

AN EXPERIMENTAL STUDY OF THE METABOLISM  
AND PATHOLOGY OF DELAYED CHLOROFORM  
POISONING.<sup>1</sup>

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PLATES VIII-X.

In the course of some investigations which have occupied our attention during the past four years, we used prolonged and repeated inhalations of chloroform in an attempt to diminish the oxidative power of the cells and thus to inhibit or prevent the transformation of certain substances with which we were experimenting. We found, however, that a number of control animals which received chloroform alone, were profoundly affected and died after a lapse of hours or days after their return to consciousness, and that they all exhibited severe parenchymatous lesions, especially in the liver. In other words, we produced typical delayed chloroform poisoning. It appeared to us advisable in view of the great importance attaching to delayed poisoning to study the metabolic changes occurring as a result of this, and in comparison with the pathological lesions produced. Dogs, upon which metabolic experiments are most easily carried out, can also regularly be so anæsthetized as to cause their death many hours after the anæsthesia.

The conception of delayed chloroform poisoning has been of slow development. Although Casper (1) and Langenbeck (2), in 1850, and Mouat (3), in 1856, spoke of a postponed fatal effect of chloroform, it was not until more than thirty years later that the condition began to be generally recognized and accepted. Thiem and Fischer (4), in 1890, reported a fatal case due, they believed, to the delayed effect of chloroform. In the following year Bastianelli (5) reported three cases, and in 1892 Fränkel reported several more. Thereafter,

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reports appeared in rapid succession by Ambrosius, Guthrie, Bandler, Marthen, Heintz, Steinthal, etc. In England, Guthrie's paper first called attention to the condition, and in this country cases have been reported by Brewer, Bevan and Favill, Holmes, Cushing, Ballin, Wells and others. It is undoubtedly true that some deaths have been reported as due to chloroform that could, with greater probability, be referred to some other cause, such as the original disease or antiseptics, but this does not effect the validity of the majority of reported cases.

Nothnagel (6), in 1866, was the first to study the lesions produced by chloroform in animals. In his experiments chloroform was given by mouth and subcutaneously, and not by inhalation. The amount employed was, moreover, so large that most of the animals died in a few hours, or he killed them at that time. Nevertheless, he produced fatty changes in the heart, liver and kidneys.

The first attempt to explain delayed poisoning was made in 1883 by Ungar with the help of Junkers (7). Ungar had been impressed with the occasional, unfavorable outcome after operation when everything apparently had been progressing favorably until a short time before the patient's death. He appreciated the great difficulties in the way of separating symptoms and pathological changes due to chloroform from those due to the primary disease or injury necessitating operation, and therefore decided to use animals. His experiments were carefully planned and carried out, and his results of great importance. He attempted first to repeat Nothnagel's experiments, but could obtain no satisfactory results. He then administered chloroform by inhalation to rabbits and dogs. Rabbits were difficult to use on account of their great susceptibility to chloroform during its prolonged administration, but in dogs he was able to produce great fatty changes in the liver and heart and less in the kidneys. He observed that prolonged administration was necessary and that the lesions were much more severe when repeated inhalations were employed. Unlike most subsequent investigators, he let his animals die and so obtained more severe lesions than have those who after twenty-four, forty-eight or more hours killed their animals. Like many who followed him, it is evident that he began with a preconceived idea as to the organ most probably responsible for death, and wrote his descriptions of lesions from that standpoint. He found great change in the cardiac muscle and

inclined to this as the cause of death. From his description of the appearance of the livers, both macroscopical and microscopical, it is apparent, however, that in them very severe lesions were present. He says that the center of the lobules was made up of fatty detritus only; the cell outline was indistinguishable.

In 1889, Ostertag (8) and Strassmann (9) independently confirmed Ungar's findings. Ostertag experimented on many different animals: rabbits, guinea-pigs, pigeons, rats, cats and dogs, and obtained fatty changes in all. Some of his animals died of late poisoning, but he was unable to account for this, and recognized in them no necrotic lesions. He made the important observation that dogs exhibited great differences in resistance to the chloroform.

Thiem and Fischer (10), in 1890, used rabbits and dogs and produced marked fatty changes, especially in the livers. In 1896 Bandler (11) and Heintz (12), independently, recognized necrosis in the livers of fatal cases and produced necrotic lesions in the livers of dogs and rabbits. In the same year Marthen (13) reported a fatal case with liver necrosis and Ajello (14) caused experimental necrosis in the livers of animals. From this year dates the recognition of necrosis as the most important of the changes produced by chloroform. In fact, hardly an autopsy since that time has been reported that has not shown necrosis, and in many it has been so marked that the cases have been denominated acute yellow atrophy.

Lengemann (15), in 1900, was unable to produce delayed chloroform poisoning in a dog by repeated inhalations when the amount of chloroform was carefully measured and just enough given to produce anæsthesia and no more. Other experimenters, whose work should be mentioned, are Stiles and McDonald (16) and Müller (17). There has been practically complete agreement among all writers during the last ten years that the most extreme lesions are produced in the liver, and that here is to be found the chief cause of death. Offergeld (18) alone dissents from this view and thinks the kidneys are the organs chiefly involved.

There is no record in the literature, so far as we know, of a study of the metabolism in animals in which such a condition of delayed chloroform poisoning has been experimentally induced. The metabolic effects of chloroform given by mouth, by subcutaneous injection or by inhalation have been widely studied, but in every case, normal, uneventful recovery apparently occurred. Strassmann (19) made estimations of the urinary nitrogen before and after chloroform inhalation in an effort to determine whether the fatty change in the liver was an infiltration or degeneration. He concluded that fatty degeneration had occurred because of a marked increase in the nitrogen excreted. This increase amounted to twenty per cent. on the day on which chloroform was given and continued

for the two following days. In Salkowski's (20) experiment a dog in nitrogenous equilibrium was given fifteen cubic centimeters of chloroform in two hundred cubic centimeters of water on three successive days. Narcosis was not produced but the nitrogen elimination increased, the increase being noticeable on the day following the end of the chloroform period. Taniguti (21) under Salkowski's direction showed in one experiment that inhalation anæsthesia by chloroform caused an increased nitrogen excretion lasting for two days after the inhalation. He also noted an increased elimination of phosphoric acid which was not, however, parallel to the elimination of nitrogen. Kast and Mester (22) examined changes in the sulphur excretion after anæsthesia with chloroform in human subjects. The earlier results by Kast (23), which showed a marked increase in the elimination of chlorine in the urine of fasting dogs anæsthetized with chloroform, led them to the belief that metabolic disorders of a very fundamental nature were induced by that drug. They found marked changes in the ratio of sulphate sulphur to neutral sulphur—the latter form being increased greatly in amount, the former being little affected. They found also that the acidity of the urine increased after chloroform, in some cases remaining high for three days after the anæsthetic was administered. This result was confirmed in 1898 by Thomas (24) who demonstrated a decrease in the alkalinity of the blood during chloroform anæsthesia. Rudenko (25) examined the urines of Salkowski's dog (26) with regard to the sulphur distribution after administration of chloroform by mouth for four consecutive days. An increase of sulphate sulphur appeared only on the last three days; an increase of neutral sulphur on the last two days and on the two days following. There was therefore a marked percentage increase in neutral sulphur on these days, and it was most noticeable on the days following chloroform administration. An attempt to determine whether the neutral sulphur so eliminated was capable of being oxidized by the normal animal failed to give decisive results.

Savelieff (27) confirmed Rudenko's results and those of Salkowski by a single experiment on a dog in whose urine nitrogen and sulphur were estimated before and after chloroform water was given by mouth for four consecutive days. On the first chloroform day,

sulphate sulphur increased, on the second, total nitrogen began to increase; neutral sulphur did not increase until the fourth day of chloroform administration. The increase of sulphate sulphur continued during the first day after the chloroform period, that of total nitrogen during two days after, and that of neutral sulphur during three days after. He considered his figures to indicate that chloroform increased protein destruction but diminished oxidations. Becker (28) and Abram (29) found acetone to be present in the urine after chloroform anæsthesia in the majority of human cases studied, a fact since confirmed by Baldwin (30).

In 1897, Vidal (31) published the results of a very comprehensive study of the effect of chloroform on metabolism. Some of the methods which he used are now open to serious criticism. Urea, for example, was estimated by the hypobromite method after precipitation by basic lead acetate or by phosphotungstic acid; creatinin by the old Neubauer method. The results of a number of observations on human subjects, showed total nitrogen to undergo a marked increase, usually greatest on the second day after anæsthesia. These results were confirmed in experiments on dogs and rabbits. His results on the distribution of nitrogen in the urine and the experiments which he made to determine the causes of variations in the different urinary constituents cannot be regarded as convincing. His results on sulphur elimination confirmed those of Rudenko, Kast and Mester, and Savelieff. The increase in total sulphur elimination was parallel to the excretion of total nitrogen.

