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Electron Microscope Observations on Elastic Fibers. (18540)

C. M. FRANCHI AND E. DE ROBERTIS.

From Departamento de Ultraestructura Celular Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay.

Elastic tissue generally considered both from chemical and submicroscopic analysis (1) as a relatively homogeneous entity, has been recently described as a two component system(2). Gross(2) in a very documented electron microscope analysis of elastic tissue from a variety of sources concluded that the elastic fibers are "composed of bundles of trypsin resistant threads of characteristic form and size plus a trypsin sensitive, heat-resistant 'amorphous' binding matrix." This conclusion was based on experiments in which digestion of either boiled or unheated elastic tissue with crystalline trypsin caused the "release" of characteristic thin coiled threads. These threads were observed in the surrounding of the partially digested fibers, but could not be observed with certainty within the fiber itself. In some recent experiments of digestion with crystalline trypsin on nerve material we were surprised to find threads which looked very similar to those described by Gross on elastic tissue. Furthermore, in control experiments in which the enzyme alone was incubated, similar threads could be observed, simultaneously with the presence of few bacteria and bacterial flagella.

These findings led us to reinvestigate the structure of the elastic tissue with the electron microscope, and to the demonstration that the threads described by Gross(2) are not elastic constituents.

Material and methods. The elastic material was obtained from the swimming bladder of the fish micropogon opercularis (Quoy and Gaimard). The preparation was similar to that described by Gross with minor modifications. The swimming bladder was cut into small pieces and stored overnight in 1% acetic acid at 4°C. The viscous mass obtained was diluted with more acetic acid and fragmented in a Waring Blendor. The elastin was separated from the ichthyocol (collagen) by repeated centrifugations and washing with dilute acetic acid. To complete the collagen extraction the suspension was boiled for 30 minutes and washed once more in acetic acid. The purified elastin was finally suspended in distilled water and boiled once more for sterilization. For this last operation glass beads had to be added to the suspension to overcome the strong tendency to stick of the purified elastin. Crystalline trypsin (Armour) dissolved to 0.1% in borate or phosphate buffer of pH 8 was used. Some digestion experiments were made under toluene, as described by Gross. In other cases a crystal of thymol was added to the fresh solution.

Digestion experiments under complete aseptic conditions were also performed. The trypsin solution was filtered through a Seitz microfilter. The buffer solution was sterilized by boiling and a final check of the pH value was made. Sterile glass material was used and all the operations were performed aseptically. The tubes containing the elastic material together with the controls were incubated at 37°-38°C for the same amount of time. After digestion the suspension was slightly disintegrated with a 10 Kc sonic magnetostriction oscillator. Specimens for electronmicroscopy

1. Wolpers, C., *Klin. Woch.*, 1944, v23, 169.
2. Gross, J., *J. Exp. Med.*, 1949, v89, 699.

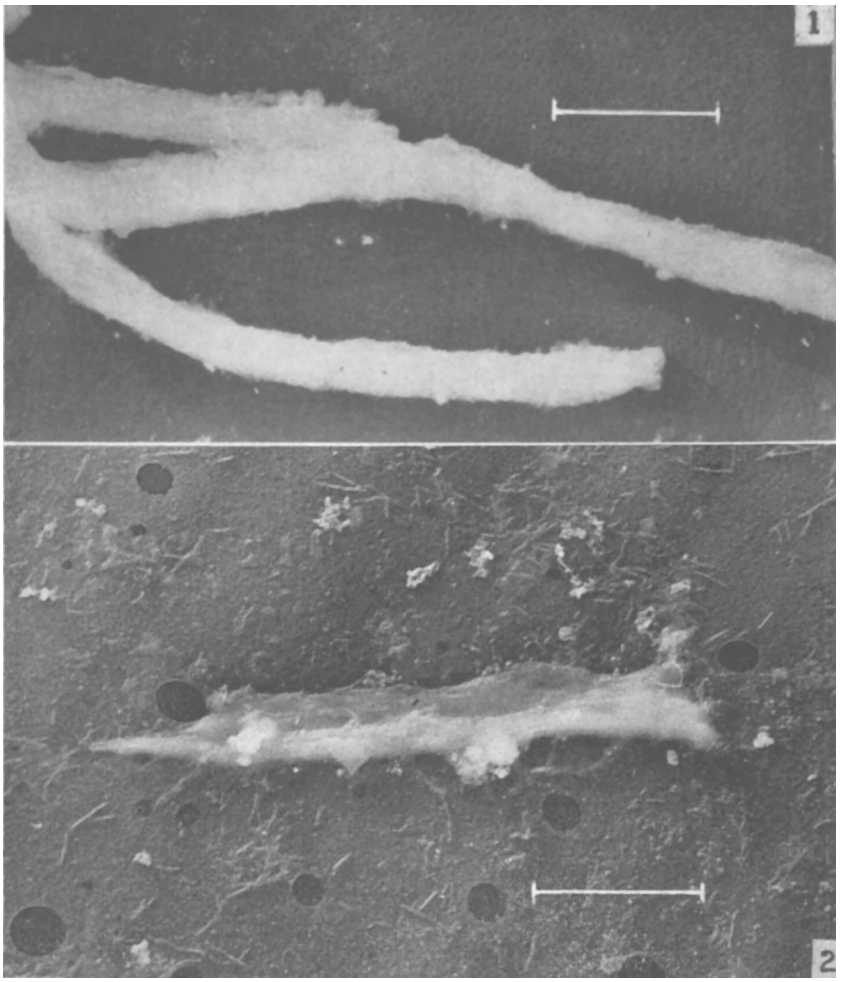

FIG. 1. Elastic fibers from fish swimming bladder. Longitudinal fibrillation within the fibers is apparent. Control material incubated for 6 hr in sterile buffer of pH 8. 22,000×.

