 c.c. per 100 c.c. of blood. These clots offered practically no resistance to a glass rod. In order to determine the thrombin, 50 c.c. of clear serum were added to 950 c.c. of 95 per cent. alcohol. The thrombin was subsequently shown to be present.

Autopsy.—The autopsy was performed at once. Thorax, lungs, and spleen were normal. The heart contained one large filaria in the conus of the right ventricle. In the pancreas there were a few small inconspicuous fat necroses just below its peritoneal covering and uniformly distributed throughout all its parts. The medullary rays of the kidneys contained many tubules which were full of fat droplets. The condition was more marked than in the normal dog's kidney.

Liver.—The organ was considerably enlarged. The centers of the lobules were bright red and rather sunken. The periphery of each lobule was translucent, but the middle zone was decidedly opaque and yellow. There were no hemorrhages. On microscopical section each lobule showed evidence of a central necrosis involving about one-half of the cells. Much of the necrotic material had been dissolved and removed, leaving an empty reticulum containing a good many phagocytes and dilated capillaries. Many of the dead liver cells remained as spherical or irregular oval granular hyaline masses in which

<table>
<thead>
<tr>
<th>Table III. Dog 101.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date.</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>March 19</td>
</tr>
<tr>
<td>March 20</td>
</tr>
<tr>
<td>March 21</td>
</tr>
<tr>
<td>March 22</td>
</tr>
<tr>
<td>March 22</td>
</tr>
</tbody>
</table>

March 22 Autopsy: liver necrosis invaded one-half of each lobule.
no nuclei were seen. Polymorphonuclear cells were not very numerous. The liver cells about the portal spaces were much swollen and contained numbers of fat vacuoles. Mitotic figures were very numerous, and repair by the remaining intact liver cells was evidently going on with great rapidity. The portal structures and bile ducts were normal.

The bone marrow of the femur was red and cellular in places, but was in great part made up of fat. Microscopical section showed a definite grade of patchy hyperplasia. This had involved particularly the polymorphonuclear leucocytes and their precursors. There was no evidence of cell injury. The giant cells of the bone marrow were fairly numerous and quite normal in appearance.

**Dog C-6.**

April 12. Mongrel male; weight 24½ lbs. Chloroform anesthesia for two hours; 1 oz. given. The anesthetic was well taken.

April 13. Chloroform anesthesia for two hours; 1 oz. given.

April 14. Chloroform anesthesia for one hour; ½ oz. given. The anesthetic was poorly taken. The animal bled easily from an ear prick, and the bleeding continued for twenty to thirty minutes.

April 15. From small ear pricks which were still bleeding, the dog had lost during the night about 20 to 30 c.c. of blood. No clots were formed at the edges of these small incisions. The animal appeared weak and restless, but did not vomit. At 10 a. m. under ether anesthesia the dog was bled from the carotid. The serum was colored a clear amber. The fibrinogen was 0.0184 gm. per 100 c.c. and the calcium was 0.0141 gm. per 100 c.c. Blood which was collected in cylinders clotted in the usual time, but the blood clots were very flabby and settled rapidly to the bottom. The clots constituted about 10 per cent. of the volume in the cylinder.

Table IV (dog C-6) shows a remarkable leucocytosis, and these counts may be considered accurate because of repeated examinations to confirm the unusual rise from normal to over 300,000 white blood cells.

**Autopsy.**—The autopsy was made immediately after death. The serous cavities were normal, as were also the heart, spleen, kidneys, stomach, and intestines.

**Lungs.**—Both the lower lobes of the lungs showed greenish patches of consolidation. In the pleura, over these areas, were numerous ecchymoses. The lung tissue was decidedly moist. In the microscopical section were patches...
Liver Necrosis of Chloroform Poisoning.

