162, and are very similar in composition to recent sediments at the crest of the East Pacific Rise. Boström (*5*) has reported Fe concentrations in excess of 20 percent on a carbonate-free basis in East Pacific Rise deposits, and Boström and Peterson (*6*) have found that these sediments contain concentrations of Mn, Cu, Zn, Ni, and Co similar to those occurring in the sediments described here (Table 1). The high metal concentrations in East Pacific Rise sediments are thought by Boström (*5*) to result from submarine hydrothermal activity associated with the generation of new ocean floor at the rise crest. Corliss (*7*) has proposed that these hydrothermal solutions originated by the leaching of the newly extruded basalt. However, Turekian and Bertine (*8*) have suggested that some metal enrichments in ocean ridge sediments may result from their deposition under anaerobic conditions. Such a mechanism is unlikely to apply to the sediments described in this report, since they are oxidized throughout and contain the bulk of their Fe in the ferric state. In any event, according to current theories of sea-floor spreading, new ocean floor moves away from ridge crests, thereby carrying away sediment deposited on it irrespective of its origin. The ferruginous sediments described here are probably the Tertiary equivalents of those forming at the crest of the East Pacific Rise at the present time (*9*).

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Alpha$_1$ Antitrypsin in the Livers of Patients with Emphysema

Abstract. *Parenchymal liver cells from emphysema patients with an inherited deficiency of α_1-antitrypsin contain globules of glycoprotein that bind fluorescent antibody to α_1-antitrypsin. The globules can be seen after hematoxylin and eosin-staining or on electron microscopy, but are more readily demonstrated by PAS stain of amylase-treated liver sections. It appears that an inappropriately large amount of α_1-antitrypsin is found in the liver even when there is a deficiency in the serum. Genetic variants of the normal antitrypsin molecule may be unable to leave their site of synthesis in the liver cell because of some molecular aberration.*

Individuals with an inherited deficiency of α_1-antitrypsin (A_1AT) in their serums are predisposed to the development of pulmonary emphysema (*1*, *2*). The concentration of A_1AT in the blood is controlled by alleles of a pair of codominant genes that produce variants of the normal antitrypsin molecule (Pi system) (*3*). Certain variants are associated with a reduced concentration of A_1AT and a parallel reduction of its activity in serum (*4*). Thus, a deficiency of A_1AT results from its reduced concentration rather than from reduced activity of the antitrypsin variant.

Alpha$_1$-antitrypsin is synthesized primarily by the liver (*5*) but no consistent abnormality of liver structure or function has been described in genetically deficient subjects with lung disease (*6*). However, instances of inherited infantile cirrhosis of the liver have been observed in children with severe antitrypsin deficiency associated with the Pi^{ZZ} homozygous state (*7*, *8*).

Sharp *et al.*, using both light and electron microscopy examined the livers of such children and described parenchymal liver cells containing unusual globules whose contents were antigenically related to A_1AT (*9*). Their observation suggests that the deficiency in these children may be related to an abnormality of hepatic storage or release of the A_1AT. Our study was undertaken to determine whether abnormal quantities of A_1AT are also present in the livers of adults with pulmonary emphysema and antitrypsin deficiency.

Liver tissue was obtained from ten patients by percutaneous liver biopsy and from four patients at autopsy. Eight of these specimens were from patients with a severe deficiency of A_1AT due to Pi^{ZZ} phenotype [less than 0.4 unit (milligrams of trypsin inhibited by 1 ml serum)] and six were from patients with an intermediate deficiency (one Pi^{SS}, four Pi^{MZ}; their activity was 0.4 to 0.85 unit). All 14 patients had lung disease, but only one patient had evidence of liver disease related to metastatic carcinoma. None of the other 13 patients had clinical or laboratory evidence of liver disease.