The experiments of Doyen and Billet (32) may be mentioned here on account of the relation which they bear to the later work of Paton. By giving two grams of chloroform per kilo dissolved in oil by mouth, they were able to induce in dogs intense necrotic changes in the liver with diminished coagulability of the blood. They considered that chloroform under the conditions of their experiments exercised a selective action upon the liver. Paton's (33) recently published series of experiments was directed specifically toward the elucidation of the question of delayed chloroform poisoning. Dogs in a condition of nitrogenous equilibrium were poisoned with chloroform by inhalation, by mouth, and by subcutaneous injection. When given by inhalation for one, two or three hours, there was a slight increase in urinary nitrogen, accompanied by either no change or a percentage increase in urea nitrogen. He attributes these effects to a stimulation of the urea-forming function in the liver. Changes in ammonia nitrogen (Schlössing's method) were not constant, creatinin excretion was parallel with total nitrogen, and sulphur was not changed. When chloroform (20

grams in 75 cubic centimeters of oil) was given by mouth on two consecutive days, greater changes were produced. The increase in total nitrogen was accompanied by a relative decrease in urea, especially marked on the second chloroform day and the two succeeding days. Ammonia nitrogen was increased both absolutely and relatively. Creatinin increased in absolute figures but suffered a relative decrease. Uric acid and undetermined nitrogen were increased. Sulphate sulphur suffered a relative decrease, neutral sulphur a relative increase. He considers that these experiments show a direct toxic action on the liver, and believes that the greater metabolic toxicity of chloroform given by mouth indicates that if chloroform given by the lungs is not eliminated with normal rapidity, serious injuries might be effected which would lead to symptoms of late chloroform poisoning. He suggests that delayed elimination occurred in his experiments in which chloroform was given by mouth because of unusually firm fixation of chloroform by the proteins of the tissues, this in turn being dependent upon slow absorption of chloroform from the alimentary tract into the blood. He expressed the belief that late chloroform poisoning may arise as the result of chloroform given by inhalation, if elimination of chloroform by the lungs is delayed owing to respiratory deficiency or to unusually firm fixation of chloroform by the tissues. His hypothesis is not convincing because the records fail to show that respiratory deficiency is a constant feature in anæsthesia followed by delayed poisoning in man; no suggestion is offered as to the possible nature of an "unusually firm fixation" of chloroform by the tissue proteins, and the experiments of Moore and Roaf (34) show that this fixation is dependent only upon the vapor pressure of chloroform.

Further chemical evidence regarding late chloroform poisoning is furnished by Taylor (35) and by Wells (36) who analyzed livers, obtained at autopsy, of cases in which death was due to this condition. Taylor was able to isolate leucin, tyrosin and arginin in relatively large amounts; Wells separated histidin, leucin, tyrosin, glycocoll and glutamic acid. In Well's case, a loss of about one-third of the total solids of the liver had occurred and an increase of ether-soluble material was due to infiltrated fat.

On the basis of a consideration of the part played by autolysis in tissue disintegration and of the selective action of chloroform upon metabolic processes, Wells (37) has offered an explanation of the production of tissue changes and of late poisoning by this substance. He emphasized the fact that chloroform is a protoplasmic poison which does not affect the activity of autolytic enzymes but prevents syntheses and oxidations—processes most closely connected with the vitality of cells. As a result of autolysis, tissues subjected to the influence of chloroform may undergo disintegration, the products of their disintegration accumulate and become toxic. Toxic products, in his view, may also accumulate because of impaired function of the liver and kidney. Predisposition to late chloroform poisoning he believes to be connected with influences which decrease oxidations.

The experimental results which we have cited lead to the conclusion that a marked increase of protein catabolism is caused by the action of chloroform upon the tissues which is independent of

the narcotic properties of the substance. The increased catabolism persists for a relatively long time after the administration of chloroform. When chloroform is given by mouth, its effect upon metabolism is more intense than when given by inhalation. It is logical to believe that this fact is due to the greater concentration in which chloroform reaches the liver when given by this path. Other manifestations of the toxic action of chloroform upon metabolism are the increase in uric acid and ammonia, relative decrease in urea, increase in sulphur excretion in which neutral sulphur is chiefly concerned, and decrease in alkalinity of the blood with increased acidity of the urine.

It is a striking fact that in none of the experiments upon which these conclusions are based has late poisoning actually been produced. In the papers in which attention has been directed toward this condition, it is assumed that a mere intensification of the observed effects of chloroform will lead to the condition of late poisoning. Direct evidence concerning the nature of the condition is necessary in testing the truth of this assumption. The observations that necrotic changes in the liver may constantly be found after chloroform anæsthesia in dogs, and that dogs severely poisoned may die from the late effects of the anæsthetic, led us to the hope that by producing the condition experimentally, by studying the morphological changes and the urinary excretions and by comparing these results with those similarly obtained in animals in which recovery from severe poisoning was normal, we could discover in what respects the phenomena of late chloroform poisoning are to be differentiated from those of a normal anæsthesia. We hoped also that such results might point to the nature of the predisposition which is a necessary condition in the production of late poisoning in human subjects. We have succeeded in making such a study in two dogs in which fatal, late poisoning was developed, and in one dog in which severe poisoning, followed by normal recovery, occurred. The description and results of these experiments follow the account of five of the preliminary observations which were the direct impetus to them.

## PRELIMINARY OBSERVATIONS.

DOG XVI (A).—Medium sized mongrel bitch. This animal was very resistant to the delayed effect of chloroform.

Mar. 1, '08. Chloroform by inhalation for five hours, twenty-five minutes.

Mar. 2, '08. Chloroform by inhalation for five hours, thirty minutes.

Mar. 3, '08. Chloroform by inhalation for four hours, ten minutes.

Mar. 4, '08. Was perfectly intelligent and bright and only a little weak.

Mar. 5, '08. Seemed well. Killed with chloroform. Autopsy at once. Liver: very yellow and mottled with small red points. Kidneys: in cortex yellowish lines leading down to pelvis. Intestines contained altered blood. Few hemorrhages in ileum.

*Micros.*—Liver: almost all cells heavily loaded with fat. Central zones of lobules contain a moderate number of necrotic cells that have not shrunk but whose protoplasm stains intensely with eosin and whose nuclei do not stain. No hemorrhage. Kidneys: large amount of fat in the cells of the collecting tubules.

DOG XVI (C).—Small bull dog.

Mar. 13, '08. Given chloroform for five hours. Recovered promptly.

Mar. 14, '08. Seemed well but a little weak. Given chloroform for one and three-fourths hours in A. M., and for one and one-fourth hours in P. M. Came out of anæsthetic slowly.

Mar. 15, '08. Found dead, still warm, and no rigor mortis. Autopsy. Liver: very yellow and studded with numerous dark red areas evidently in center of lobules. Kidneys: apparently unaltered. Intestines: contained altered blood and many small hemorrhages in mucous membrane of ileum. Mesentery: a few small pinhead hemorrhages.

*Micros.*—Liver: a few islands of cells scattered about the portal spaces are heavily loaded with fat and stain normally with hematoxylin and eosin. The whole center of the lobule is hemorrhagic, the cells are shrunken and stain deep pink and their nuclei are pyknotic.

DOG XVI (D).—Small mongrel fox terrier. This dog was very susceptible to the delayed action of chloroform.

Mar. 16, '08. Chloroform for two hours in A. M. and two hours in P. M., a total of four hours. Came out well from the anæsthetic.

Mar. 17, '08. Very weak and prostrated in A. M.; gradually grew feeble in P. M.; respirations very slow (9 per minute) and had several convulsions. Was moribund and was killed with chloroform. Autopsy at once. Liver: profoundly altered. Very yellow and mottled with dark red areas. Kidneys: apparently unaltered. Intestines: contained bloody feces. Few small hemorrhages in ileum.

*Micros.*—Liver: a few cells at the periphery of the lobule contain fat and stain in a normal manner. The rest of the cells of the lobule, while not diminished in size, stain deep pink with eosin and their nuclei either do not stain or are pyknotic. There is moderate congestion in the lobules, but no hemorrhage.

DOG XVI (E).—Small black and tan terrier.

Nov. 27, '08. Anæsthetized lightly with chloroform for two hours. Thereafter seemed perfectly well. Killed sixty hours later and autopsy performed



at once. Liver: very yellow. Moderate congestion. Kidneys: normal, except for yellowish lines leading down to the pelvis. Other organs normal.

*Micros.*—Liver: the cells in the intermediary zone of the lobules are full of fat, those at the periphery contain none. In the central zone the cells are shrunken, the protoplasm forms only a narrow rim around the nuclei, and from many intercapillary spaces the cells have entirely disappeared. A few cells are found staining deep pink with eosin. Kidney: the cells in the collecting tubules contain a large amount of fat.

Dog XVI (F).—Small black and tan mongrel.

Dec. 3, '08. Given chloroform by inhalation for one hour. Not under deeply and came out very promptly.

Dec. 4, '08. To all appearances quite normal.

Dec. 5, '08. Seemed quite normal. Killed with chloroform. Autopsy at once. Liver: very yellow. No other change. Kidneys: normal.

*Micros.*—Liver: very fatty throughout. Moderate congestion in center of lobules. There is undoubted necrosis of a few cells in the center of each lobule. Their nuclei do not stain and the protoplasm itself takes a deep pink stain with eosin. Kidneys: marked amount of fat in the straight tubules. Other organs normal.

#### METABOLISM EXPERIMENTS.

*Methods.*—The animals used were dogs. The urine was tested before the beginning of each experiment and in each of the animals used was found to be acid in reaction and practically free from albumin. On account of the frequency with which vomiting occurs after anæsthesia with chloroform, no food was given during any of the experiments. Osterberg and Wolf (38) and Underhill and Kleiner (39) have shown that the metabolism of fasting dogs is practically constant from a day or two after the beginning of starvation for many days. The first day of each experiment began twenty-four hours after the last feeding, except in the case of Dog I, in which it was over forty-eight hours. Water was given *ad libitum*. The animals were kept in well ventilated cages suited to the quantitative collection of urine. Most of the urine was collected by catheter. Aseptic precautions were observed in the process of catheterization and the bladder was washed daily with four per cent. boric acid solution. In none of the experiments was there evidence of an infection of the bladder. When urine was passed voluntarily in the cage it flowed immediately into a jar containing powdered thymol.

Vomiting occurred on the days following the administration of chloroform. During the first half of each twenty-four hour period contamination of urine with vomitus was always prevented. During the second half this was not always possible. The vomitus, however, was ejected immediately after drinking water and consisted of clear watery fluid, containing a little bile and mucous. The mixture of vomitus and urine after filtration gave no reaction for albumin with heat and acetic acid and we are disposed to think that accidents of this sort were of little significance. When this occurred, the uncontaminated urine of the first half of the day was analyzed separately from the contaminated urine of the second half.