FIG. 2. Elastic fiber partially digested with unfiltered trypsin. On the background and surrounding the fiber many thin threads are present. Incubation of 11 hr (with addition of thymol). 22,000×.

were prepared by depositing drops of the suspension on standard nickel grids. The preparations were shadowed with palladium at grazing angles of 9° to 11°. An R.C.A. type EMU 2C electron microscope was used and the microphotographs were generally taken at 6,000 X.

Results. Digestion of elastic material with unfiltered trypsin. The above described elastin suspension was added to the unfiltered trypsin solution in the proportion of 1 to 10 and was incubated for 11 hours. The results were similar to those obtained by Gross, *i.e.*, partially digested, flattened elastic fibers with moth-eaten edges and a coarse longitudinal fibrillation and on the background and surrounding the fibers the presence of a considerable number of fine short threads (Fig. 2).

The action of trypsin on the elastic fibers can be best appreciated by comparison with the control experiments in which sterile elastic suspension was incubated with buffer of pH 8. Characteristic long branching fibers flattened on the film and of variable diameter could be observed (Fig. 1). Usually they show a

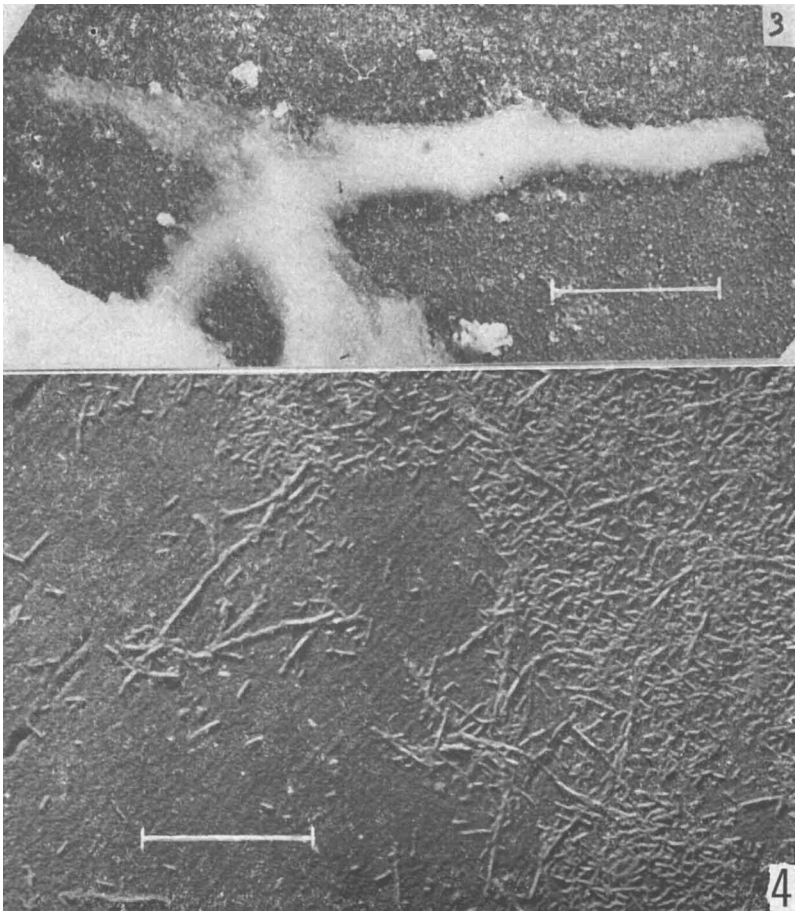

FIG. 3. Elastic fibers partially digested with filtered (sterile) trypsin. No threads are found. Incubation for 6 hr. 22,000×.

FIG. 4. Trypsin solution incubated for 11 hr (with addition of thymol). A considerable number of thin threads, similar to those found in Fig. 2 can be observed. 22,000×.

longitudinal fibrillation with tightly packed fibrils of about 400 to 700 Å in width. In these control experiments the background is completely clean, and threads are not found.

Digestion of elastic material with filtered trypsin. In this series of experiments complete aseptic conditions were insured as described above. Incubation periods ranged from 4 to 12 hours. Elastic fibers with different degrees of digestion were observed, but no threads could be found (Fig. 3).

Incubation of trypsin solutions under different conditions. In order to insure that the threads did not come from the elastic tissue the following control experiments were performed: (a) incubation of the trypsin solution (b) incubation of trypsin in sterile tubes (c) incubation of trypsin under toluene or with the addition of thymol (d) incubation of trypsin filtered through Seitz filter under aseptic conditions. All the tubes were incubated for 2 to 12 hours.

Samples of freshly prepared unfiltered trypsin solution were also examined. This fresh solution showed no threads, a few bacteria were present and constantly a finely granular and amorphous material was found which seemed characteristic for all dried samples of trypsin.

Tubes a, b, and c showed in all cases variable amounts of threads. A few bacterial bodies often showing attached flagella were

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)

J. W. REINERTSON AND PAUL E. THOMPSON.* (Introduced by A. C. Bratton, Jr.)

From the Research Laboratories, Parke, Davis and Co., Detroit, Michigan.

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.