<table>
<thead>
<tr>
<th>Time</th>
<th>Chloroform</th>
<th>Platelets</th>
<th>Calcium in c.c.</th>
<th>White blood cells</th>
<th>Bile pigment</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 12</td>
<td>0.0164 gm.</td>
<td>320,000</td>
<td>100 c.c.</td>
<td>220,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 13</td>
<td>0.0141 gm.</td>
<td>434,000</td>
<td>100 c.c.</td>
<td>20,000</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>April 14</td>
<td>Abundant</td>
<td>320,000</td>
<td>100 c.c.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 15</td>
<td>Abundant</td>
<td>330,000</td>
<td>100 c.c.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

April 15 Autopsy: liver necrosis invaded about two-fifths of each lobule. Normal cells were few.

of bronchopneumonia in which polymorphonuclear leucocytes and red blood cells were the predominant features. Very little fibrin was present. It was compact, glassy, and hyaline, as though of considerable age. No strands of fresh fibrin were present.

The pancreas was quite normal. The bone marrow (femur) had a mottled yellowish-pink color. Microscopical section showed an active hyperplasia affecting particularly the polymorphonuclear leucocytes. The bone marrow giant cells were numerous, but of rather small size. There was no cell necrosis and no nuclear fragmentation.

Liver.—The liver was large, tense, and friable. The lobules were very distinct with bright red centers and with thin, swollen, opaque margins. In microscopical sections, each lobule showed evidence of a central hyaline necrosis involving about two-fifths of the cells. There was a well-marked middle zone in which the liver cells contained large and small fat droplets. This zone was estimated to be about one-fifth of the volume of each lobule. The cells immediately surrounding the portal structures were practically normal, and mitotic figures were very numerous. Mononuclear phagocytes and polymorphonuclear cells were very numerous in the areas of necrosis.

Dog C-9.

April 26. Large mongrel male pup; weight 23 lbs. Chloroform anesthesia for two hours; ½ oz. given; anesthetic well taken.

April 28. The dog was quite sick and vomited frequently. Urine contained a large amount of bile pigment.

April 29. Dog was quite sick. Bleeding time was prolonged to four to eight minutes.

April 30. There was a little improvement. Under ether anesthesia the dog was bled from jugular vein. The fibrinogen was 0.1504 gm. per 100 c.c.

May 1. Dog was not well and would not eat.

May 10. Improvement had been slow; weight 19 lbs.

May 17. Under ether anesthesia the animal was bled from jugular. The fibrinogen was 0.6890 gm. per 100 c.c.

June 9. Dog was well. Weight 22 lbs.

June 13. Chloroform anesthesia for two hours; 1 oz. given; anesthetic well taken.
G. H. Whipple and S. H. Hurwitz.

Table V. Dog C-9.

<table>
<thead>
<tr>
<th>Time</th>
<th>Chloroform</th>
<th>Fibrinogen in 100 c.c.</th>
<th>Calcium in 100 c.c.</th>
<th>Platelets</th>
<th>Jaundice</th>
<th>Bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 26</td>
<td>2 hrs.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>April 27</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>April 28</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>April 29</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>++</td>
<td>—</td>
</tr>
<tr>
<td>April 30</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>May 17</td>
<td>—</td>
<td>0.350 g.</td>
<td>228,000</td>
<td>+</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>June 13</td>
<td>2 hrs.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 14</td>
<td>2 hrs.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 15</td>
<td>2 hrs.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>June 16</td>
<td>—</td>
<td>0.100 gm.</td>
<td>0.0102 gm.</td>
<td>—</td>
<td>++</td>
<td>—</td>
</tr>
</tbody>
</table>


June 14. Animal appeared well; no vomiting. At 11 a.m. chloroform anesthesia for two hours; 1 oz. given.

June 15. Animal refused food at 3 p.m.; chloroform for two hours; 1 oz. given.

June 16. Dog appeared pretty well. Urine contained a large amount of bile pigment. At 2 p.m. the animal was killed accidentally at the beginning of anesthesia.

