found in choline deficiency.[13,14,15]

Most investigators reported the occurrence of cirrhosis in rats only after a longer period of feeding the experimental diets. Daft, Sebrell, and Lillie[16] found, however, moderate to marked cirrhosis in weaning rats on a diet containing 4% casein and 0.5% cystine, after an average period of 83 days. In the experiments here reported cirrhosis can be produced in a short time even by giving large amounts of protein, deficient in methionine and enriched by cystine.

If the diet used in these experiments contained 2% cod liver oil, very large amounts of ceroid are deposited. If, however, the cod liver oil intake is restricted to 1 drop weekly, markedly less ceroid is formed. Hepatic cirrhosis is also observed in rats which are fed the experimental diets without any cod liver oil. However, the cirrhosis seems less severe in the majority of cases. In some instances, no ceroid was found in these cirrhotic livers, thus confirming the results of Endicott and co-workers. In other cases, however, ceroid deposition occurred, although in much smaller amounts than in the controls receiving cod liver oil. Cod liver oil can, therefore, not be the only reason for the occurrence of ceroid.

So far ceroid has only been observed in the livers of rats fed a choline-free, protein-deficient diet. It has, however also been found in the livers of mice in which cirrhotic changes had been produced by means of carbon tetrachloride[17] and O-aminoazotoluene.[18] These animals were on a full normal diet without added cod liver oil.

Summary. Regular and severe liver cirrhosis can be induced in male weaning rats on a choline-free, protein-deficient diet after 90 days, if the protein source is either fibrin or peanut meal plus casein. Early cirrhosis is occasionally observed in less than 60 days. Very large amounts of ceroid are deposited by the use of these diets, containing 2% cod liver oil. The amount of the deposited ceroid is markedly reduced if the intake of cod liver oil is restricted to one drop weekly. On the same diet without any cod liver oil, the produced cirrhosis is less severe. Some of these livers contain no ceroid, others small amounts of this pigment. Cod liver oil is therefore not the only cause for ceroid pigmentation in nutritional cirrhosis. It enhances, however, its formation very markedly.

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14 Christensen, K., *J. Biol. Chem.*, 1940, **133**, xx; *Arch. Path.*, 1942, **34**, 633.

15 Wachstein, M., *Arch. Path.*, 1944, **38**, 297.

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14988

Serial Peritoneoscopic Examinations as a Means of Studying Experimental Liver Damage in Dogs.*

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Most experimental observations of the histological changes occurring in the liver as the result of the administration of toxic agents have been based upon postmortem findings. Such a method necessitates either sacrifice of the animal or waiting until the effects of the agent are lethal.

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Experiments designed to study the degree of liver protection afforded by various diets for dogs receiving repeated doses of carbon tetrachloride have been under way in this laboratory for the past 14 months. Methods that would permit inspection and biopsy of the livers of these animals at intervals were desired. Many investigators have secured liver biopsies in dogs during the course of experimentally induced damage by performing a laparotomy. This has certain disadvantages. The use of a general anesthetic agent is necessary, and the number of abdominal incisions that can be performed is limited. Of more importance, however, is the intolerance for major surgical procedures of animals suffering from induced liver damage. Davis and Whipple[1] observed that even aseptic precautions did not prevent postoperative infections and that the incisions in such animals had a tendency to open superficially. Bollman and Mann[2] stated that many of their dogs subjected to carbon tetrachloride exposure would "die following excision of a small piece of liver for histological examination, a procedure that is relatively harmless to the normal animal." We have been gratified to observe in the course of such experiments that dogs tolerate repeated peritoneoscopic examinations and liver biopsy very well. This is a minor surgical procedure and can be performed with local anesthesia.

Peritoneoscopy is of considerable value in the clinical study of liver disease. Benedict[3] has suggested the use of peritoneoscopy in patients with cirrhosis to observe the progression or regression of the process and evaluate therapeutic procedures. So far as is known the method has not been widely employed in the experimental laboratory. It is of interest to note that Kelling, a German surgeon, first introduced the method to the 73rd Congress of German Naturalists and Physicians in 1901, by demonstrating a living dog into whose abdomen he had inserted a cystoscope for the purpose of examining the viscera. The procedure is said to have been used in the Veterinary Institute of Dresden before 1910 in certain physiological experiments (Nadeau and Kampmeier[4]). Horan[5] recommended preliminary trials of the method in dogs as a means of gaining familiarity with the instrument before clinical use.

