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LIVER CIRRHOSIS is of great interest to the surgeon. Its complications, particularly those of portal hypertension and of impending liver failure, continue to stimulate the imagination and ingenuity of surgical investigators in a search for more effective means of management.

Many conditions can result in liver cirrhosis. Recently, we observed two children with liver cirrhosis and a highly unusual associated abnormality (18). The ability of the plasma of these patients to inhibit trypsin was markedly decreased. Subsequently, it was noted that these patients lacked a specific plasma protein fraction that inhibits trypsin. Since the formation of plasma proteins is under genetic control, the absence of such a plasma protein fraction is inevitably the expression of a genetic abnormality. Evidence suggests a relationship between the genetic protein defect and a rapidly progressive type of liver cirrhosis. Its exact nature is, at the present time, unknown; however, it is believed to result from a decreased antiproteolytic activity of plasma.

The major share of the antiproteolytic activity of blood resides in the alpha globulin fraction. The responsible components recognized so far have been designated by Schultze and his associates as alpha$_1$-antitrypsin (15), a low molecular weight glycoprotein that moves on electrophoresis close to albumin, and alpha$_2$-macroglobulin (16), a protein with a sedimentation coefficient of 19 S and a molecular weight of approximately one million. Recently, a number of patients were found by Ericksson and Laurell (12, 13) to be deficient in this alpha$_1$-trypsin inhibitor. Those whose serum neutralized less than 0.4 milligram of trypsin per milliliter, in contrast to the 1 to 1.25 milligrams per milliliter neutralized by a normal individual, curiously enough, presented with pulmonary emphysema. In addition, Laurell and Ericksson (13) observed that the plasma of several family members neutralized trypsin concentrations that ranged between the former value and the normal one, from 0.45 to 0.85 milligram per milliliter of serum, a decidedly subnormal value. These members, currently regarded as heterozygotes for alpha$_1$-antitrypsin deficiency, were all free of disease, in contrast to the patients with pulmonary emphysema who are homozygous for the deficiency. Besides deficient antitrypsin activity, we found that their plasma also showed a decreased capacity to neutralize two other proteolytic enzymes, that is, plasmin, or fibrinolysin, and thrombin (8).

The two children with liver cirrhosis observed by us had homozygous alpha$_1$-antitrypsin deficiency. Sharp and his associates found in six families seven children with liver cirrhosis and homozygous alpha$_1$-antitrypsin deficiency. The mode of inheritance of the protein deficiency was autosomal recessive, similar to that previously described by Ericksson for familial pulmonary emphysema. Three other infants in these kindreds died of liver disease before the beginning of this study. Our study concerns two patients

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with this syndrome who are still alive. This report concerns their history and pathologic features, including findings on the anti-proteolytic activity of their serum, the anti-protease activities of a highly purified bovine alpha$_1$-antitrypsin fraction, and its possible relationship to the disease process; information concerning the effect of currently available treatment, as well as reflections on the future prevention and management of this syndrome, is also presented.

PATIENT REPORTS

PATIENT 1. This patient was the product of an uncomplicated pregnancy and weighed 3,750 grams at birth. Her first stool was white, but it became lightly colored by the third day. Jaundice, observed on the first day of life, persisted for two to three weeks. There was no obvious cause for these abnormalities. When she was three and one-half years of age and being seen for urinary frequency, an enlarged spleen which descended 10 centimeters below the left costal margin was palpated. Liver function studies showed a serum glutamic pyruvic transaminase value of 83 units and a cephalin flocculation of 3 plus. A percutaneous liver biopsy specimen revealed fibrosis and bile duct proliferation. During the interval before admission to the hospital, the patient experienced unexplained transient, acute abdominal pain, nose bleeds, as well as easy bruising.

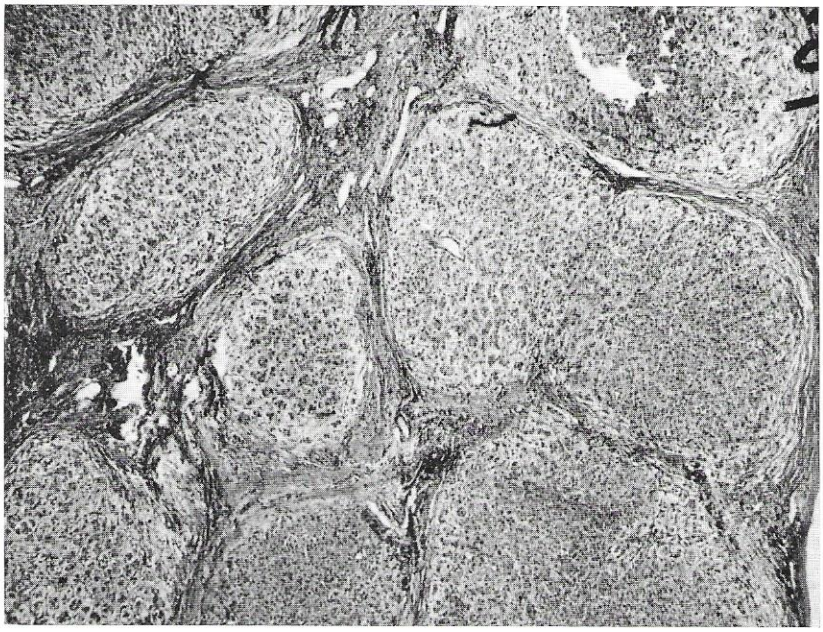
Initial evaluation when the patient was four and one-half years of age showed that she was of normal size and adequate intelligence. Numerous ecchymoses were present at the time of examination. The abdomen was mildly distended. A hard, nodular liver was palpated just below the xyphoid process. The spleen extended 12 centimeters below the left costal margin. Ophthalmologic examination revealed negative results.