Tissues were prepared for microscopy as follows. (i) The liver tissues were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin (HE) or with periodic acid schiff (PAS) reagent both before and after digestion with alpha amylase. (ii) Alternatively they were fixed in 2.5 percent glutaraldehyde in cacodylate at *p*H 7.4 followed by fixation in 1.0 percent osmic acid in barbital-acetate buffer, *p*H 7.4; such preparations were prestained with 0.5 percent uranyl acetate, dehydrated in acetone, and embedded in Epon. Ul-

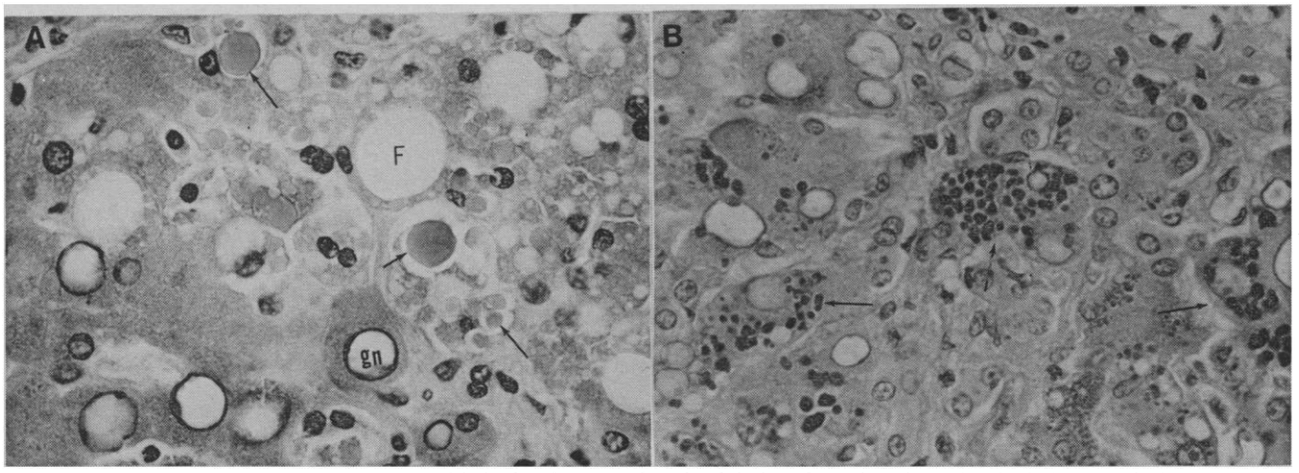

Fig. 1. Sections of liver obtained at autopsy (Pi^{zz}) showing cytoplasmic globules of varying size. (A) Globules contain homogeneous pink staining substance which is occasionally membrane bound (arrows). Glycogen nuclei (*gn*) and fatty vacuoles (*F*) are also present (hematoxylin and eosin; × 264). (B) Cytoplasmic globules (arrows) stain intensely with PAS (periodic acid–Schiff stain and hematoxylin after amylase digestion; × 125).

trathin sections were examined in the Hitachi HS-8 electron microscope after staining with uranyl acetate and lead citrate. (iii) Fresh tissues were frozen at −70°C (isopentane and Dry Ice bath) for approximately 10 minutes and sectioned by cryostat at 6 to 10 μm. The frozen sections were treated with fluorescein-conjugated specific antiserums (Hyland; Behring Diagnostic) to human albumin, human A_1AT, human fibrinogen, and human gamma globulin, according to the direct method of Coons (*10*); these sections were examined with the AO Fluorolume and AO Fluorestar microscope. Formalin fixed and frozen sections were also subjected to various histochemical procedures such as PAS preceded by enzyme digestion and blocking reactions, performic acid-Schiff stains preceded by boiling in chloroform, and protein stains such as the Ninhydrin Schiff.

Seven liver specimens from patients without A_1AT deficiency, but with severe emphysema, were also examined by light microscopy after HE and PAS staining of paraffin embedded tissues and by electron microscopy.

The serum trypsin-inhibitory capacity was assayed with benzoyl-L-arginine ethyl ester substrate (*2*). Antitrypsin phenotypes were determined by acid-starch electrophoresis and crossed-antigen antibody electrophoresis as described by Fagerhol (*11*).