Method. The instrument used is the Ruddock peritoneoscope;‡ the procedure as recommended by Ruddock[6] for clinical purposes has been followed. A preliminary sedative dose of morphine (5 mg per kilo of body weight) is given to the dog by hypodermic one hour prior to the examination. The animal is strapped to an operating table, the abdomen is shaved, scrubbed and prepared with a suitable antiseptic agent. The abdomen is draped with sterile towels and a laparotomy sheet. Sterile technic is used throughout the operative procedure. Sterilization of the peritoneoscope and its accessory parts is carried out for 20 minutes in a mercury oxycyanide solution of 1-500 concentration. All instruments are rinsed in sterile water before transfer to the instrument table. The point chosen for insertion of the instrument is the midline, one-third the distance from the xiphoid to the symphysis. Infiltration of 1% procaine hydrochloride in the skin and underlying tissues produces satisfactory local anesthesia. Care is taken to see that a small portion (2 cc) is infiltrated just at the parietal peritoneal surface. A small skin incision of 5 mm suffices for introduction of the blunt pneumoperitoneum needle. Air is pumped into the peritoneal cavity until the abdomen is tense. This is accomplished by means of a small hand bulb, room air being used. Filtration through cotton is unnecessary; the amount of air introduced is not important. The initial skin incision is enlarged to 1 cm and the larger trocar is inserted into which the peritoneoscope itself fits snugly.

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2 Bollman, J. L., and Mann, F. C., *Ann. Int. Med.*, 1931, **5**, 699.

3 Benedict, E. B., *New England J. Med.*, 1944, **230**, 125.

4 Nadeau, O. E., and Kampmeir, O. F., *S. G. and O.*, 1925, **41**, 259.

5 Horan, T. N., *Mich. St. Med. Soc. J.*, 1937, **36**, 634.

‡ American Cystoscope Makers, Inc., New York, N.Y.

6 Ruddock, J. C., *Western J. Surg.*, 1934, **42**, 392.

The presence of pneumoperitoneum or the manipulation of the instrument causes the animal no apparent discomfort. Inspection of the peritoneal cavity can readily be carried out by the illumination furnished by the small bulb just distal to the lens. Due to the anatomical arrangement in the dog a more complete inspection of the liver and spleen can be accomplished than is true in the human subject. Specimens of liver tissue can be secured by means of a biopsy forceps. The tissue specimen that can be secured is a triangular shaped block measuring about 5 mm in length and 4 mm at the base. This represents 40 to 50 mg of liver tissue. Specimens can be secured from various lobes as desired. Serious hemorrhage from the biopsy site has not been encountered; animals having severe liver damage as judged by the histological and gross appearance and altered liver function tests exhibited only minimal bleeding. Cautery can be employed; we have used it occasionally, more to gain familiarity with the maneuver than to control hemorrhage. Application of a thrombin solution§ to the biopsy site and application of small amounts of a soluble cellulose substance (Oxycel§) have recently been tried; again this has been done as a preliminary to future clinical trial rather than as a necessity for the control of hemorrhage from the dog's liver. Upon completion of the observation, the air is released from the abdominal cavity and the trocar is removed. The skin edge is closed by means of a single Michael clip which is left in place 5 days. No other closure is made. The animal, when released from the operating table, exhibits no ill effects and scampers about the room. Feeding schedules are resumed immediately postoperatively. Herniation of a piece of omentum at the site of the 1 cm long incision was observed in but one animal, this occurred after the seventh examination. Recovery was uneventful and did not interfere with further examination in this same animal.

Material. The first series of experiments in which this method was employed concerned a correlation study of the histology of the liver and the results of multiple liver function tests performed on the day that the biopsy was secured. In a group of 18 adult dogs, 145 peritoneoscopic examinations and liver biopsies were carried out in a 10-month period; 8 of the group were subjected to peritoneoscopy 10 or more times. Carbon tetrachloride was administered repeatedly to 15 of the animals. Three were maintained on a high-fat, low-protein diet. In a second series 9 animals receiving carbon tetrachloride have been subjected to 36 peritoneoscopic examinations and liver biopsy in a 2-month period. During each peritoneoscopic examination 3 or more biopsies were taken from different lobes of the liver.