Autopsy.—The autopsy was performed immediately after death. Unclotted blood was obtained from the right auricle and mixed with oxalate solution, as usual, for the determination of fibrinogen. This amounted to about 0.100 gm. per 100 c.c. The serum was canary yellow in color and slightly milky, due to an alimentary lipemia. Blood drawn into cylinders clotted in the usual time and pretty firmly. The clots were broken up with considerable ease, but retained their shape well and did not shrink. Extreme reduction in fibrinogen was not suggested. One hundred cubic centimeters of blood, analyzed for calcium, yielded 0.0102 gm.

The heart, spleen, kidneys, stomach, duodenum, intestines, and pancreas were normal. In the lungs were large areas of consolidation having a greenish red color. They measured 1 or 2 cm. in diameter and were present particularly in the lower lobes. The bronchi were full of creamy material. The pleural surface was smooth and moist. On microscopical examination the...
bronchi were found to be full of an exudate made up in great part of well preserved polymorphonuclear leucocytes. The alveoli were full of an exudate of polymorphonuclear cells, mononuclear cells, a few red blood cells, and a granular coagulum.

Liver.—The liver was large and friable. As usual, the lobulation was conspicuous. Microscopically the liver necrosis was not very striking in this case. This is rather surprising considering the duration of chloroform anesthesia. More than half of the liver cells in each lobule were uninjured or, at most, showed only a few small fat droplets. Mitotic figures were numerous, and evidently the liver cells repaired the injury very effectually and with great rapidity. The centers of the lobules showed almost complete removal of the necrotic liver cells. A few small hyaline masses remained, some of which were undergoing calcification. Large pigmented phagocytes were numerous.

The resistance of this animal (dog c–9) to chloroform anesthesia is difficult to explain. It is evident, however, that its liver was very resistant to the drug and that the injury was repaired with greater rapidity than normally. The findings in the liver sections corresponded exactly with the fibrinogen content of the blood, which, although considerably reduced, was not dangerously low. Undoubtedly the bronchitis and bronchopneumonia were the most important factors in the animal’s death.

In the preceding experiments several interesting points are brought out. The chart and table of dog 101 show that chloroform anesthesia given at short intervals, can reduce the fibrinogen fraction of the blood to a minimum, causing at the same time extensive liver necrosis. The experiment with dog c–6 shows much the same as regards the fibrinogen, and supports the other observations. In two dogs (Nos. 85 and 88) the thrombin and calcium were found to be normal. In dogs 104 and c–9 the formed elements of the blood, especially the platelets, were not involved except in a secondary manner, for the liver necrosis calls for many leucocytes. This caused a rise in the white cells in the blood for two to four days following the liver injury, and effected some hyperplasia of the bone marrow. The bone marrow giant cells, as well as all the other marrow cells, respond to this hyperplasia, and platelets are more numerous than normally in the circulating blood. The bone marrow shows no signs of injury by the drug.

Table IV (dog c–6) is of particular interest in connection with
the view of Müller that fibrinogen is produced by the leucocytes. In this experiment the chloroform anesthesia caused extensive liver necrosis and reduced the fibrinogen content of the blood to about 5 per cent. of the normal. Instead of a leucopenia we had a tremendous leucocytosis due probably to the combination of liver necrosis and bronchopneumonia, which stimulated the bone marrow to hyperactivity. It is difficult to explain the great decrease in fibrinogen which took place in this case simultaneously with the tremendous increase in the leucocytes, if the leucocytes are in any way concerned with the production of fibrinogen.

Chart 4 and Table V (dog c-9) show a fibrinogen curve similar to that in Chart I. About six weeks after the first anesthesia, this animal manifested a greater resistance to chloroform than in the beginning. The liver was able to repair the injury very actively; the blood contained a fair amount of fibrinogen (0.100 gms. ± per 100 c.c.); the dog gave few signs of poisoning; and it did not bleed from small cuts. This observation suggests again the possibility that the primary injury to the liver conferred some immunity. Nevertheless, animals vary so greatly that, to establish this point, much evidence must be accumulated.

Dog c-3.

