also found. Threads were of variable length and of about 120-150 Å in width (Fig. 4). A few of the threads showed a definite coiling.

Tubes with unfiltered trypsin stored in the refrigerator at 5°C for a period of a week also developed threads and many bacteria. In the experiment with filtered aseptic trypsin (tube d) neither threads nor bacteria were found even after long incubation periods.

Discussion. The experiments reported here clearly demonstrate that the "trypsin resistant threads" described by Gross as a characteristic component of the elastic fiber ultrastructure do not belong to this tissue. These threads appear in all cases in which incubation of the elastic tissue with trypsin is not done under absolutely sterile conditions. Neither toluene, as used by Gross, nor thymol are sufficient to prevent bacterial development and the appearance of thin threads. These threads have been also found by incubating other tissues with trypsin, and even trypsin alone, when complete aseptic precautions are not taken. These results lead to the conclusion that the thin coiled threads described by Gross belong to material digested from bacteria and probably from bacterial flagella. Experiments are under way in order to solve this problem.

Summary. Purified elastic material from fish swim bladder was fragmented and observed under the electron microscope. Elastic fibers were digested with unfiltered trypsin and also with trypsin sterilized by filtering through a Seitz microfilter. When digestion is not done under absolutely sterile conditions, "fine threads" described by Gross(2) as a component of the elastic tissue are observed.

If digestion is performed under complete sterile conditions no threads appear, and only partially digested elastic fibers can be found. Incubation of trypsin alone also shows the presence of threads when sterilization is not used and their absence in sterile conditions. The conclusion is reached that the threads described as a component of elastic tissue(2) belong to material digested from bacteria and probably from bacterial flagella.

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Experimental Amebic Hepatitis in Hamsters. (18541)

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While human infection with *Endamoeba histolytica* is most often manifested clinically as enteritis of varying severity, this pathogen frequently produces serious extra-intestinal lesions, particularly in the liver. Methods are available for producing experimental intestinal infections with *E. histolytica* in such animals as cats, dogs, rats, rabbits and guinea pigs. These infections under optimal conditions cause severe intestinal pathology with sufficient regularity to be useful in studies on intestinal amebiasis. However, such amebic infections are commonly restricted to the intestinal tract and hence are of very limited use for observations on extra-intestinal amebiasis. Cleveland and Sanders(1,2) were able to induce amebic hepatitis in cats by intrahepatic inoculation of amebae and associated bacteria but many early deaths, presumably from bacterial infection, and much variability in the incidence of hepatitis were encountered.

The foregoing considerations stimulated search for a more efficient experimental extra-intestinal amebic infection. When amebae were inoculated similarly into the liver of

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1. Cleveland, L. R., and Sanders, E. P., *Science,* 1930, v72, 149.

2. ———————*Amer. J. Hyg.*, 1930, v12, 569.

small numbers of dogs, rats, mice, guinea pigs and hamsters, the most encouraging results occurred with the latter animal. The apparent superiority of the hamster from the standpoint of susceptibility to amebic hepatitis or of relative resistance to early death from bacterial infection lead to the studies which permit this preliminary report.

Experimental animals. Golden hamsters *(Cricetus auratus)* of either sex, weighing between 40 and 55 g, and approximately one month old were routinely used as experimental hosts. The animals were maintained in individual cages on a diet of Purina Laboratory Chow and fresh lettuce. During exploratory phases of the study differences in susceptibility did not appear to be associated with age. Statistical examination† of 188 subsequent, routinely infected animals revealed no evidence of sex influence on lesion size. Lesion weight tended to vary in proportion to initial body weight but the correlation was too weak ($\rho = 0.18$, significant at 5% level) to be useful in refinement of lesion weight data in the narrow weight range employed.

Infecting organisms and infection procedures—Endamoeba histolytica of human origin, designated as strain 200 or BNIH(3-5), were maintained in culture at 37.5°C with 3 species of bacteria from the *coliform, Proteus* and *Streptococcus fecalis* groups, respectively, and hereafter referred to collectively as strain 200. Cleveland-Collier medium (Difco) was used with addition of a finely-milled preparation of Belgian rice starch (Stein-Hall Co.). The infecting inoculum, 25,000 or 50,000 amebae, from a 24-hour culture, was suspended in a menstruum of 0.05 ml of the culture medium overlay and infections were induced by direct injection into the liver following laparotomy under ether anesthesia. The operation site was prepared by cleansing with a germicidal agent, Phemerol Chloride.‡ A single injection was made into the ventral surface of the right hepatic lobe so as to produce a visible bleb beneath the hepatic capsule. Injections were made with a 27-gauge hypodermic needle with precautions to minimize seepage of the inoculum from the liver. A solution of Thrombin Topical (Parke, Davis and Co.) was applied to bleeding surfaces in an effort to control hemorrhage. The body wall was closed with skin clips, and the incision was covered with a solution of parlodion in ether.