Results. In no instance has a fatality occurred as a direct or indirect result of the procedure. The occurrence of hepatic necrosis and the development of nodular cirrhosis can be detected grossly by this method of observation. The validity of the observed gross appearances has been checked by subsequent autopsies.

In Fig. 1, representative photomicrographs of serial liver biopsies in 3 of the animals are shown. Dog No. 17 (Fig. 1 A, B, and C) was maintained on a high-fat, low-protein diet and received repeated doses of carbon tetrachloride via stomach tube. Fig. 1-A represents the histological appearance prior to the administration of the toxic agent; 1-B represents the third biopsy taken 49 days later after 39 cc of carbon tetrachloride in divided doses had been administered. Fig. 1-C represents the fifth biopsy taken on the 93rd day of the experiment at which time a total of 111 cc of carbon tetrachloride had been given.

In Fig. 1-D, E and F the photomicrographs represent biopsies secured in dog No. 5, maintained on a high-protein, low-fat diet. Fig. 1-D is the appearance at the start of the experiment and 1-E the third biopsy taken 83 days later by which time 91 cc of carbon tetrachloride had been given in divided doses. In Fig. 1-F the fifth biopsy is shown taken on the 125th day by which time 239 cc of carbon tetrachloride had been administered. Serial biopsies from Dog 3 are shown in Fig. 1-G, H, and I. This animal was maintained on a high-fat diet, no toxic agent had been given. Photomicrographs shown represent the control liver biopsy, Fig. 1-G, the fourth biopsy,

§ Parke, Davis and Co., Detroit, Michigan.