April 7. Old mongrel, male; weight 64 lbs. Chloroform anesthesia for two hours; 1½ oz. given. During the anesthesia 200 c.c. of blood were obtained from the jugular vein and 400 c.c. of 0.8 per cent. salt solution, containing 16 gms. of calcium lactate, were infused subcutaneously. The fibrinogen determination was 0.4492 gm. per 100 c.c.

April 8. Dog was very sick, and at 3.30 p. m. appeared to be fatally poisoned. The animal was bled under ether anesthesia from the carotid. The fibrinogen was 0.2340 gm. per 100 c.c. and the calcium was 0.058 gm. per 100 c.c. The blood drawn in cylinders clotted rather slowly, but the clot was pretty firm. A large amount of blood was defibrinated in a flask, and 180 c.c. of this defibrinated blood was injected intravenously into dog C-4.

Autopsy.—This was performed at once. All the tissues showed a delicate eteroid tint. The serous cavities, as well as the heart, lungs, spleen, and kidneys, were normal. In all parts of the parenchyma of the pancreas were great numbers of fat necroses, some of the larger ones measuring 2 mm. in diameter. There were no hemorrhages.

Liver.—A large amount of blood was present, giving the liver a deep purple color. The lobules were sharply outlined. In microscopical sections all the liver cells contained fine fat droplets, which were of larger size and more con-
Liver Necrosis of Chloroform Poisoning.

Spicuous in the central part of each lobule. There was extensive necrosis involving about one-half of each lobule. The necrosis was quite recent (thirty hours), and the liver cells retained their contour very well. The protoplasm, however, in these cells was rather glassy and uniform, and the nuclei showed pyknosis and fragmentation. There were very few wandering cells. The capillaries were congested.

Dog C-4.

April 8. Fox terrier, male; weight 16 lbs. Under ether anesthesia 100 c.c. of blood were obtained from the jugular vein and infused intravenously with 180 c.c. of defibrinated blood from dog C-3. The animal was under anesthesia for only twenty minutes and recovery was rapid. Fibrinogen was determined (0.600 gm. per 100 c.c.). There was a little vomiting during the night.

April 9. Dog was quite lively, but did not eat.

April 11. Dog was improving; was killed with ether. The fibrinogen determination gave 0.6712 gm. per 100 c.c. The thorax, heart, lungs, and spleen were normal. The peritoneal cavity showed a little fresh fibrinous exudate over the dome of the liver and particularly about the gall bladder, which had a deeply injected, purplish, rough, serous covering. There were a good many ecchymoses in this region. The gall bladder contained normal bile, and the mucosa showed no change. The rest of the peritoneal cavity was practically normal. The pancreas and kidneys were normal, and the stomach and intestines were full of food and showed a normal mucosa.

Liver.—The liver lobulation was definite and rather conspicuous. The lobules seemed somewhat swollen and the centers were congested. In the microscopical sections, all of the liver cells contained a few tiny fat droplets which were brought out only by fat stains. There was no evidence of cell necrosis.

We are unable to explain the early peritonitis, which, however, was not of a severe grade and did not prevent the animal from taking food. It is possible that this followed the introduction of bacteria at the time of the infusion. This experiment, together with others to be reported later, indicates beyond any doubt that blood removed from an animal poisoned with chloroform is not poisonous to another animal when given intravenously. The slight rise in fibrinogen may be explained by the
peritonitis which had begun, for it is known that the fibrinogen content of the blood is high in pneumonia, peritonitis, etc.

**Dog 96.**

March 5. Small young female; weight 17 lbs. The dog was bled, under ether anesthesia, from the jugular vein. The fibrinogen was 0.3120 gm. per 100 c.c.

March 10. The dog is quite well and is bled again, under ether anesthesia. The fibrinogen was 0.2872 gm. per 100 c.c.

May 4. Chloroform anesthesia for one hour and fifty minutes; ¼ oz. given.

May 5. Animal bled from jugular vein, under ether anesthesia. The fibrinogen was 0.0150 gm. per 100 c.c.