The hemoglobin value was 9.5 grams per cent and the white blood cell count 6,200, with 61 per cent neutrophils, 35 per cent lymphocytes, 3 per cent monocytes, and 6 per cent eosinophils. Platelet count was 127,000 per cubic millimeter. Reticulocyte count was 2.6 per cent, and erythrocyte sedimentation rate was 14 millimeters per hour. Serum iron was 12 milligrams per cent and iron binding capacity 570 milligrams per cent. Prothrombin time was 15.6 seconds, control 13.8 seconds, and partial thromboplastin time was 51 seconds, control 37 seconds. The prothrombin time did not respond to vitamin K. Urinary copper excretion in 24 hours was 8.3 micrograms, normal up to 30. The urinary amino acid excretion pattern was normal. Galactose-1-PO_4 uridyl transferase was 23.8 units, normal 14 to 25 units. Sweat chlorides were 5 milliequivalents per liter. Liver function tests showed a bilirubin value of 0.4/1.2 milligram per cent Bromsulphalein® (sulfobromophthalein) retention of 11 per cent, ornithine carbamyl transferase of 93 decaunits, and an alkaline phosphatase value of 45 King-Armstrong units. Following spontaneous correction of the coagulation defects, percutaneous liver biopsy was repeated. Sections showed proliferation of bile ducts in a dense, fibrous stroma which widely separated adjacent liver parenchymal cords from the ducts. Focal areas showed a dense, chronic infiltrate of lymphocytes.

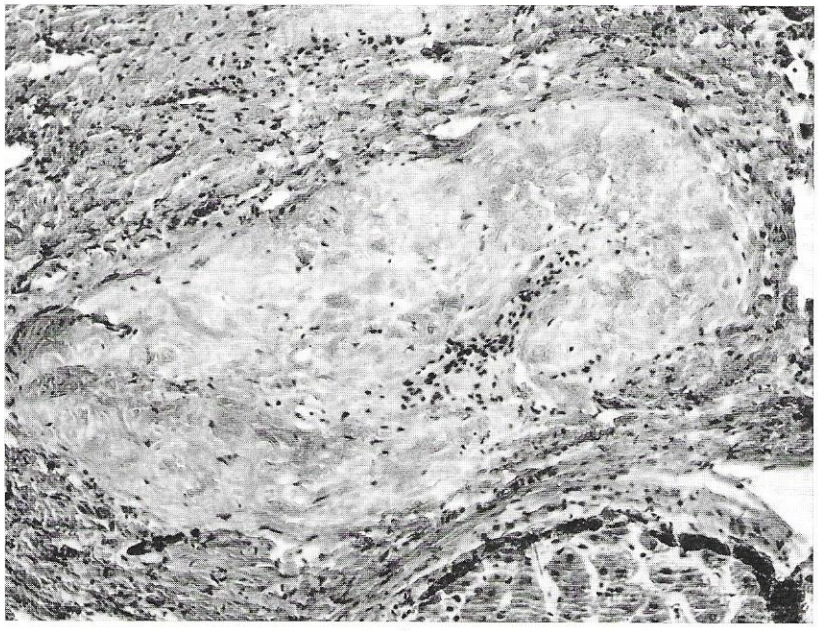
Protein electrophoresis revealed a total serum protein value of 6.6 grams per cent, with 4.5 grams per cent of albumin, 0.0 grams per cent of alpha$_1$-globulin, 0.5 gram per cent of alpha$_2$-globulins, 0.79 gram per cent of beta globulin, and 0.9 gram per cent of gamma globulin, and an antitrypsin content of less than 40 per cent of normal.

PATIENT 2. A ten year old girl was first admitted in 1957 at one month of age because of fever and obstructive jaundice which had been present since the third day of life. The infant was discharged from her first admission after the jaundice had cleared but was readmitted at six months of age because of hematemesis. Physical examination revealed hepatosplenomegaly. Liver function values included a bilirubin of 0.4/1.0 milligram per cent, alkaline phosphatase of 82 King-Armstrong units, and Bromsulphalein® retention of 6 per cent. The patient was rehospitalized in August of 1961 for further evaluation of the hepatosplenomegaly which was now accompanied by marked abdominal venous distention. Liver function values at that time included a bilirubin of 1.4/3.8 milligrams per cent, alkaline phosphatase of 90 King-Armstrong units, serum glutamic oxalacetic transaminase of 173 units, and cholesterol of 400 milligrams per cent. An upper gastrointestinal series showed probable esophageal varices.

