as the bone marrow reverted to normoblastic activity.

It is possible that ascorbic acid therapy alone over a long enough period of time would produce a remission. In Case 1 a suggestive increase in reticulocytes occurred but therapy was not continued long enough to warrant any conclusions. It is probable that megaloblastic anemia in pregnancy can be prevented by adequate ascorbic acid intake during pregnancy.

Summary. Five cases of megaloblastic anemia in pregnancy are reported. The relation of this anemia to a deficiency in ascorbic acid is suggested by the effectiveness of combined ascorbic acid and vit. B_{12} therapy where vit. B_{12} alone was ineffective. Two patients were refractory to adequate trial with vit. B_{12}. One of these patients later had a complete remission when ascorbic acid was added to the therapy. The other responded adequately to folic acid therapy and blood transfusions. Two patients were treated for short periods of time with ascorbic acid alone. A minimal response in reticulocytes was observed in one. Subsequent addition of vit. B_{12} to the therapy after saturation with ascorbic acid produced a complete hematologic remission. One patient had a spontaneous remission after delivery. In 3 patients combined therapy with ascorbic acid and vit. B_{12} produced a complete remission.

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Fiber Formation in Trypsinogen Solutions: An Electron Optical Study.* (19034)

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Recently the author(1) reported a study of the structure of elastic tissue with the electron microscope which emphasized a seemingly specific effect of trypsin on elastic fibers in releasing large numbers of characteristically coiled threads. These were composed of pairs of smooth filaments each about 70 A in diameter coiled in a tight, regular helix with a pitch of 500 A and a width of 120 A. Numerous unwound filaments were also observed. It was also reported that electron micrographs of freshly prepared trypsin solutions dried on the supporting film did not show the coiled threads. More recently Franchi and DeRobertis(2) reported the appearance of coiled threads, resembling those described above, in solutions of Armour's crystalline trypsin after incubation—in the absence of elastin—whereas, after sterilization of the trypsin solution by Seitz filtration, no such elements were found. They also reported fresh trypsin solutions to be devoid of fibers. Because a few bacteria and flagella were found in the non-sterilized preparations, they suggested that the coiled threads observed by Gross and themselves were degradation products of tryptically digested flagellae. In a second paper(3) these authors described the appearance of coiled threads in a culture of *Bacillus brevis* incubated in Armour's crystalline trypsin. This organism was found as a contaminant of the enzyme.