May 6. Animal found dead in cage, the result of bleeding from the neck, which, however, contained some very soft clots.

**Autopsy.**—The serous cavities, lungs, spleen, and kidneys were normal. The heart was full of purple, soft, almost semi-fluid clots.

**Liver.**—The liver showed the usual characteristics seen in chloroform poisoning. In the microscopical sections was found an extreme grade of central necrosis involving more than half of every lobule. Only three or four rows of normal liver cells surrounded the portal spaces. There was a thin mid-zone of fatty degeneration. The mitotic figures were very few. Evidently this dog was severely and probably fatally poisoned by the single administration of the anesthetic. Examination of the blood showed a remarkably rapid and almost complete disappearance of the fibrinogen at the end of twenty-four hours. This in itself indicated a very high grade of poisoning, as the usual diminution at the end of twenty-four hours is not more than 50 per cent.

**Dog 92.**

February 22. Fox terrier, male pup; weight 17 lbs. Bled, under ether anesthesia, from jugular vein. The fibrinogen was 0.7896 gm. per 100 c.c. It is possible that this determination is too high as it was one of the earliest in the series, and the determination was made without the use of the centrifuge to throw down the precipitate.

March 15. Weight 16½ lbs. The dog was bled, under ether anesthesia, from the jugular vein into oxalate solution. The fibrinogen was 0.5396 gm. per 100 c.c.

May 13. Chloroform anesthesia for two hours; ¼ oz. given.

May 14. The dog was bled, under ether anesthesia, from jugular vein. The fibrinogen was 0.3776 gm. per 100 c.c.
Liver Necrosis of Chloroform Poisoning.

May 15. The animal seemed quite well.

June 22. Chloroform anesthesia for two hours; 1 oz. given. During this time the blood of the animal was defibrinated. Cannulas were inserted into the carotid artery and jugular vein. About 200 to 300 c.c. of blood were allowed to flow into a defibrinating flask. The blood was defibrinated by shaking with beads; it was then strained through gauze, and allowed to flow back slowly into the jugular vein. The defibrinating bottle was then rinsed out with a little 0.8 per cent. salt solution, and the rinsing solution, also, was introduced through the canula. This procedure was repeated six times during the two hours of anesthesia. The blood removed at the last bleeding remained fluid, and practically no fibrin was formed after shaking for five minutes. No clot formed in the carotid cannula during a period of ten minutes after the vessel was clamped, and it is pretty clear that the circulating blood contained very little fibrinogen.

June 23. The dog was very quiet, but did not look particularly ill. At 11 a.m. the bandages were removed and some large soft clots were found in the neck wound from which a little blood was still oozing. Under ether, the animal was bled from the same carotid into oxalate solution. The fibrinogen was 0.2368 gm. per 100 c.c. The serum had a clear rose pink color due to hemolysis. The volume of the red cells was about one-half the normal for, after centrifugation, the red blood cells made up only about one-fourth of the total volume. Urine obtained during this time showed a definite hemoglobinuria resulting from the hemolysis and hemoglobinemia.

June 25. The dog ate well and appeared to be in good condition. It was killed with ether.

Autopsy.—The autopsy was performed at once. The serous cavities, heart, lungs, spleen, pancreas, duodenum, and stomach, were perfectly normal. The kidneys showed a pale greenish coloration of the cortex, but were otherwise normal.

Liver.—The liver was practically normal. Microscopical sections showed no fatty cell changes or necrosis.

We are not prepared to explain the lack of necrosis observed in the liver following chloroform anesthesia. This is an isolated observation and evidently has to do with the defibrination procedure. It is possible that the repeated withdrawals of the blood
permitted the escape of much of the chloroform which it contained, for, presumably, the anesthetic injures the liver by circulating through it. Enough chloroform, however, was present in the blood to produce light anesthesia. The first part of the experiment indicates that the animal was more than normally resistant to chloroform poisoning, and this initial anesthesia may have still further raised the animal's resistance or natural immunity. The experiment supports the work of Matthews as to the rapidity of production of fibrinogen by the normal liver.