Chloroform (Squibb's, for anæsthesia) was administered by an open cone

and enough was given to produce and maintain surgical anaesthesia, *i. e.*, complete muscular relaxation with absence of the conjunctival reflex. The actual amounts of chloroform used, or the percentage strengths of the chloroform vapor inhaled, were not estimated. During the periods of narcosis, the bodily heat of the animals was maintained by an electric lamp suspended several inches above the body. No fall of temperature occurred in any experiment. The total urine of the twenty-four-hour period, except where otherwise specified, was combined with washings of the bladder and of the collecting tray of the cage and made up to known volume with water. Total nitrogen was estimated by the Kjeldahl method; urea, ammonia, creatinin and creatin by the methods of Folin. The different forms of sulphur were estimated as barium sulphate following the procedures recommended by Folin.

The tissues were fixed in 5 and 10 per cent. formalin for frozen sections and in Müller's fluid and formalin (10 per cent.) equal parts for parafine and celloidin sections. Fat was stained with Scharlach R. The parafin and celloidin sections were stained with hematoxylin and eosin, with Weigert's hematoxylin and with eosin and methylene blue.

#### DESCRIPTION OF EXPERIMENTS.

EXPERIMENT I.—White, bull bitch pup; weight, 7.65 kilos.

*Normal period, June 11 and 12.*—Catheterized daily at 9.45 A. M. and bladder washed with boric acid solution.

*Chloroform day, June 13.*—Catheterized at 9.45 A. M. Chloroform administered from 10.30 A. M. to 12.30 P. M. Urine collected at 12.30 by catheter, 10 c.c. Total urine till 2.30 P. M., 75 c.c. Chloroform again administered from 3.15 to 4.45 P. M. Total period of anaesthesia, three and one-half hours. At end of second period, 35 c.c. of urine were obtained by catheter. Recovery from the second anaesthesia was slow; for over an hour the animal remained in the second stage of anaesthesia and during the evening was very weak. The following morning she appeared normal except for weakness and general depression. No vomiting occurred.

*After period, June 14–16.*—No noteworthy symptoms occurred. There was no vomiting and feces passed on June 14 were normal in appearance and consistency.

At the end of the experiment, the animal was killed with ether and the autopsy immediately performed.

*Autopsy.*—Animal much emaciated, adipose tissue practically absent. Heart and lungs normal. Stomach and intestines normal. A small amount of bile-stained fluid was found in the stomach, duodenum and lower end of the ileum. Pancreas and spleen appeared normal. Liver apparently of normal size; surface smooth, consistency about normal; color strikingly yellow; cut sections showed lobulations plainly; centers of lobules appeared grayish and periphery very yellow. No congestion. Gall bladder was full of greenish bile. Kidneys were of normal size and consistency; surface normal. On section, the periphery of the cortex was rather lighter in color than the remainder.

*Microscopical Examination. Liver.*—This is very fatty. The fat is chiefly, but not entirely, confined to the periphery of the lobules. Stained with hema-

toxylin and eosin, it is seen that around the interlobular spaces in the periphery of the lobules the liver cells retain well their form and staining properties. Within the cells appear numerous larger and smaller round, clear areas from which fat has evidently been dissolved. In the intermediary and central zones great changes have taken place. The liver cells are in all stages of degeneration. Some show only a faintly staining nucleus and the reticular structure of the cell; others stain very badly—both the nucleus and protoplasm. Other cells consist only of a shrunken and distorted and deeply staining nucleus surrounded by a very narrow rim of protoplasm. From some intercapillary areas the cells have entirely disappeared. Confined almost entirely to the intermediary and central zones of the lobules are scattered giant cells containing within themselves masses which stain dark blue with hematoxylin. These masses are soluble in dilute acetic acid without the production of gas bubbles, stain black with a dilute solution of nitrate of silver, and are therefore composed of calcium phosphate. Only in a few instances are they found among the apparently healthy cells. The number of these in each lobule varies between ten and fifty. The capillaries in the central zones contain a few red blood cells. There are no thromboses.

*Kidneys.*—There is a moderate fatty deposit (not much more than is ordinarily found in dogs) in the cells of the limbs of Henle's loop and in the collecting tubules. The glomeruli are normal. There is a marked degenerative change in the cells of the tubules, especially of the convoluted tubules. The cells are in places swollen so as almost to occlude the lumina of the tubules, their borders are irregular and fragments of them have been broken off. In other tubules nothing but nuclei surrounded by a narrow rim of protoplasm remain, and in still others the cells are retained but the nuclei do not stain. Many tubules contain fragments of cells and casts.

*Heart.*—The muscle retains its transverse striations.

*Gastro-Intestinal Tract.*—Normal.

EXPERIMENT 2.—Full-grown fox terrier bitch; weight, 6.78 kilos.

*Normal period, June 23 and 24.*—Catheterized daily at 9.00 A. M., and bladder washed with boric acid solution.

*Chloroform day, June 25.*—After catheterization at 9.00 A. M., administration of chloroform was begun at 9.38 A. M., and continued till 12.08 P. M. Twice during this period artificial respiration was necessary. Chloroform was again given from 4.10 to 6.10 P. M. Artificial respiration was necessary twice during this period. Total duration of anæsthesia, four and one-half hours. Recovery from the effects of the anæsthetic was slow but uneventful.

*After period, June 26–28.*—Catheterized and bladder washed twice daily at twelve-hour intervals. Vomiting occurred after water was taken on June 26. During the day contamination of the urine from this cause was prevented, but the large volume of urine for the twenty-four hours indicates that urine was mixed with vomitus during the night. Except for the presence of a little frothy mucus the urine appeared normal, and after filtration gave no reaction for albumin. No vomiting occurred on June 27 and 28. Feces containing old blood were passed on June 27.

*Second chloroform day, June 29.*—Chloroform was administered from 10.15 to 11.45 A. M., and again from 1.45 to 3.15 P. M. Total duration of anæsthesia, three hours. The course of the anæsthesia was uneventful but recovery was

slow, the animal being completely prostrated until about 7.00 P. M. Urine secreted from 9.00 A. M. to 7.00 P. M., was collected by catheter and analyzed separately (29 a). On the following morning, at 7.30 A. M., the animal could stand and walk about and, except for weakness and general depression, appeared to be normal in behavior. From 8.30 A. M. till death occurred, at about 10.00 A. M., she was completely prostrated, and, during this time, frequent convulsions occurred. They began with increase in depth and frequency of respiration, twitching and rigidity of the neck muscles; then followed clonic spasms of the legs, and finally a tetanic condition of the whole body. The seizures resembled the convulsions produced by potassium cyanide. The mucous membranes of the mouth, nose and eyes were noticeably bright pink in color. Respiration was markedly increased after each convulsion. In the intervals between the convulsions, prostration was very great, the animal was unconscious but the reflexes were retained. During this time black fluid feces which apparently consisted of old blood were passed. Vomitus of a similar character was ejected. Death occurred at 10 A. M.

The urine which had been passed during the night (7.00 P. M. to 7.00 A. M.) was found mixed with feces or vomitus or both, similar in character to that just mentioned. To this mixture was added the urine collected by catheter at 9.00 A. M., the whole heated to boiling, acetic acid added to complete coagulation, the mixture cooled, made up to 500 c.c., and filtered. The filtrate gave a fairly intense biuret reaction. Aliquot portions of this filtrate were used for the analyses (29 b).

*Autopsy.*—Made immediately after death. The animal was not greatly emaciated, subcutaneous fat being fairly abundant. A curious, vivid, pinkish hue was noticed in the subcutaneous tissues and muscles. Heart and lungs appeared normal. The peritoneum appeared smooth and glistening. Omentum was congested and in it two small areas of hemorrhage were found. It was dotted here and there with typical fat necroses in all stages of formation; none exceeded 4 mm. in diameter. These necroses were found in the fat surrounding the pancreas, within the pancreas itself and in the mesentery of the colon. The pancreas was very firm and pale. There was no sign of obstruction in its ducts. The gall bladder was full of greenish bile. The cystic and common ducts were patent. Liver was of normal size and very yellow. On the surface and on cut section it was dotted with very numerous reddish areas of pinhead size, which were evidently in the center of the lobules. The spleen was normal. Kidneys: cortex slightly yellowish in the neighborhood of the pyramids; otherwise they appeared normal. The mesenteric glands were enlarged and soft. The stomach contained hemorrhagic material, but the mucosa appeared normal. The whole intestinal tract was full of hemorrhagic material. The mucosa of the entire small intestine was hemorrhagic, less marked in the upper third of the ileum than in the duodenum and lower two-thirds of the ileum. The colon was congested near the ileo-cæcal juncture.

*Microscopic Examination. Liver.*—There is not a normal parenchymatous cell to be found in any of the sections. About the portal spaces are a few small islands of cells that retain their approximate form and staining properties but are very fatty. There are hemorrhagic areas occupying the center of the lobules. Within these areas the liver cells are in all stages of dissolution.

Many are swollen, their nuclei are disintegrated or absent and they stain deep pink with eosin. Others have degenerated to amorphous masses. The remainder of the lobules are composed of swollen cells of which only the nuclei and the reticular structure of the cell may be seen. Scattered among these hydropic cells are others that stain deep pink and are without nuclei. In the hemorrhagic areas only a few small masses stain red with Scharlach R, while in the periphery most of the cells stain deep red. No thromboses are found.

*Kidneys.*—These contain rather more fat than in Dog 1 but in the same situation. The same changes are to be found in the tubules as in Dog 1 but they are not quite so marked.

*Pancreas.*—The substance of the pancreas itself is normal. In the fat immediately surrounding the pancreas and in the omentum and mesentery are numerous small hemorrhages and typical fat necroses. The necrotic areas are surrounded by hemorrhage and accumulations of polymorphonuclear leucocytes in great numbers.

*Heart.*—Striations are present in the heart muscle.

*Gastro-Intestinal Tract.*—The mucous membrane is congested throughout. In the stomach and large intestine a few hemorrhages are seen; in the small intestine they are almost continuous throughout its whole extent.

EXPERIMENT 3.—Young white bull-bitch; weight, 8.52 kilos.

*Normal period, July 4 and 5.*—Catheterized and bladder washed daily at 9.30 A. M.