Prevention of death from bacteria. When animals that had not been immunized against strain 200 bacteria were injected with the ameba-bacteria inoculum approximately 30% died within 72 hours. Heart blood cultures from animals dying during this interval revealed an overwhelming bacteremia, with *Proteus* as the predominant organism. It was found that intraperitoneal vaccination of animals, with living strain 200 bacteria, conferred virtually complete protection against the bacterial deaths following hepatic injection of amebae and bacteria. Immunization was then incorporated as part of the routine procedure and was conducted as follows: The bacterial component of the ameba strain was maintained free of amebae in Cleveland-Collier medium with added rice starch. The overlay from 24-hour cultures incubated at 37.5°C was diluted with an equal volume of sterile medium overlay, and 0.05 ml was injected intraperitoneally 6 days and 3 days before intrahepatic inoculation with amebae and bacteria. This immunizing procedure very rarely caused death, but approached the maximum tolerance of the animals since deaths were encountered in substantial numbers of animals when the total vaccine was given in a single injection.

Observations on the nature and etiology of the infections. Titration of the ameba-bacteria inoculum using 2-fold increments in numbers of amebae from 25,000 to 200,000, revealed no consistent relationship between the number of organisms and the size of the

† Dr. C. V. Winder.

3. Tobie, J. E., *Am. J. Trop. Med.*, 1949, v29, 859.

4. Thompson, P. E., and Lilligren, B. L., *Am. J. Trop. Med.*, 1949, v29, 323.

5. Thompson, P. E., Dunn, M. C., Bayles, A., and Reinertson, J. W., *Am. J. Trop. Med.*, 1950, v30, 203.

‡ Parke, Davis and Co. trademark for benzethonium chloride. Used here as a 1:1000 aqueous solution.

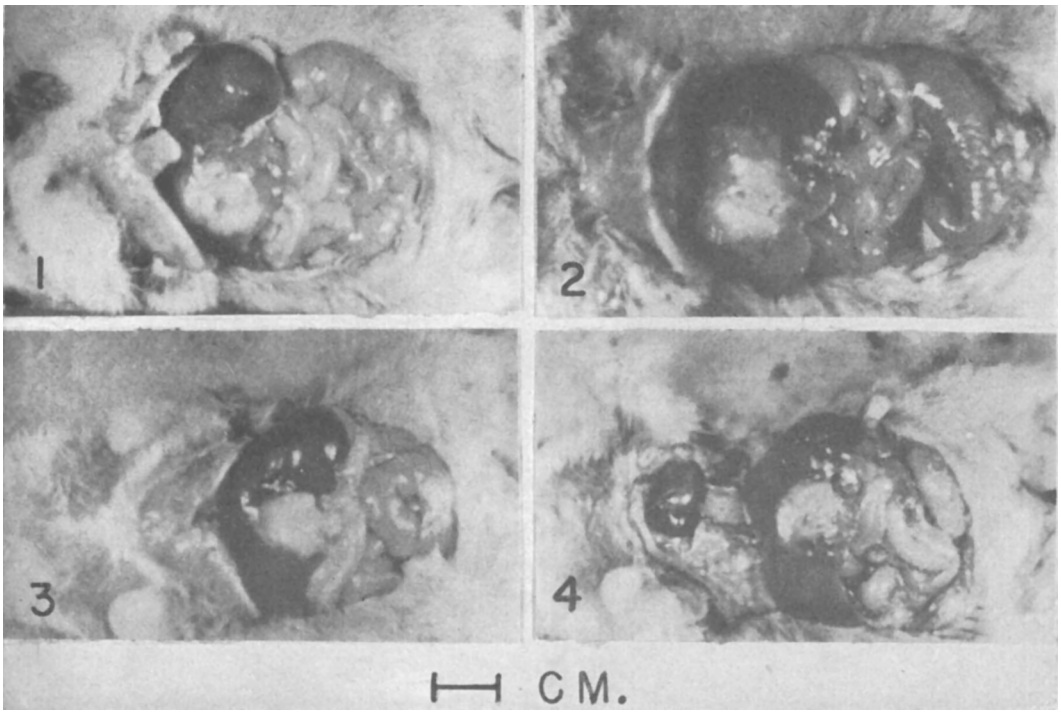

FIG. 1-4.
Amebic hepatitis in hamsters 92 hr after intrahepatic injection with 50,000 amebae.