Dog 91.
February 22. A lean, female pup; weight 27 lbs. The animal was bled, under ether anesthesia, from the jugular vein. The fibrinogen was 0.7488 gm. per 100 c.c.
February 24. Chloroform anesthesia for two hours: 8 oz. given
February 25. Animal seemed well and did not vomit.
February 26. The dog was bled from the jugular vein under ether anesthesia. The fibrinogen was 0.4444 gm. per 100 c.c. Twenty-five cubic centimeters of blood were analyzed for calcium. The calcium was 0.01248 gm. per 100 c.c.
March 3. The animal had a little distemper, but was fairly well.
March 10. The dog was bled from jugular vein under ether anesthesia. The fibrinogen was 0.3784 gm. per 100 c.c.
March 24. The animal appeared well and weighed 24 lbs.
April 7. Under ether anesthesia the common bile duct was ligated.
April 8. The dog seemed pretty well. Urine contained bile pigment.
April 11. Jaundice had developed as usual; the urine was very highly colored; and the stools were pasty and free from bile.
April 20. The picture was typical of obstructive jaundice.
April 23. The animal weighed 21 lbs. All the tissues were deeply jaundiced. Under ether anesthesia the dog was bled from the carotids. The calcium was 0.0133 gm. per 100 c.c. and the fibrinogen was 0.4158 gm. per 100 c.c. The blood clotted in a normal manner. The clots were very tough and firm.
Liver Necrosis of Chloroform Poisoning.

Autopsy.—The peritoneal cavity was normal except for old adhesions about the site of operation on the common bile duct. The tissues were everywhere deeply bile-stained. The heart, lungs, spleen, pancreas, stomach, and intestines were normal. There was a small punched out ulcer in the duodenum measuring 1 cm. in diameter. The gall bladder and the common cystic and hepatic ducts were all enormously dilated and a little thickened. They contained fluid bile. All the body fluids contained bile pigments.

Liver.—In the microscopical sections the liver cells in the central portion of each lobule showed some large and small fat droplets and contained, in addition, some greenish yellow granular pigment. There was an increase in wandering mononuclear cells about the tissue in the very centers of the lobules, but the greater part of all the lobules was practically normal; the cells had a normal appearance, and there was scarcely any evidence of the injury presumably caused by the chloroform anesthesia.

The curve of fibrinogen in this experiment (Chart 8) is of considerable interest. Possibly the first determination is too high, as this is one of the earliest, and was made before the value of centrifugalizing the precipitate was appreciated. The chloroform anesthesia was very well borne and probably caused only a slight drop in the fibrinogen. The determination of March 19 showed a slight diminution, and may possibly be explained by the poor condition of the animal, the result of distemper. The total obstructive jaundice, which lasted for a period of two weeks, caused no drop in the fibrinogen, but rather a slight increase. The calcium content of the blood likewise increased slightly.

Table VI.

<table>
<thead>
<tr>
<th>Normal Dogs. Fibrinogen in 100 c.c. of blood.</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 3. Dog 95 .......... 0.3820 gm.</td>
</tr>
<tr>
<td>March 3. Dog 95 .......... 0.3664 gm.</td>
</tr>
<tr>
<td>March 15. Dog 93 .......... 0.3348 gm.</td>
</tr>
<tr>
<td>March 5. Dog 97 .......... 0.3064 gm.</td>
</tr>
<tr>
<td>June 6. Dog 98 .......... 0.3272 gm.</td>
</tr>
</tbody>
</table>

A review of Table VI and some of the other tables indicates that a normal dog may have a fibrinogen content averaging from 0.300 to 0.600 grams per 100 cubic centimeters of blood. Under fixed conditions an animal may have a pretty constant fibrinogen content and this may be half or double that of another healthy animal. We are not prepared to explain these differences, which we feel sure are not due to errors in the determinations. Perhaps some satis-
factory explanation may be reached when more evidence has been accumulated.