*Chloroform day, July 6.*—Chloroform given from 9.40 A. M. till 12.40 P. M., and from 3.20 till 5.50 P. M. Total duration of anæsthesia five and one-half hours. Urine secreted between 9.30 A. M. and 9.30 P. M. measured 82 c.c., and was analyzed separately (6 a). Urine passed during the night (9.30 P. M. to 9.30 A. M.) was contaminated with clear watery vomitus and contained some mucus. This portion was analyzed separately (6 b).

*After period, July 7 and 8.*—During July 7, vomiting occurred very frequently after water was taken. The vomitus did not contain blood. Contamination of the urine was prevented. Feces passed on July 7 were of fairly firm consistency and contained both fresh and old blood. Aside from weakness and these gastro-intestinal symptoms, the animal appeared normal.

July 8.—Until 4.00 P. M. the animal was conscious and apparently normal except for great muscular weakness, and gastro-intestinal symptoms. She stood or walked with great difficulty. Vomiting was frequent; the vomitus contained bloody mucus. At about 4.00 P. M. she became prostrated and nearly unconscious. She lay on her side and at intervals whined in a semi-conscious manner, snarling and biting aimlessly at the floor of the cage. The respiration was very rapid, sometimes deep and labored. The mucous membranes of the mouth, nose and eyes were very noticeably bright pink in color. In the evening at about 9.30 she was prostrated and unconscious, though the reflexes were retained. Respiration, 134 per minute; pulse, 220 per minute; rectal temperature, 41° C. (normal for this animal, 38.8° C.). Her stupor was interrupted by periods of delirious crying, aimless and unconscious or semi-conscious attempts at biting and incoördinated muscular movements. She was catheterized at 9.30 P. M. Death occurred during the night and on the following morning she was found in a state of rigor. The jaws were smeared with bloody vomitus, the hind-

quarters with bloody feces, and the urine receiver contained a mixture of urine with bloody vomitus and feces. The urine collected between 9.30 A. M. and 9.30 P. M. was analyzed. The results obtained, as well as the volume of urine, have been doubled in order to make the figures comparable with those obtained on previous days.

*Autopsy.*—Made soon after the death of the animal was discovered. Emaciation was not extreme. An unusual vivid pinkish hue was noticed in the subcutaneous tissues and muscles. Hemorrhagic extravasations were found in the tissues of the mediastinum and pleura. The lungs were normal. The pericardium and epicardium contained hemorrhagic areas. The myocardium was pale. The parietal peritoneum contained no hemorrhages. The omentum showed areas of marked congestion and contained a few scattered points of hemorrhage. There were numerous scattered areas of hemorrhage in the mesentery, close to its intestinal attachment. The spleen was somewhat shrunken; no follicles were seen on section. The pancreas was firm and pink in color. The liver was apparently somewhat smaller than normal. Its substance was soft and easily friable. In appearance it resembled that of Dog 2, being very yellow with reddish areas apparently at the center of the lobules. Such areas were larger than in the liver of Dog 2. The gall bladder was filled with dark green bile. The kidneys were of normal size, the tissues somewhat soft and the cortex pale in color. The stomach contained bloody material of fluid consistency; its mucosa was slightly congested. The intestines were filled with blackish fluid material, evidently consisting chiefly of blood. An accurate judgment concerning the condition of the intestinal mucosa was difficult on account of post-mortem change which was considerable. It appeared congested and hemorrhagic, especially in the duodenum and lower third of the ileum. The mesenteric lymph nodes were large and soft.

*Microscopic Examination. Liver.*—This is greatly altered. At the periphery of the lobules the cells stain well but are very fatty. The inner two-thirds of each lobule are hemorrhagic. In the hemorrhagic areas the cells are swollen, stain deeply with eosin, and are without nuclei. Giant cells containing calcium phosphate are present in the hemorrhagic areas, but are confined to their periphery and are not found in the center. No thromboses are to be seen.

*Kidneys.*—These are greatly congested. There are small hemorrhages in the cortex as well as the pyramids, and one or two within the glomeruli themselves. In other respects the changes are like those that have been described.

The results which have been obtained are included in the tables on pages 367, 368, 369 and 370. Some of the results have been plotted in the form of curves and may be found on pages 359, 361 and 362. Other data not included in the tables are as follows: a faint reaction for albumin was obtained in the urine of each dog on the days on which the chloroform was given. The urine showed a slight reducing power when tested with Fehling's solution on these days. The urine of each dog on the days on which chloroform was administered was highly colored and gave strong reac-

tions for bile pigment. This reaction persisted until the end of the experiment and in the cases of Dogs 2 and 3 was very intense. Gerhardt's test for diacetic acid and Legal's test for acetone was negative in every urine. Every urine was acid to litmus.

The limits of this paper do not include a consideration of the symptoms exhibited by human cases, but a word in regard to the similarity to them of those observed in our dogs may be allowed. In the so-called "cholæmic" or adult type of delayed poisoning, a longer or shorter uneventful period is the rule. And so it was with both our dogs. With Dog 3 at least 28 hours intervened after the anæsthetic before untoward symptoms appeared, and during this time it was impossible to detect any difference from the recovery from any severe narcosis. Human cases become wildly delirious, occasionally have convulsions and coma ends the scene. The signs of mental disorder or the equivalent of mental disorder in a dog were seen in both of ours. In Dog 2 there were convulsions and in Dog 3 purposeless attempts to bite and snarling and howling. Coma supervened in both instances. The vomiting of blood and the bloody stools and the large amount of bile pigment in the urine are common to both animals and human beings.

#### SUMMARY OF RESULTS.

*Total Nitrogen.*—In every experiment a greatly increased nitrogen elimination occurred on the day on which chloroform was given and on the following days. On the day of poisoning, it amounted to 48 per cent. in Experiment 1; to 65 per cent. in Experiment 2; and to 108 per cent. in Experiment 3. On the next day after, in every experiment, a still greater increase occurred: 54 per cent. in Experiment 1; 76 per cent. in Experiment 2; and 185 per cent. in Experiment 3. In the two following days, in Experiment 1, a gradual decrease to normal took place. In Experiments 2 and 3, the nitrogen elimination remained high until the end.

*Urea and Ammonia.*—The curve of excretion of nitrogen in the form of urea and ammonia follows the curve of total nitrogen excretion very closely in Experiments 1 and 3. In Experiment 2 the sum of these forms of nitrogen diminished on the day following chloroform while the total nitrogen increased. A gradual increase then occurred during two days, but on the second chloroform day the increase in total nitrogen was again accompanied by a decrease in urea and ammonia. In every experiment the *percentage* of nitrogen in the form of urea and ammonia decreased on the day of chloroform. This percentage diminution became greater on the next following day in Experiments 1 and 2 and remained the same in Experiment 3. In every experiment it then rose,

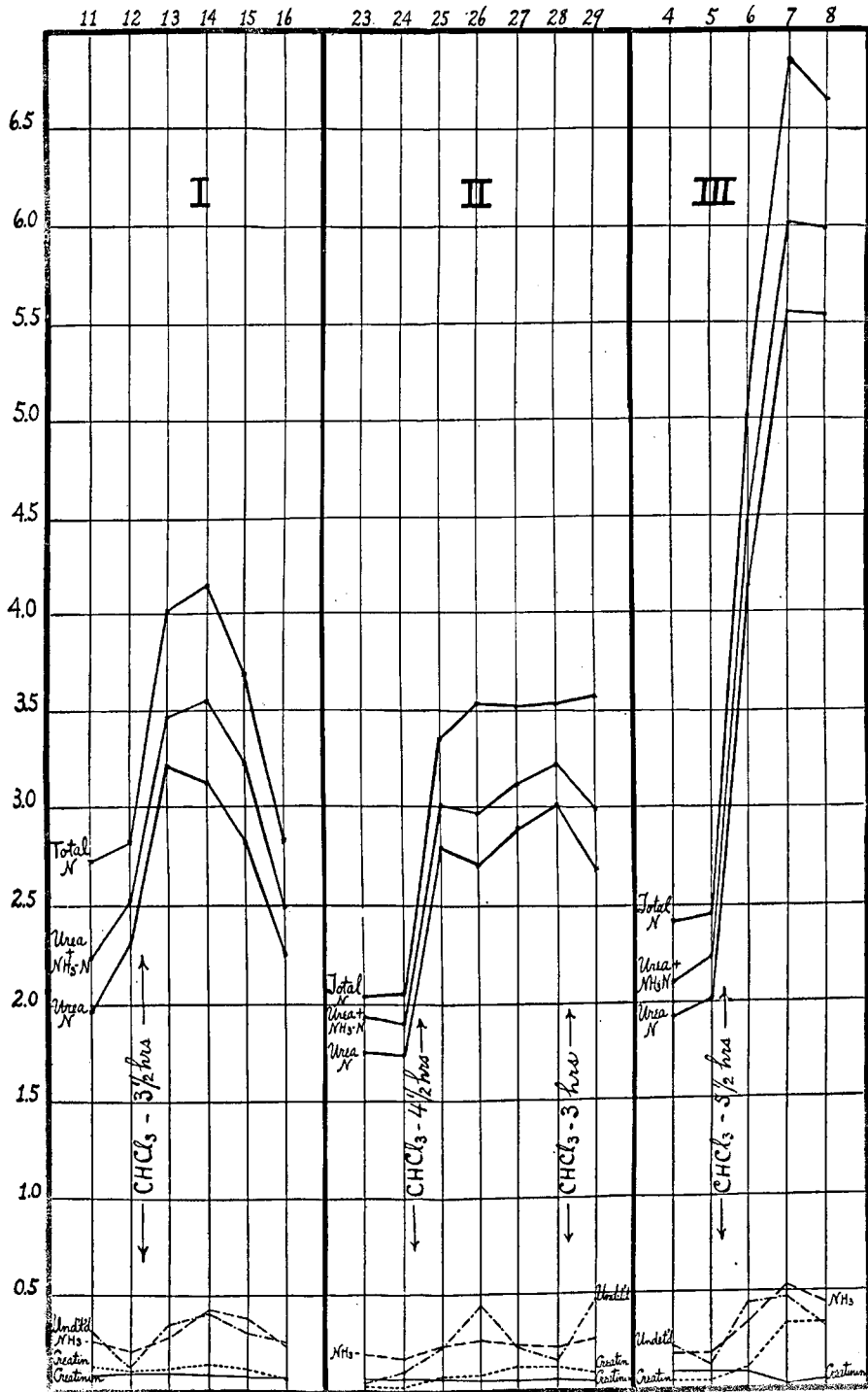


DIAGRAM I. Excretion of nitrogen in the three metabolism dogs. Ordinates = grams of nitrogen; abscissas = days of experiments.



returning to normal in Experiments 1 and 2. In Experiment 2 the second administration of chloroform again caused a decrease in the percentage of nitrogen as urea and ammonia.