This paper describes a reinvestigation of the

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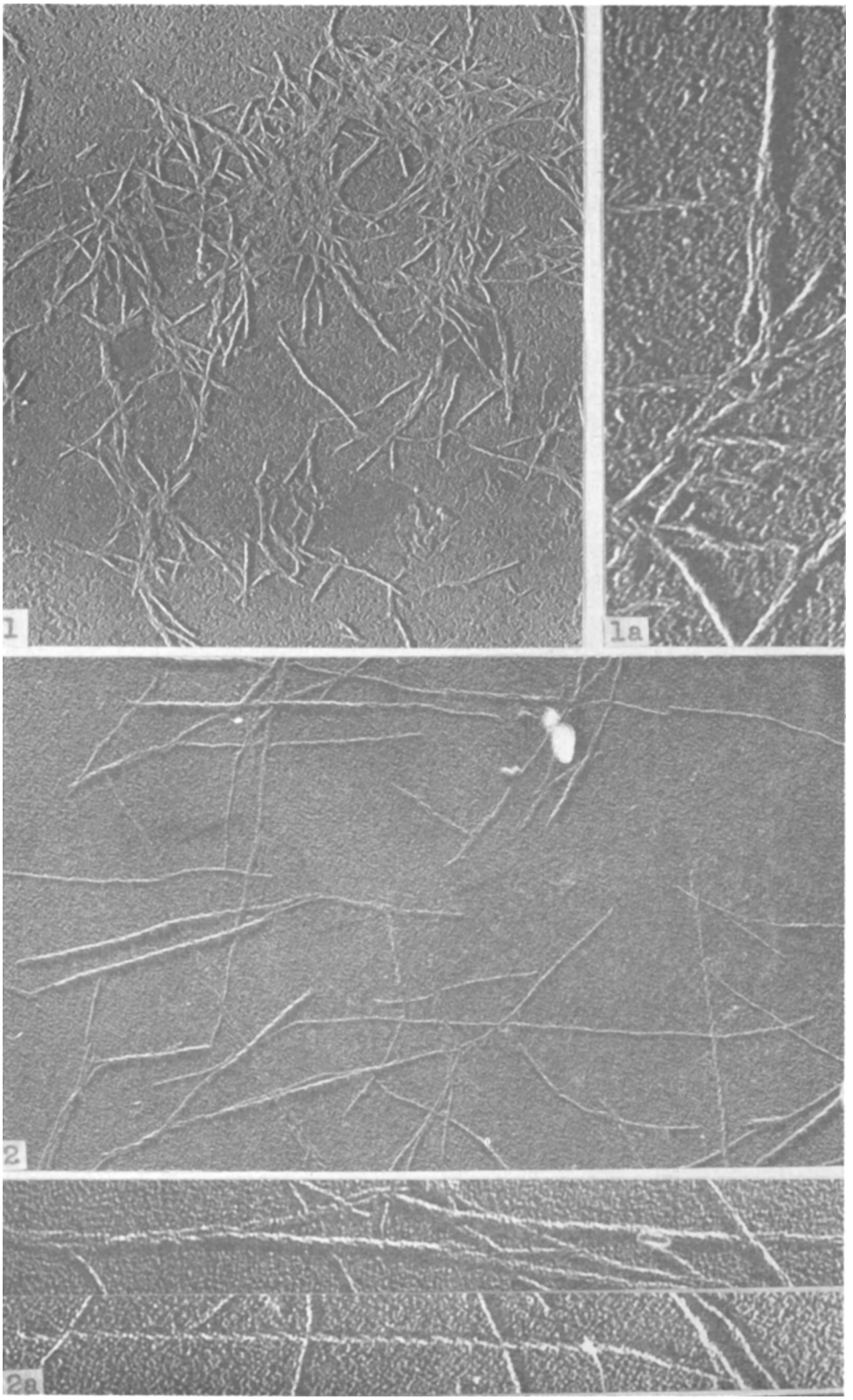
1
1a
2
2a

FIG. 1. Electron micrograph of coiled threads and filaments present in fresh crystalline trypsin solutions. 24,600 ×.

FIG. 1a. Enlargement of small area of Fig. 1. 129,500 ×.

FIG. 2. Electron micrograph of coiled threads and filaments produced in trypsinogen solution. 41,000 ×.

FIG. 2a. Enlargement of two regions of Fig. 2. 70,000 ×.

origin of the coiled threads previously observed after tryptic digestion of elastin.

Experimental procedures and results. Careful reexamination of specimens prepared by depositing a drop of fresh 0.1% water solutions of Armour's crystalline trypsin on the collodion supporting film of the specimen grid, draining after 5 minutes and shadowing with chromium, revealed the vague outlines of what might be fibrous material covered with a thick amorphous layer. Centrifugation of such trypsin solutions for 20 to 30 minutes at 4000 RPM in the International centrifuge produced a small amount of white sediment. This was drained of supernatant, redispersed in water and prepared for electron microscopy as described above. Examination showed large numbers of helically-coiled threads identical with those described previously by the author (Fig. 1). Thus, the controls of the trypsin solutions, as performed both by Gross and by Franchi and DeRobertis, were obviously inadequate; the coiled threads and filaments were pre-existent in the unincubated "crystalline" trypsin and required concentration and freeing from the amorphous material before they could be detected. Five separate batches of Armour's crystalline trypsin contained large numbers of coiled threads and filaments. Visible sediments were obtained on centrifugation of both water and bicarbonate-buffered solutions in all instances. These structures were not found in Armour's "Tryptar", a highly purified and Seitz-filtered crystallized trypsin.

Few, if any, bacteria were seen in the electron micrographs, and in fact one of these trypsin preparations was proven sterile by thioglycollate and blood plate cultures (kindly prepared by Dr. L. Dienes) while the other 4 batches produced very few colonies.