It may be worth while to say a few words about the parallelism between the liver necrosis and the drop in blood fibrinogen. Probably the injury to the liver is accomplished during the chloroform anesthesia, for after the anesthesia one is quite unable to protect an animal against the liver necrosis by bleeding, infusions, etc. The necrosis does not increase in extent during the second twenty-four hours, yet there is a rapid drop in fibrinogen, which usually reaches its minimum on the second day and may remain very low during the next two days. The hyaline appearance of the dead liver cells—a typical hyaline coagulative necrosis—is most striking during the second day.

Why does the blood fibrinogen drop in this manner or why does it not drop immediately after the chloroform anesthesia? Liver extirpation causes the fibrinogen to disappear in two hours. Nolf considers that it is anchored by the vaso- and leucothrombins which normally are held in check by the hepato-thrombin. Pawlow circulated the blood through the head, heart, and lungs, and observed that the blood would not clot after a few hours. One possibility is that only a part of the liver cells are injured and the rest are able to form some fibrinogen. But this does not explain the very low blood fibrinogen during the second and third days, when the necrosis is stationary and the repair has commenced in the uninjured liver cells and is moving ahead rapidly. It may be argued that the coagulative liver necrosis contains fibrin and uses up a good deal of the blood fibrinogen. Again, it is possible that the acute inflammatory reaction, which is at its height on the second and third days, and is naturally accompanied by much edema and swelling of the tissues, causes sufficient compression of the intact liver cells to embarrass their action.

We have been unable to discover a convincing argument to explain the rapid disappearance of the blood fibrinogen, although some of the factors mentioned above may explain it in part. This problem is bound up with that of the life history and general utility of the fibrinogen. It is hard to believe that the coagulation of blood is the only function of the blood fibrinogen. Is it not possible
Liver Necrosis of Chloroform Poisoning.

that the blood fibrinogen is taken up by the various organs to replace their tissue waste? If it can be shown that fibrinogen is available as a tissue building proteid, then it is easy to explain all the phenomena under discussion. When all of the fibrinogen is removed by repeated blood defibrination (Chart 7), it was restored in twenty-four hours to more than half the normal, thus indicating the great reserve power of the liver. When the liver has actively regenerated following a severe chloroform poisoning, we find a rise of fibrinogen much above normal. This seems to indicate that the production of fibrinogen is an essential part of liver cell activity.

SUMMARY.

1. Chloroform anesthesia for two hours or more will cause more or less central liver necrosis in dogs, depending on the length of the anesthesia and the susceptibility of the animal.

2. If the fibrinogen of the blood of such an animal be estimated at intervals, it is found that this proteid shows a drop corresponding to the amount of liver necrosis.

3. By administering chloroform, the fibrinogen may be almost eliminated from the circulating blood, and the poisoned animal may bleed for hours from small skin pricks or cuts.

4. The liver can recover from a grave injury due to chloroform and return practically to a normal condition in about ten days.

5. The fibrinogen reappears in the blood as the liver effects its repair. It seems that the quantity of fibrinogen present is a good indicator of the liver efficiency and a fairly accurate index of the amount of liver injury.

6. Shortly after the recovery of the liver from an injury due to chloroform, one may find an excess of fibrinogen in the blood.

7. In severe cases of chloroform poisoning, the calcium of the blood was normal or slightly increased, and the thrombin was normal.

8. These experiments give no evidence that the formation of thrombin or prothrombin is dependent upon liver activity.

9. The hemorrhages of chloroform poisoning are due not to lack of blood clotting but to inefficient coagulation. The clot has not the body and toughness supplied by the fibrinogen, and is, therefore, unable to check even capillary hemorrhage.
G. H. Whipple and S. H. Hurwitz.

161

10. Fibrinogen is either formed in the liver or is wholly dependent upon liver activity for its production.

In conclusion we desire to express our appreciation to Dr. W. H. Howell for his stimulating interest and invaluable assistance, and to Dr. W. W. Duke for observations on the formed elements of the blood.

BIBLIOGRAPHY.