In each experiment on the day of poisoning, the curve of urea nitrogen excretion runs almost exactly parallel with those of total nitrogen and urea and ammonia nitrogen. On the day following poisoning there is a divergence of the urea curve from that of total nitrogen, the percentage of urea nitrogen being further decreased. Although an increase in ammonia nitrogen occurred on each day on which chloroform was given, it was not proportional to the increase in total nitrogen, and hence in every case a percentage decrease was found. A greater increase in ammonia nitrogen occurred in every experiment on the day following chloroform, so that in Experiment 2 the percentage returned to normal, and in Experiments 1 and 3 it exceeded normal. In Experiment 1 and in Experiment 2 till the second chloroform day, a gradual decrease then took place, the percentage figures remaining high only in Experiment 1.

*Creatin and Creatinin.*—In Experiments 1 and 2 the absolute values for creatinin were constant throughout; the percentage figures are, therefore, decreased on the chloroform days and those following. In Experiment 1 creatin nitrogen increased on the chloroform day and the day following, so that a fairly constant percentage of this form of nitrogen was maintained. In Experiment 2 a greater absolute increase in creatin occurred, so that the percentage was higher than normal on and after the chloroform day. The second administration of chloroform in Experiment 2 did not cause as great an increase in creatin as did the first.

In Experiment 3, creatinin was constant on the two normal days and on the chloroform day. It disappeared altogether on the day following anaesthesia, but reappeared in small amount on the final day. These changes produced a marked decrease in the percentage values. Creatin, constant on the two normal days, rose slightly on the day of anaesthesia, and on the two following days was increased by 200 per cent. This caused a very marked rise in the percentage values.

*Undetermined Nitrogen.*—The undetermined nitrogen in each experiment increased on the day of anaesthesia. The percentage on this day is high in all and much above normal in Experiment 2. In every experiment a still greater absolute increase took place on the day following chloroform. On this day, in Experiments 1 and 2, there was a percentage increase. In Experiment 3 a decrease in the percentage value occurred. An actual and a percentage fall in undetermined nitrogen occurred on the days of recovery in Experiments 1 and 2, and also on the day of death in Dog 3. In Dog 2, on the second day of poisoning, an actual and percentage increase in undetermined nitrogen took place.

*Total Sulphur.*—As in the case of total nitrogen, total sulphur was greatly increased on the days of chloroform poisoning in every experiment. This increase amounted to 114 per cent. in Experiment 1; 133 per cent. in Experiment 2; and 170 per cent. in Experiment 3. In Experiment 1, this rise was succeeded by a gradual fall to normal. In Experiments 2 and 3 the total sulphur underwent still greater increase on the day following poisoning; thereafter there was a decrease, but never to normal figures. In none of the experi-

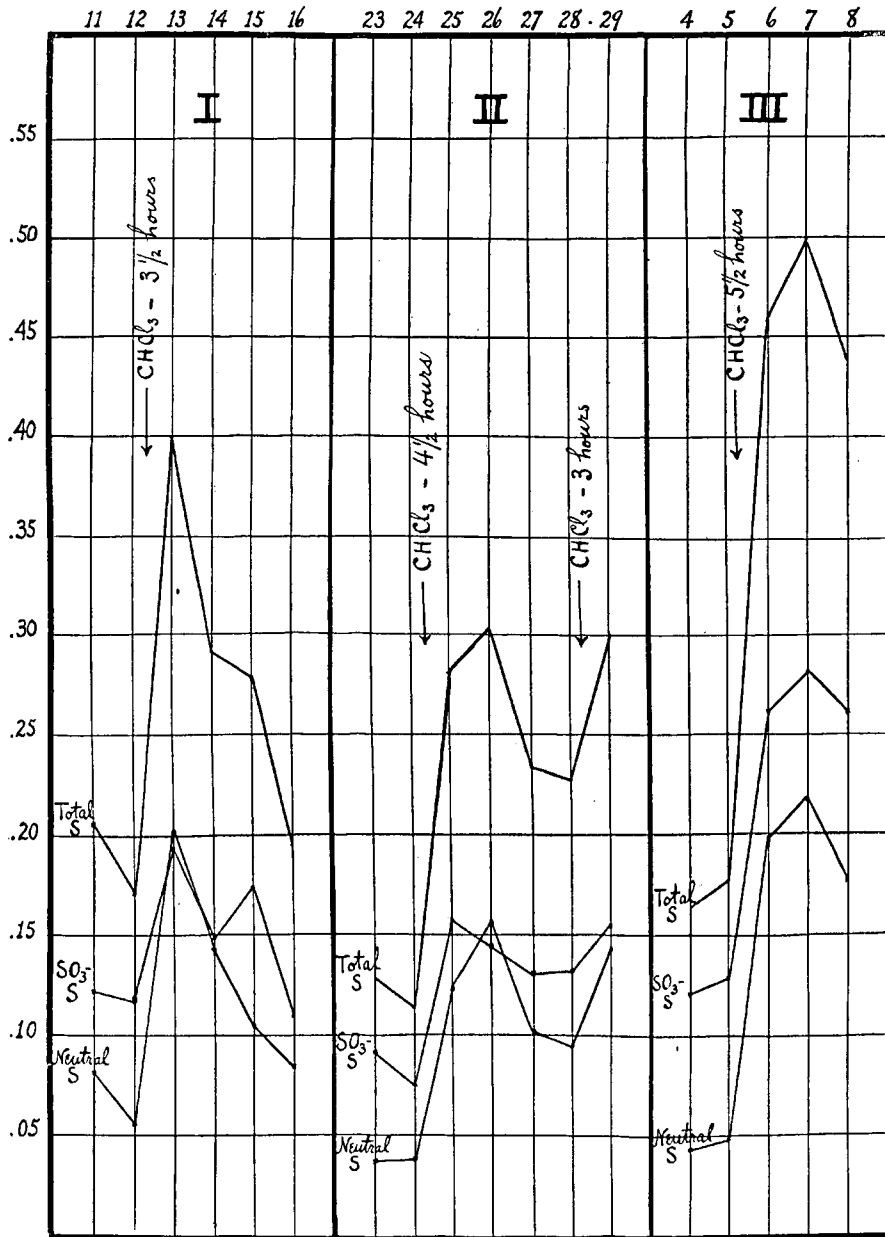


DIAGRAM 2. Excretion of sulphur in the three metabolism dogs. Ordinates = grams of sulphur.

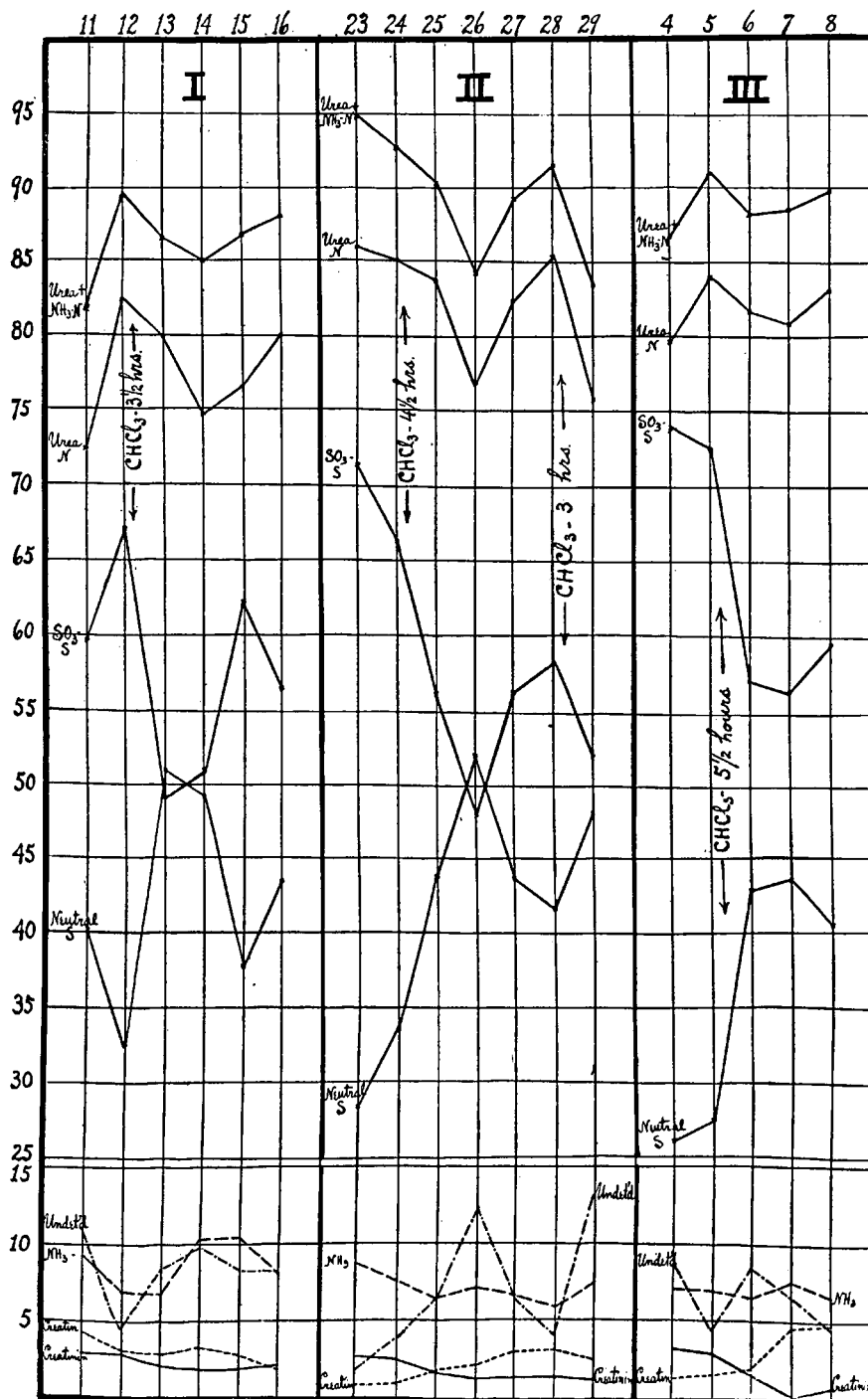


DIAGRAM 3. Distribution of nitrogen and sulphur in the three metabolism dogs. Ordinate = percentages of total nitrogen and total sulphur.

ments was there a close parallelism between total sulphur and total nitrogen elimination.