lesions when animals were examined approximately 92 hours after infection. Greater variability in success of infection occurred with smaller numbers, although infections could be induced with as few as 3,000 amebae. Minimal variability occurred when the inoculum contained as many as 25,000 to 50,000 amebae and the latter has been selected as the usual infecting dose. Injections into the portal vein resulted in numerous small hepatic lesions, showing that trauma of the hepatic parenchyma was probably unnecessary for the routine infection. Successful inoculation into the liver, without appreciable seepage from the organ, produced usually a single well-defined lesion extending from the site of inoculation (Fig. 1-4), but occasionally metastasis to other lobes of the liver occurred. The lesion rarely liquefied but developed rapidly as a relatively homogeneous pale necrotic area until it involved by gross inspection from 25 to 50% of the liver. This amount of pathology appeared to be invariably fatal, with deaths occurring mainly between 6 and 8 days after infection. Amebae were readily demonstrable in the lesions by microscopic examination and by culture in Cleveland-Collier medium. An infection rate of approximately 95% has been observed among over 1000 hamsters inoculated during a period of 17 months. Hematogenous dispersal of amebae occurred commonly and was most readily apparent from the development of numerous discrete amebic lesions throughout the lungs.

The rate of lesion development is apparent from the data in Table I, which were collected during a single experiment. The animals were vaccinated against bacteria, injected intrahepatically with 50,000 amebae and groups selected at random were sacrificed at the specified intervals after inoculation. These data reveal an approximately linear rate of lesion growth.

Microscopic study of a few hematoxylin and eosin-stained sections of lesions 56 and 92 hours after infection revealed extensive necrosis of the parenchyma, manifested by disruption of the hepatic lobule architecture, and pale staining of the cytoplasm and nuclei

TABLE I. Data Illustrating the Rate of Hepatic Lesion Development in Hamsters.

Hr. after inoculation with amebae	Mean lesion wt in g ± stand. error	No. positive for amebae / No. inoculated
48	.30 ± .02	8/8
56	.78 ± .02	8/8
74	1.18 ± .03	10/10
92	1.78 ± .03	10/10
110	*2.29 ± .05	10/10

* 3 animals died between the 92nd and 110th hr and were not included in determining lesion wt.

of hepatic and connective tissue cells. Cellular infiltration was slight and consisted only of small aggregates of leucocytes with pyknotic nuclei.

The following observations suggest that amebae rather than bacteria are the major etiologic agents in producing hepatitis. (1) When strain 200 bacteria alone were injected into 28 hamsters, progressive hepatitis had not occurred when autopsied 92 hours after infection—14 had no lesions and the remaining 14 had lesions no larger than the original implantation. (2) Typical amebic hepatitis developed among all of 4 animals when the ameba-bacteria inoculum was pretreated for 3 hours with a mixture of sodium penicillin G (0.6 mg/ml) and dihydrostreptomycin sulfate (1 mg/ml) and the animals were treated with 0.6 mg/kg/day of Penicillin SR§ and 100 mg/kg/day of dihydrostreptomycin. No apparent effect on lesion development occurred when 10 animals were infected without pretreatment of the inoculum but were given these antibiotics in doses of 4.5 and 100 mg/kg/day, respectively. In both instances the antibiotics were given subcutaneously twice daily for 4 days, with the first dose 5 hours before inoculation. (3) The incidence and rate of lesion development were entirely comparable in animals effectively immunized against bacteria and in those not immunized. (4) Lesions were rarely purulent by either gross or microscopic examinations. In contrast to these observations bacteria persisted in the lesions and were readily demonstrable by culture. For this reason it would seem that bacteria cannot be completely exonerated until lesions have been produced with bacteria-free amebae.

§ Parke, Davis and Co. trademark for a mixture containing 3 parts procaine penicillin G and 1 part sodium penicillin G and having a potency of 1167 International units/mg.

Discussion. The procedures and materials described here seem to provide a convenient means for studying various aspects of extra-intestinal amebiasis. Chief emphasis so far has been to explore their usefulness in evaluating the action of drugs against amebic hepatitis. Chemotherapeutic data which will be reported elsewhere indicate that the clinically established drugs for this entity—emetine dihydrochloride and chloroquine diphosphate—are active in hamsters. In addition, it has been found that two clinically useful antimalarial drugs—quinacrine hydrochloride and amodiaquin (Camoquin‖)—have therapeutic action in amebic hepatitis in hamsters.

Summary. Progressive and usually fatal amebic hepatitis can be induced readily in golden hamsters by the intrahepatic injection of a strain of *Endamoeba histolytica* and its bacterial associates from cultures. Amebae rather than bacteria appear to constitute the major etiologic agents in producing the hepatitis.

‖ Parke, Davis and Co. trademark.

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