Twelve of the 14 liver specimens from patients with antitrypsin deficiency had discrete, rounded, membrane-enclosed cytoplasmic liver globules. These were not seen in any of the seven emphysema patients with normal or elevated A_1AT concentrations. The globules measured 3 to 20 μm in diameter and were faintly eosinophilic with HE staining and moderately to strongly PAS-positive after amylase digestion (Fig. 1). The number of these globules was highly variable from cell to cell and inconspicuous enough to be overlooked on HE staining in most instances, especially in the tissues obtained by percutaneous liver biopsy. The cells containing globules did not have any particular lobular localization in the biopsied liver tissues, although they tended to cluster around portal areas and hepatic veins in the livers obtained at autopsy. More cells also appeared to contain globules in the specimens from autopsy. The bat-

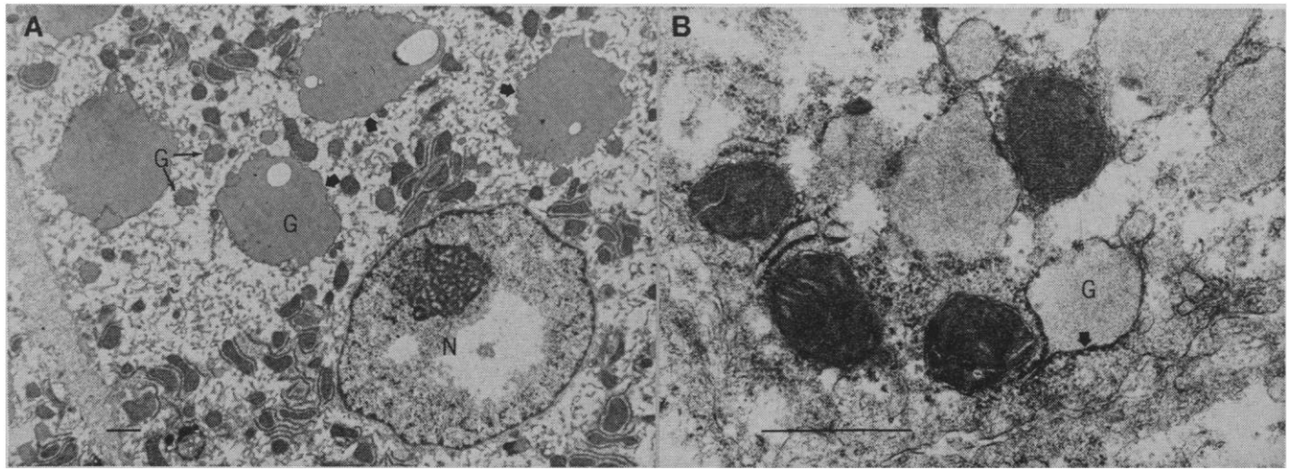

Fig. 2. (A) Electron microscopy of liver biopsy (Pi^{zz}) shows cytoplasmic globules (*G*) measuring approximately 0.5 to 3 μm in diameter filled with homogeneous moderately electron dense material. They are enclosed by a single irregularly bosselated membrane (dense arrows). Nucleus is present below (*N*) (uranyl acetate and lead citrate; × 9360). (B) Higher magnification showing dilated rough endoplasmic reticulum (arrow) surrounding moderately electron dense amorphous substance (*G*) (uranyl acetate and lead citrate; × 41,400).

tery of histochemical tests indicated that the hepatic globules had a nonlipid, glycoprotein composition. Other inconstant features of the liver seen on light microscopy were (i) mild focal steatosis, (ii) glycogen nuclei, and (iii) mild portal fibrosis and bile duct proliferation.

On electron microscopy, structures were identified in 5 of 11 cases (three biopsy, two autopsy) which measured 0.5 to 20 μm and matched those seen on light microscopy in shape and internal detail. Their ultrastructural characteristics were those of cytoplasmic bodies, namely, they were single-membrane bound, roughly oval, occasionally irregular and completely or incompletely filled with a homogeneous, moderately electron-dense material (Fig. 2). Some of the membranes had a beaded appearance suggesting rough endoplasmic reticulum. Occasional failure to find the globules by electron microscopy may have been due to their nonuniform cellular distribution within the liver lobule. In four of the six cases where globules were not seen on electron microscopy, they were seen by light microscopy after PAS staining.