*Sulphate Sulphur and Neutral Sulphur.*—On the day of chloroform poisoning in each experiment the increase in neutral sulphur was greater than the increase in sulphate sulphur. On this day, therefore, a marked decrease in the percentage of sulphate sulphur and a corresponding increase in the percentage of neutral sulphur was found. On the day following chloroform in Experiment 1 recovery of the normal sulphur relations began. In Experiment 2, a decrease in sulphate sulphur occurred while neutral sulphur underwent a still further increase. In Experiment 3 on this day both increased. In both experiments (2 and 3), the decrease in percentage of sulphate sulphur and the increase in percentage of neutral sulphur which began on the day of anaesthesia were intensified on the following day. On the second and third days after chloroform in Experiment 2, which were days of recovery, the sulphur values tended to regain normal, but on neither day was the return to normal nearly complete. This is also true of the final day (day of death) in the case of Dog 3. The second day of chloroform in Dog 2 is marked by changes in the sulphur excretion similar to those on the first chloroform day.

#### DISCUSSION OF CHEMICAL RESULTS.

In the first of the three metabolism experiments which have been described, severe poisoning by chloroform followed by normal recovery has been induced. This experiment may therefore serve as a control for the second and third experiments in which fatal delayed poisoning resulted. In all three experiments the excretion of total nitrogen is greatly increased on the day of poisoning. In Experiment 3 this increase was fifty per cent. greater than in Experiments 1 and 2. In all three the distribution of the nitrogen as urea and ammonia is normal: the absolute values for creatinin are strictly normal. Percentage figures for undetermined nitrogen in Experiments 1 and 3 are within normal limits. The quantitative change in total sulphur excretion varies among the three, being greatest in Experiment 3, but the change in distribution between sulphate sulphur and neutral sulphur is alike in all. It is obvious, then, that on the day of anaesthesia no distinction can be made between the animal which recovered and those in which fatal poisoning ensued on the basis of percentage differences in the distribution of nitrogen as urea, ammonia, creatinin, and undetermined, or in the sulphur distribution. Only in the case of creatin nitrogen is there a difference between the control (1) and the other two dogs. In the control on the day of poisoning it is normal, in the other two a

marked increase occurs. The change in amount of this substance is not proportional to the severity of poisoning, for it was greater in Experiment 2 than in Experiment 3. So far, then, as a comparison of the data for the day of poisoning is concerned, we may conclude that there is nothing in the analytical differences to show which animal will succumb to late poisoning, although a rough index of the severity of the acute effects is given by the total nitrogen and sulphur figures and the changes in creatin.

A similar examination of the figures for the days following the poisoning shows no difference in distribution of nitrogen as urea and ammonia between control Dog 1 and fatally poisoned Dog 3. In both, the percentage figures are normal throughout. Considered separately an apparent difference exists in that the percentage of ammonia nitrogen rises in Experiment 1 and remains nearly constant in Experiment 3; it is also fairly constant in Experiment 2. It is difficult to say whether any importance should be attached to this result or not. The percentage of undetermined nitrogen is within normal limits throughout in Dogs 1 and 3.

Creatinin, which in the control animal was constant up to the second day after poisoning and then suffered only a slight decrease, in Dog 2 was somewhat decreased after the poisoning and markedly decreased after the second poisoning (29 *b*). In Dog 3, it disappeared entirely on the day after poisoning, but reappeared in the half-day preceding death. Creatin, which in the control (1) was normal on the chloroform day, on the day after was moderately increased, and during recovery sank to below the initial figures. In the dogs suffering the severer poisoning, creatin, increased on the days of poisoning, is very strikingly increased on the days after. So far as the nitrogen distribution is concerned, then, the fatally poisoned dogs vary from the control mainly in respect to changes in creatin and creatinin. Recovery of the normal ratio between the sulphate sulphur and neutral sulphur is delayed for a day after poisoning in the control dog; in neither of the other two is the normal relation reached after the poisoning. In this respect, the animals suffering late poisoning differ from the one in which recovery is normal.

These results show that quantitative differences only exist be-

tween the fatally poisoned dogs and the control, and that aside from variations in the amounts of nitrogen and sulphur excreted, the greatest differences are to be found in creatin, creatinin and neutral sulphur.

Definite conclusions as to the significance of these changes are difficult. It is most reasonable to assume that the intense destruction of body protein which the excreted nitrogen and sulphur represent is the result of autolysis. The known property of chloroform of interfering with synthetic and oxidative processes without inhibiting autolytic processes (as pointed out by Wells (40)), together with the great similarity between the late fatal chloroform poisoning and phosphorus poisoning in which increased autolysis has been demonstrated, bear out this view. It is a striking fact and worthy of especial emphasis that the distribution of nitrogen as urea, ammonia and undetermined, so closely approximated the normal. There could have been no excessive elimination of amino-acids, and it is obvious that death could have not been due to accumulation of these bodies, or to an acid intoxication.

In view of our present ignorance concerning the physiological significance of creatin, creatinin and neutral sulphur, attempt to fix the responsibility for the fatal effects of chloroform upon changes in processes having to do with the elaboration of these substances, would be hazardous. Folin is the author of the conception of the intimate relationship between creatinin excretion and endogenous metabolism. Our figures show—as has already been shown in the case of potassium cyanide—that an intense increase of endogenous catabolism may be consistent with markedly diminished creatinin excretion. They might be considered as supporting in a measure the view of Mellanby (41) that the liver is the organ chiefly concerned in the formation of this substance. It is also impossible for us to decide at present whether the increase in neutral sulphur is the result of decreased oxidation of sulphurized substances which under more nearly normal conditions would have been excreted as sulphuric acid or whether its excretion in increased amount represents an increase in processes of metabolism, the specific end products of which include sulphur in this form.

We believe we are justified in the belief that the death of the

two fatally poisoned animals was not due to acid intoxication or to the accumulation of products (amino-acids) of cell digestion. We believe also that death was not due solely to the excessive amount of protein lost, *i. e.*, tissue disintegrated. Under the influence of starvation alone an animal could lose the amount of protein equivalent to the amounts of nitrogen excreted by our dogs without serious consequences.

We are driven to the quite indefinite and unsatisfactory view that death is due to the presence of toxic substances of an unknown nature, which owe their presence either to excessive formation by abnormal metabolic processes, or to the failure on the part of the organism to neutralize in the normal manner toxic substances normally formed.

#### DISCUSSION OF PATHOLOGICAL RESULTS.

From the experiments of others and those that we have ourselves performed, it is plain that a single anæsthesia with chloroform of a duration of more than half an hour induces fatty changes that are demonstrable in the liver in from twelve to twenty-four hours, reach their maximum in about forty-eight hours and then gradually retrograde, but the excess of fat may be demonstrated for about two weeks. The fat appears first in the intermediary zone and eventually may spread to the periphery and the whole lobule be intensely fatty. With more prolonged, and especially with repeated anæsthesias which are very much more destructive than single ones, necrosis appears. But, as is shown by two of our experiments, anæsthesia of only one or two hours is sufficient, at least at times, to cause necrosis. The necrosis also begins in the center and extends centrifugally, so that almost all the cells may be necrotic and only a few around the portal spaces be still living and these are full of fat. The amount of protoplasm in the necrotic cells varies according to the length of time intervening before death. When this occurs promptly the cells may remain of almost normal size and contour. When longer delayed, the protoplasm diminishes markedly until there may be only the narrowest margin around degenerated nuclei that lie in the intercapillary spaces. The amount of hemorrhagic extravasation varies from none at all to a very con-

siderable amount. Some of our specimens show a degree of this greater than we have seen reported.

TABLE I.  
*Excretion of Nitrogen in Experiment 1.*

Date, June, 1908.	Body Weight.	Volume of Urine.		Urea N.	NH <sub>3</sub> N.	Urea + NH <sub>3</sub> N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.	Urea N.		NH <sub>3</sub> N.	Urea + NH <sub>3</sub> N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.
		c.c.	gms.								per cent.	per cent.						
11	7.18	315	2.7170	1.9710	0.2520	2.2230	0.0809	0.1131	0.1940	0.3000	72.54	9.28	81.82	2.98	4.16	7.14	11.04	
12	7.00	240	2.8210	2.3270	0.1950	2.5220	0.0791	0.0914	0.1705	0.1285	82.49	6.91	89.40	2.80	3.24	6.04	4.56	
13	6.78	400	4.0170	3.2136	0.2704	3.4840	0.0816	0.1150	0.1966	0.3364	80.00	6.73	86.73	2.03	2.86	4.89	8.37	
14	6.68	315	4.1730	3.1226	0.4264	3.5490	0.0772	0.1378	0.2150	0.4090	74.82	10.22	85.04	1.85	3.30	5.15	9.80	
15	6.48	705	3.6920	2.8262	0.3848	3.2110	0.0674	0.1052	0.1726	0.3084	76.55	10.42	86.97	1.83	2.85	4.68	8.35	
16	6.32	495	2.8210	2.2568	0.2262	2.4830	0.0582	0.0507	0.1089	0.2291	80.00	8.02	88.02	2.06	1.80	3.86	78.12	

Very striking in two of our metabolism dogs were the calcium deposits and the giant cells surrounding them. These deposits were always in the necrotic areas and chiefly in the periphery of these. Their presence in less than forty-eight hours after poisoning indicates the rapidity of calcium deposition and engulfing cell formation. There is no mention of such deposits in pathological or experimental literature relating to chloroform. That they follow as a result of the chloroform is shown by the fact that we produced them in two instances.