Seitz-filtered water solutions of Armour's crystalline trypsin were free of threads and filaments and, in fact, were shown to be devoid of tryptic activity (gelatin digestion) when small quantities (10 cc of 0.1% solution) were filtered, probably because of adsorption of the enzyme. This method of sterilization was used by Franchi and DeRobertis and probably accounts for their failure to find coiled threads in sterilized preparations; they were filtered out.

Fragmented ligamentum nuchae, purified and native, was incubated with the clear supernate of centrifuged Armour's crystalline trypsin and Armour's "Tryptar" in bicarbonate-buffered (pH 7.8) solution at 37°C. The enzyme solutions were also tested for proteolytic activity. Electron microscope examination of the centrifuged sediments of such preparations after shadowing revealed no coiled threads. It was therefore concluded that purified, crystallized trypsin does not liberate these structures from elastic tissue.

Because of the existence of these characteristic fibrous elements in the crystalline trypsin, it seemed desirable to test the precursor substances.

Water solutions of crystalline trypsinogen were centrifuged as described above, and both supernatant and sediment (extremely small) proved to be devoid of the specific fibrous elements.† One per cent solutions of trypsinogen (same batch) in 0.4 M borate buffer at pH 8.0 were seeded with a few crystals of "Tryptar" (to activate the trypsinogen)‡ and incubated at 5°C for 3 to 7 days and their sedi-

† Since submission of this manuscript, two recent lots of Worthington's crystalline trypsinogen were shown to contain large numbers of coiled threads. These preparations were centrifuged and passed through ultra-fine sintered glass filters and reexamined prior to incubation. The filtrates were clear of threads. An Armour preparation of crude crystallized trypsinogen contained no demonstrable threads and gave large masses of them after incubation.

‡ Subsequent work indicates that trypsin seeding is not necessary for this process and, in fact, coiled threads will form in the presence of large quantities of crystallized pancreatic and soy bean trypsin inhibitors.

ment examined after centrifugation at 30,000 RPM for 30 minutes in the Spinco preparative centrifuge.§ Large numbers of typically coiled threads and uncoiled filaments were observed (Fig. 2). Examination of similar sediments prepared within 15 minutes of solution of the trypsinogen in buffer revealed a rare coiled thread or filament. Incubation of trypsinogen at 5°C in 0.4 borate buffer at pH 5.2 failed to produce any fibrous structures. This experiment was then repeated under sterile conditions. The "Tryptar"-seeded trypsinogen solution buffered at pH 8.0 was passed through an ultra-fine, sterilized, sintered glass filter and checked by thioglycollate and blood plate cultures for bacterial contamination immediately following filtration and after five days of incubation at 5°C. The solution proved to be sterile throughout the experiment. No coiled threads or filaments were found in the fresh filtrate. After incubation for 5 days the solutions were centrifuged and the sediments examined. Large numbers of long, typically coiled threads and filaments were observed in all fields.

Thus, it would appear that the characteristically coiled threads, described as components of elastic tissue by this author and as components of bacterial flagellae by Franchi and DeRobertis, are in fact fibrous structures arising from solutions of purified, crystallized trypsinogen during incubation under certain conditions. There exists the interesting possibility that this phenomenon may represent a fibrous transformation of trypsinogen or a component or contaminant thereof. Such transformations are known to occur in solutions of insulin(4), actin(5), and tropomysin(6). Further studies on the characterization of these fibrous units and the conditions for their production will be reported in detail elsewhere.

Summary. The coiled threads and filaments described by Gross as essential components of elastin, liberated from the tissue by tryptic digestion, and later described by Franchi and DeRobertis as products of tryptically digested bacterial flagellae have been demonstrated to be present in Armour's crystalline trypsin, but not in the more highly purified product "Tryptar," and can be produced by incubation from clear, sterile solutions of crystalline trypsinogen. This process probably represents a fibrous transformation of trypsinogen, a component or contaminant thereof.

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§ Because of the very small (oft-times invisible) sediment, the walls of the centrifuge tube had to be scrubbed thoroughly in 1 cc distilled water. Higher trypsinogen concentrations produced visible sediments.

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Evidence for a Sodium Retaining Factor in Toxemia of Pregnancy.* (19035)

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Toxemia of late pregnancy is a clinical problem which still remains to be defined in terms of etiology and pathological physiology. Practical management has taken diverse forms and has evolved from one plan to another, but

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