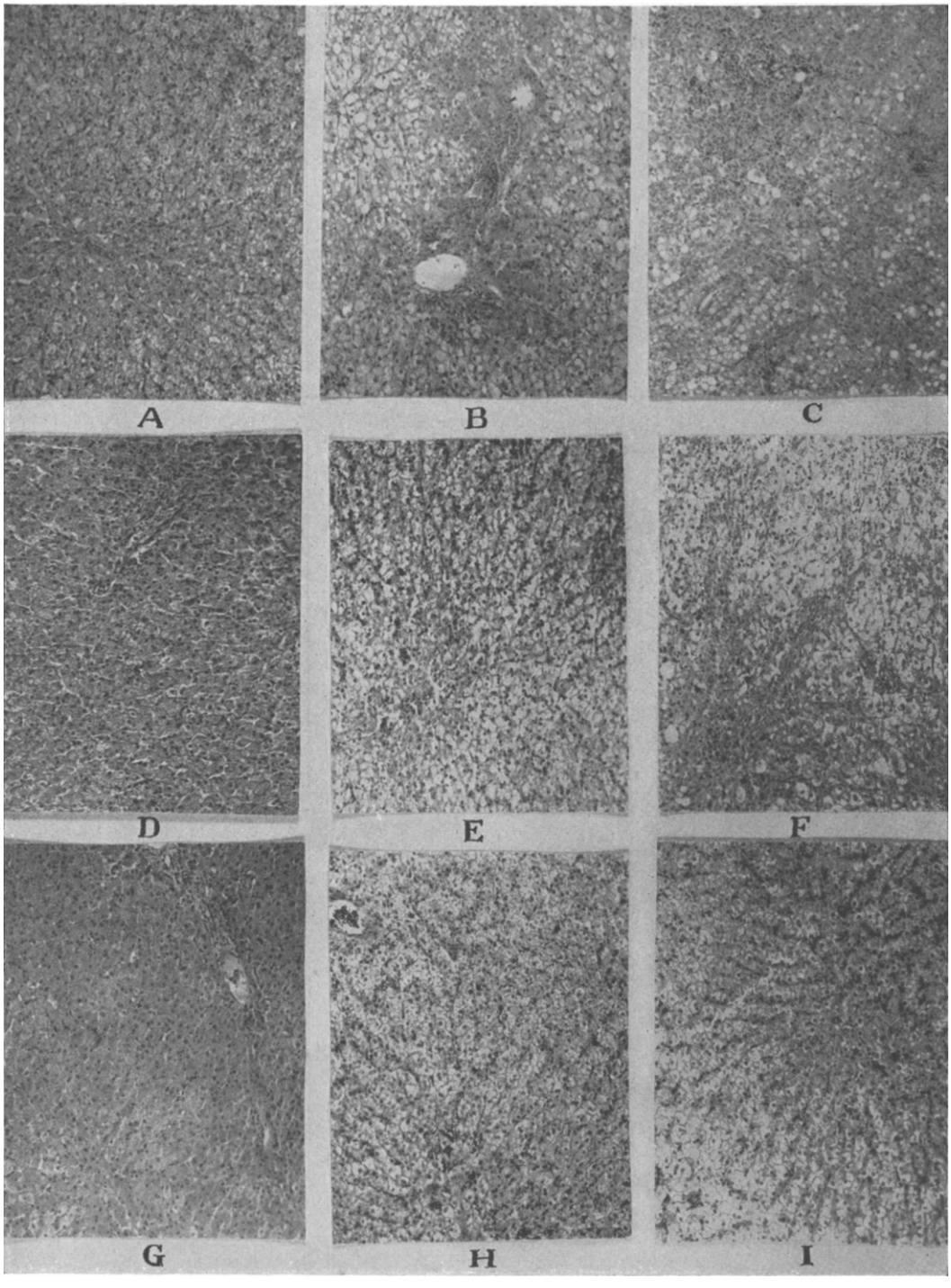

FIG. 1.

Photomicrographs of liver biopsies secured at intervals by means of repeated peritoneoscopic examination. For details see text.

Fig. 1-H taken on the 104th day, and Fig. 1-I the eighth biopsy taken on the 227th day.

Summary and Conclusions. 1. The procedure of peritoneoscopy, as described by Ruddock, is a satisfactory means of inspecting the liver and of securing liver biopsy specimens in the dog. The examination can be accomplished without pain to the animal using local anesthesia, after morphine sedation. Repeated examinations with inspection and biopsy of the liver can be made over a period of time in the same dog.

2. A series of 145 peritoneoscopic examinations in a group of 18 dogs with toxic or dietary liver damage was carried out in a 10-month period. Eight dogs in this group were subjected to peritoneoscopy 10 or more times. A second similar group of 9 dogs were subjected to 36 such examinations in a 2-month period. In each instance multiple liver biopsies were taken.

3. No fatalities attributable to this procedure have occurred even when performed on dogs exhibiting severe liver damage.

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The Intracranial Toxicity of Influenza Virus for Mice.*

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A study of the properties of the influenza A virus, designated IC strain, isolated during the 1943 epidemic at Iowa City, involved the injection of the virus into the brains of Swiss mice to determine its neurotropic qualities. Two strains of influenza virus are known to be neurotropic, one reported by Stuart-Harris[1] and the other by Francis and Moore.[2] These strains propagated in the mouse brain and after several passages produced convulsions and death. Henle and Henle[3] working with non-neurotropic strains showed that active virus injected in sufficient concentrations produced convulsions but they did not obtain evidence of virus propagation. Although the investigation herein reported was completed before the publication of Henle and Henle[3] this work will serve as a confirmation of their experiment. Evans and Rickard[4] found that the injection of type A and type B influenza virus in the rabbit's eye produced corneal opacity without multiplication of the virus. Rake and Jones[5] using viruses of lymphogranuloma venereum, mouse pneumonitis, and meningo-pneumonitis have shown that these viruses exert a toxic effect.

All virus and antibody titers given in this report were determined by the Hirst[6] hemagglutination test as modified by Salk.[7]

Preliminary experiments indicated that allantoic fluid containing the IC strain of influenza virus, injected in 0.03 ml doses intracranially in mice, resulted in convulsions in a majority of cases after 48 hours, with death occurring on the second or third day, rarely as late as the fifth. The mortality rate was 50-100% depending upon the concentration of the virus. That the convulsions were not caused by bacterial infection was demonstrated

* This investigation was aided in part through the Commission on Influenza, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.

1 Stuart-Harris, C. H., *Lancet*, 1939, **1**, 497.

2 Francis, T., Jr., and Moore, A. E., *J. Exp. Med.*, 1940, **72**, 717.

3 Henle, G., and Henle, W., *Science*, 1944, **100**, 410.

4 Evans, C. A., and Rickard, E. R., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 73.

5 Rake, G., and Jones, H. T., *J. Exp. Med.*, 1944, **79**, 463.

6 Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

7 Salk, J. E., *J. Immunol.*, 1944, **49**, 87.