TABLE II.  
*Excretion of Sulphur in Experiment 1.*

Date, June, 1908.	Total S.	Total SO <sub>3</sub> S.	Inorganic SO <sub>3</sub> S.	Ethereal SO <sub>3</sub> S.	Neutral S.	Total SO <sub>3</sub> S.	Neutral S.
	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.
11	0.2055	0.1228	0.1033	0.0195	0.0827	59.76	40.24
12	0.1739	0.1170	0.1030	0.0140	0.0569	67.28	32.72
13	0.3961	0.1942	0.1783	0.0159	0.2019	49.03	50.97
14	0.2920	0.1486	0.1475	0.0011	0.1434	50.89	49.11
15	0.2799	0.1739	0.1588	0.0151	0.1060	62.13	37.87
16	0.1956	0.1104	0.0926	0.0178	0.0852	56.44	43.56



In our second metabolism experiment mention was made of fat necrosis of the omentum and mesentery. There is no similar record in the literature. That fat necrosis sometimes occurs with hepatic poisons is shown by the reported observation of Wells (42) in regard to hydrazine poisoning. He produced in one dog small and scattered but typical fat necroses. Our result with chloroform stands as an isolated observation.

TABLE III.  
*Excretion of Nitrogen in Experiment 2.*

Date.	June, 1908.		Volume of Urine.	Total N.	Urea N.	NH <sub>3</sub> N.	Urea + NH <sub>3</sub> N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.	Urea N.	NH <sub>3</sub> N.	Urea + NH <sub>3</sub> N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.
	Body Weight.																	
	kilos.	c.c.	gms.	gm.	gm.	gms.	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
23	6.78	38	2.0280	1.7446	0.1794	1.9240	0.0530	0.0146	0.0676	0.0364		86.02	8.85	94.87	2.61	0.72	3.33	1.80
24	6.62	37	2.0475	1.7420	0.1560	1.8980	0.0511	0.0173	0.0684	0.0811		85.08	7.62	92.70	2.50	0.84	3.34	3.96
25	6.58	240	3.3540	2.8080	0.2210	3.0290	0.0507	0.0543	0.1050	0.2200		83.72	6.58	90.30	1.51	1.62	3.13	6.56
26	6.51	605	3.5360	2.7200	0.2570	2.9770	0.0455	0.0722	0.1177	0.4413		76.92	7.27	84.19	1.29	2.04	3.33	12.48
27	6.48	88	3.5100	2.8938	0.2392	3.1330	0.0462	0.1062	0.1524	0.2246		82.44	6.81	89.25	1.32	3.03	4.35	6.40
28	6.28	165	3.5230	3.0134	0.2106	3.2240	0.0486	0.1084	0.1570	0.1420		85.53	5.98	91.51	1.38	3.08	4.46	4.03
29 <sup>a</sup>		110	2.0540	1.6562	0.1638	1.8200	0.0223	0.0648	0.0871	0.1469		80.63	7.98	88.61	1.09	3.15	4.24	7.15
29 <sup>b</sup>			1.49175	1.0335	0.1040	1.1375	0.0147	0.0207	0.0354	0.31885		69.28	6.97	76.25	0.98	1.39	2.37	21.38
29 <sup>c</sup>	6.10		3.54575	2.6897	0.2678	2.9575	0.0370	0.0855	0.1225	0.46575		75.86	7.55	83.41	1.04	2.41	3.45	13.14

The changes produced in the kidneys and heart have been found by other investigators and by ourselves to be much less constant and severe than in the liver. There is often considerable fat in the kidneys, especially in the cells of the straight and collecting tubules. The presence of fat in this situation in normal dogs renders a conclusion somewhat difficult. Hemorrhages are sometimes found in the kidney. The chief change is in the cells of the convoluted tubules which are at times swollen, at others eroded and often a few necrotic. The lumina of the tubules contain fragments of cells and casts. It is the rule to find the glomeruli entirely unaffected.

The muscles of the heart may show loss of transverse striation

and many fat drops throughout the fibers. Changes in the nuclei have also been described. Frequently, however, no abnormality can be found. From our experience it seems that there is not enough damage done this organ to explain any interference with its functional activity.

TABLE IV.  
*Excretion of Sulphur in Experiment 2.*

Date, June, 1908.	Total S.	Total SO <sub>3</sub> S	Inorganic SO <sub>3</sub> S	Ethereal SO <sub>2</sub> S	Neutral S.	Total SO <sub>3</sub> S.	Neutral S.
	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.
23	0.1299	0.0930	0.0860	0.0070	0.0369	71.57	28.43
24	0.1155	0.0766	0.0718	0.0048	0.0389	66.32	33.68
25	0.2814	0.1579	0.1527	0.0052	0.1235	56.11	43.89
26	0.3035	0.1456	0.1390	0.0066	0.1579	47.98	52.02
27	0.2347	0.1321	0.1264	0.0057	0.1026	56.28	43.72
28	0.2292	0.1335	0.1255	0.0080	0.0957	58.25	41.75
29 <sup>a</sup>	0.2015	0.1137	0.1042	0.0095	0.0878	56.43	43.57
29 <sup>b</sup>	0.0987	0.0423	0.0368	0.0055	0.0564	42.86	57.14
29 <sup>c</sup>	0.3002	0.1560	0.1410	0.0150	0.1442	51.94	48.06

We have found congestion and small areas of hemorrhage in the gastro-intestinal tract frequently, and in one dog the whole small intestine was hemorrhagic. In this same dog were hemorrhages into the peritoneum, pleuræ, pericardium and mediastinal tissue. We have also found hemorrhages in the serous membranes of other dogs. The lesions found elsewhere are negligible.

It may thus be seen that there is a very great similarity between the experimental lesions and those reported in fatal human cases. In both they are most severe in the liver and consist of central necrosis and fatty degeneration and, allowing for a certain difference in structure of the organ, are strictly comparable. The same may be said of the kidney lesions. In both there is also a tendency to hemorrhage into the serous membranes and into the gastro-intestinal tract.

<sup>a</sup>The figures for June 29 are the sum of those obtained in the separate analyses of 29<sup>a</sup> and 29<sup>b</sup>. 29<sup>a</sup> represents the twelve hours during part of which chloroform was administered and the urine of which was uncontaminated. 29<sup>b</sup> represents the following twelve hours, the urine of which was contaminated (see page 352, *i. e.*, description of technique of experiments).

TABLE V.  
*Excretion of Nitrogen in Experiment 3.*

Date, July, 1908.	Body Weight.	Volume of Urine.								per cent.								
		Total N.	Urea N.	NH <sub>3</sub> N.	Urea + NH <sub>3</sub> N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.	Urea N.	NH <sub>3</sub> N.	Urea + NH <sub>3</sub> N.	Creatinin N.	Creatin N.	Creatinin + Creatin N.	Undetermined N.		
	kilos	c.c.	gms.	gms.	gm.	gms.	gm.	gm.	gm.	gm.	gm.	gm.	per cent.	per cent.	per cent.	per cent.	per cent.	per cent.
4	8.52	100	2.4245	1.9344	0.1716	2.1060	0.0760	0.0274	0.1034	0.2151	79.79	7.08	86.87	3.13	1.13	4.26	8.87	
5	8.32	102	2.4570	2.0644	0.1716	2.2360	0.0719	0.0382	0.1101	0.1109	84.02	6.98	91.00	2.93	1.56	4.49	4.51	
6a		82	2.0475	1.7576	0.1014	1.8590	0.0317	0.0434	0.0751	0.1134	85.84	4.95	90.79	1.55	2.12	3.67	5.54	
6b		3.0225	2.3868	0.2262	2.6130	0.0404	0.0537	0.0941	0.3154	78.97	7.48	86.45	1.34	1.78	3.12	10.43		
6 <sup>3</sup>	8.75	82	5.0700	4.1444	0.3276	4.4720	0.0721	0.0971	0.1692	0.4288	81.74	6.46	88.20	1.42	1.92	3.34	8.46	
7	7.80	255	6.8705	5.5614	0.5226	6.0840	0.0000	0.3211	0.3211	0.4654	80.95	7.61	88.56	0.00	4.67	4.67	6.77	
8 <sup>4</sup>	7.42	204	6.6560	5.5432	0.4368	5.9800	0.0482	0.3224	0.3706	0.3054	83.28	6.56	89.84	0.72	4.85	5.57	4.59	

We have previously said that from our metabolism studies we were led to believe that the differences between the changes produced by prolonged anæsthesia and those by delayed poisoning were of degree and not of kind. This view is strongly substantiated by

TABLE VI.  
*Excretion of Sulphur in Experiment 3.*

Date, July, 1908.	Total S.	Total SO <sub>2</sub> S.	Neutral S.	Total SO <sub>2</sub> S.	Neutral S.
	gm.	gm.	gm.	per cent.	per cent.
4	0.1647	0.1218	0.0429	73.95	26.05
5	0.1781	0.1292	0.0479	72.54	27.46
6a	0.2203	0.1395	0.0808	63.33	36.67
6b	0.23965	0.1232	0.11645	51.41	48.59
6 <sup>3</sup>	0.4600	0.2627	0.1973	57.11	42.89
7	0.5003	0.2821	0.2182	56.39	43.61
8 <sup>4</sup>	0.4398	0.2615	0.1783	59.46	40.54

\* The figures for July 6 are the sum of those obtained in the separate analyses of 6a and 6b. 6a represents the twelve hours during part of which chloroform was administered and the urine of which was uncontaminated. 6b represents the following twelve hours, the urine of which was contaminated by vomitus and feces (see page 352, *i. e.*, description of technique of experiments).