Application of fluorescein-conjugated antiserum for human A_1AT to the liver tissues from one Pi^{ZZ} homozygote, one Pi^{SS} homozygote and two Pi^{MZ} heterozygotes revealed positively staining structures that corresponded in size, shape, and cellular localization to the globules. The fluorescence was completely blocked by prior incubation of serial sections in unconjugated antiserum to human A_1AT (liver tissues from seven controls did not fluoresce). Specific fluorescence for albumin or fibrinogen was negative; scattered fluorescing foci of gamma globulin were seen but did not coincide with the A_1AT fluorescent structure.

These observations complement the findings of Sharp *et al.* and indicate that certain genetic variants of A_1AT result in reduced levels of inhibitor in the serum with unusually large amounts of inhibitor in the liver. Intracellular hepatic globules containing A_1AT are present not only in the livers of antitrypsin-deficient patients with infantile cirrhosis, but also in the livers of antitrypsin-deficient adults with pulmonary emphysema and no liver disease. The rarity of liver disease in individuals with homozygous antitrypsin deficiency implies that infantile cirrhosis does not result directly from the abnormal intrahepatic metabolism of antitrypsin. It is possible that an additional causative factor, such as the Australian antigen (*12*), is transmitted to the fetus in families with cirrhosis. This second factor may severely damage a liver made vulnerable by the presence of A_1AT filled globules. A search for such a factor should be undertaken in these families.

More liver cells seemed to contain globules in the autopsy cases than in those specimens obtained by percutaneous biopsy, suggesting that a greater number of globules are present in individuals who are acutely ill. Of course, larger sections of liver were prepared from autopsy material, enabling easier identification of the globules. However, A_1AT is an acute-phase reactant protein, and the stimuli that normally increase the production of A_1AT by the liver may be intact in patients with a deficient variant resulting in more hepatic globules in severely ill, terminal patients. Such stimuli would be incapable of increasing the release of the deficient A_1AT variant from the liver, since neither the administration of estrogens (*8, 13*), nor typhoid vaccine (*14*) has any effect upon the serum level of A_1AT in homozygous deficient individuals. The low levels of inhibitor in the serums of these patients may result from failure of a release mechanism in the liver, associated with certain variants of the A_1AT molecule. The inherited defect resulting in A_1AT deficiency must reside within the liver, since Sharp *et al.* obtained complete normalization of the serum concentration of A_1AT following a liver transplant in one of their subjects (*9*). A releasing enzyme in the liver cell may be ineffective, or the A_1AT molecule may be unable to pass through the cell membrane when its configuration is abnormal.

We suspect that an aberrant molecular configuration of the deficient A_1AT variants impairs the release of inhibitor from the liver cell resulting in deficient levels of serum A_1AT. Both of the A_1AT variants usually associated with a deficiency in serum (Pi^Z and Pi^S) move more slowly in an electric field, suggesting that a difference in ionic charge or molecular weight may relate to their defective release from the liver.

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Gametogony of Sarcocystis sp. in Cell Culture

Abstract. *Sexual stages and cystlike bodies of* Sarcocystis *sp., a protozoan parasite found in muscles of reptiles, birds, and mammals, including man, developed in cell culture. Motile organisms, obtained from leg muscles of wild grackles, were inoculated into cell line cultures of embryonic bovine kidney. Mature micro- and macrogametes and the cystlike forms were found 30 and 42 hours after inoculation, respectively. These observations indicate that the parasite is probably a coccidium.*

Micro- and macrogametes and cystlike bodies have been observed in cultured cells inoculated with motile *Sarcocystis* sp. organisms. Previous reports (*1*) described development of *Sarcocystis* in cultured avian and mammalian cells. However, sexual stages were not found at that time and the significance of the cystlike bodies found at 48 and 72 hours was, therefore, not understood.

Motile, banana-shaped organisms were obtained from cysts in the leg and thigh muscles of wild grackles (*Quiscalus quiscula*) and prepared for inoculation into cell cultures as previ-