<sup>4</sup>Death occurred during the second half of this day. Urine of the first twelve hours was collected without contamination and the results obtained have been multiplied by two to enable a comparison with those of other days.

the pathological examination. Dog 1 that was recovering and that served as a control had changes exactly similar to those in Dog 3, but they were not so severe. That in this Dog 1 with the extensive liver lesions the metabolism should have been normal at the close, is very remarkable and serves to show that metabolism may not be disturbed even by severe organic lesions in the liver. Furthermore, the many experiments that we have made show unmistakably that with severe liver lesions there may be apparent perfect health, but they also show that any prolonged anæsthesia with chloroform produces in the dog great alteration and even destruction in liver tissue. We believe that this is true of human beings as well as of animals.

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EXPLANATION OF PLATES.

PLATES VIII-X.

FIG. 1.<sup>5</sup> Liver of Dog XVI D. Shows almost complete necrosis. A few islands of living cells remain.

FIG. 2. Liver of metabolism Dog (2). Stained with Scharlach R. Shows periphery of lobules full of fat. The central necrotic portions do not take the fat stain.

FIG. 3. Liver of metabolism Dog (1). Low power. Shows central necrosis with crystals of calcium phosphate.

FIG. 4. Same as Fig. 3. High power. Shows giant cells engulfing calcium crystals.

FIG. 5. Liver of metabolism Dog (3). Low power. Note similarity between this and Fig. 3. Shows also calcium phosphate crystals but more extensive necrosis.

<sup>5</sup>For all these photographs we are indebted to Dr. Edward Leaming of the Rockefeller Institute.

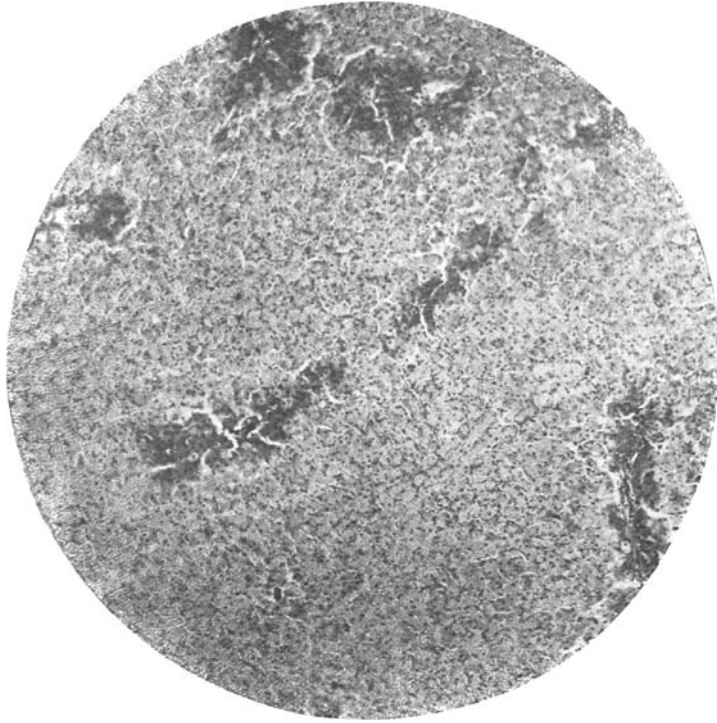


FIG. 1.

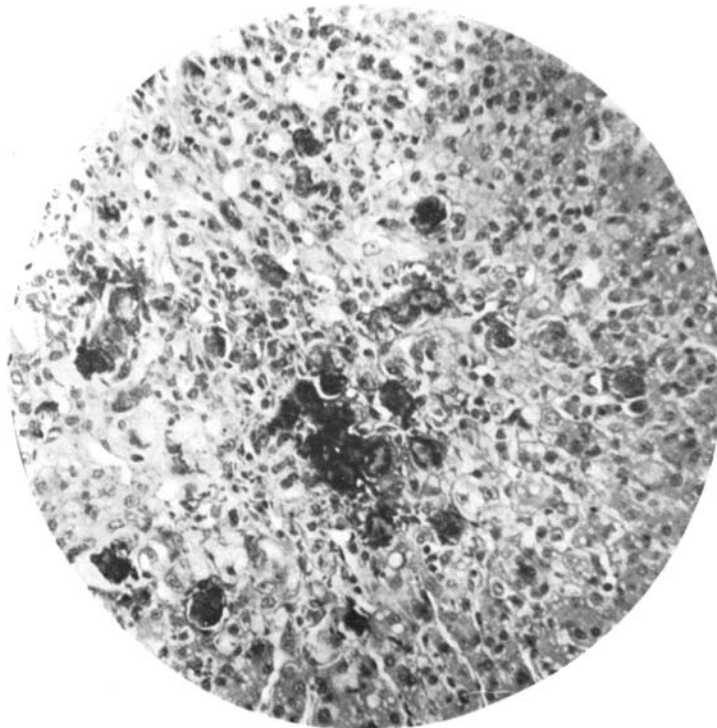


FIG. 2.

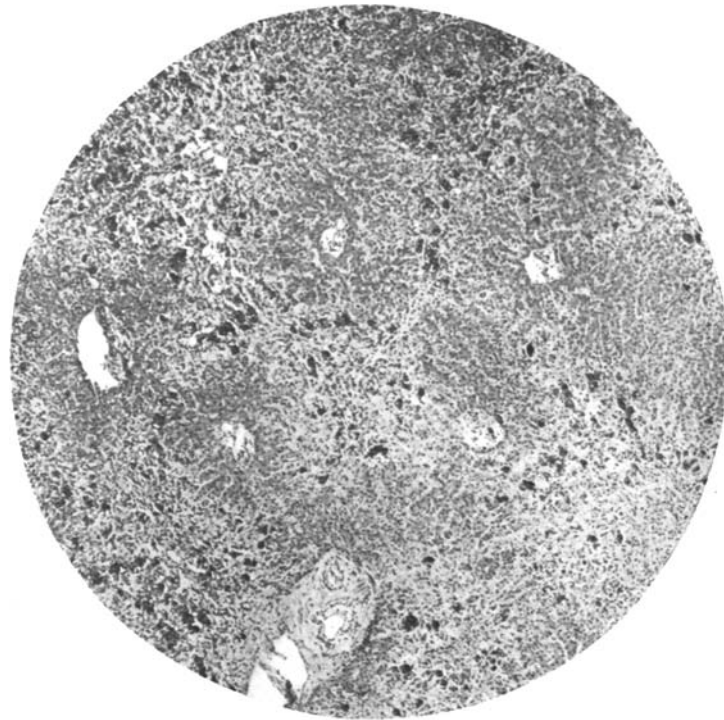


FIG. 3.

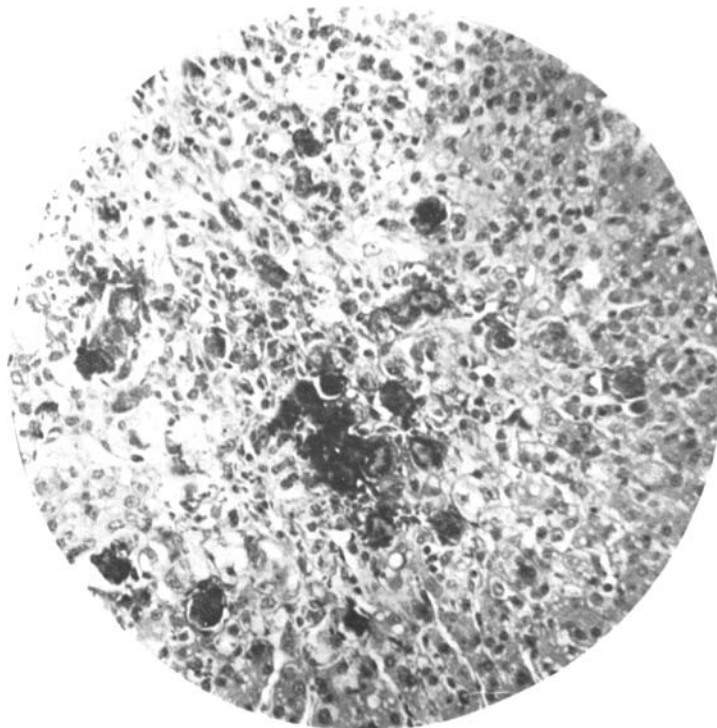


FIG. 4.

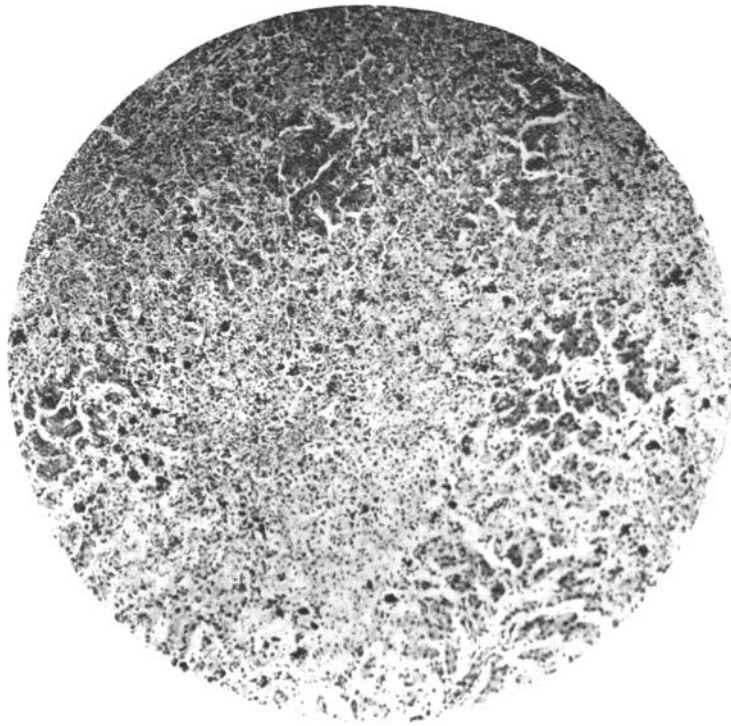


FIG. 5.