A second episode of hematemesis precipitated the next admission in 1963 when the patient was five years of age. After the bleeding had stopped, a percutaneous splenic portogram was performed in the operating room. The splenic pulp pressure was 330 millimeters of saline solution, and radiologic studies demonstrated collaterals throughout the esophageal plexus and along the wall of the chest and the abdomen without portal vein obstruction. Laparotomy was undertaken, and a finely nodular cirrhotic liver and enlarged portal vein and spleen were observed. Operative cholangiogram was negative. Liver biopsy revealed greatly distorted architecture, with individual hepatic lobules separated by dense, fibrous connective tissue (Fig. 1a). No interlobular bile ducts were seen. The hepatic cells were large and pale, with those in the periphery containing large amounts of bile pigment. Bile plugs could be found in some of the canaliculi. One relatively large mass of connective tissue harbored two circular masses of



a



b

FIG. 1. Liver biopsy. a, Greatly distorted architecture with individual hepatic lobules separated by dense, fibrous connective tissue. Hematoxylin and eosin, ×25. b, Portal triad at higher magnification. Note the thrombosed portal radical. Hematoxylin and eosin, ×100.

bright, pink-staining hyaline material, which were thought to be hyalinized thrombi in blood vessels (Fig. 1b). A splenorenal shunt reduced the operative portal pressure from 300 to 150 millimeters of saline solution. Subsequently, the patient did well for the next five years.

At the present admission, recently developed ascites was controlled by Aldactone® (spironolactone)

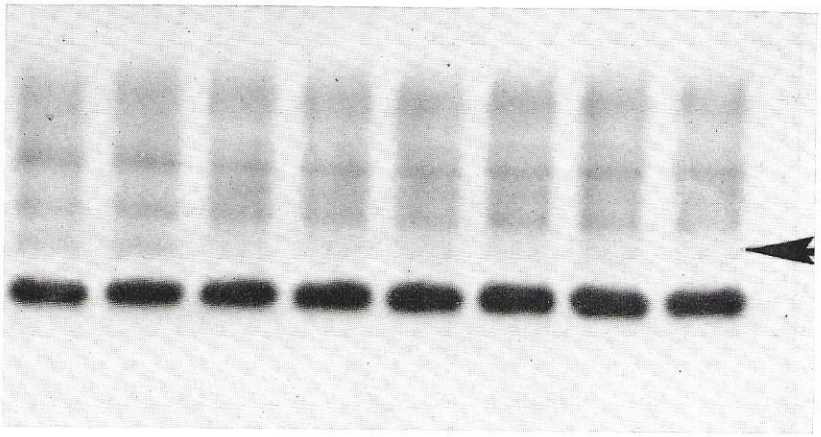


FIG. 2. Serum electrophoresis of normal serum, the two left samples, and the serum of the two patients. Note the absence of the alpha$_1$-globulin band in the serum of the patients.

and diet. Further bleeding episodes were denied. However, the patient complained of intermittent pain in the hands plus cyanosis after exercise. Her stools continued to be light-colored, bulky, and fetid. Family history disclosed two maternal great uncles who had died in infancy of liver disease. Physical examination revealed a girl with jaundice, with ascites and edema, marked clubbing, spider angiomas, palmer erythema, and distended venous pattern. An alternating esotropia was the only ophthalmologic abnormality. Upon palpation, only a hard, nodular left lobe of the liver was noted. Liver function values included a bilirubin of 4.1/8.1 milligrams per cent, ornithine carbamyl transferase of 65 decaunits, alkaline phosphatase of 79 King-Armstrong units, and cholesterol of 282 milligrams per cent. Urine examination for galactose and inclusion bodies was negative. The amino acid pattern was normal. Repeated serum copper and ceruloplasmin values had not been low. Protein electrophoresis revealed a total serum protein value of 4.5 grams per cent, with 1.3 grams per cent of albumin, 0.1 gram per cent of alpha$_1$-globulin, 0.7 gram per cent of alpha$_2$-globulin, 1.4 grams per cent of beta globulin, and 1 gram per cent of gamma globulin. Trypsin binding capacity was less than 40 per cent. A grandmother, who has emphysema, was studied and found to have normal liver function but also no alpha$_1$-globulin on cellulose acetate electrophoresis.

METHODS AND MATERIALS

Fresh human plasma samples collected at the blood bank served as controls. Blood samples were obtained from the two aforementioned patients with homozygous alpha$_1$-antitrypsin deficiency. These patients had been previously studied by Sharp and his associates and found to have an antitrypsin level of less than 40 per cent normal. The blood samples of these two patients were allowed to clot, and the serum was collected and quick-frozen and subsequently used for the various determinations of antiproteolytic activity, as previously described (8).

Determination of the leukoprotease activity of leukocytes in man was carried out on standard fibrin plates, each containing 20 milligrams of bovine fibrin. Details of the procedure for the preparation of the plates and the leukocyte samples have been previously described (6). Cellulose acetate microzone electrophoresis was performed at room temperature for 20 minutes at 250 volts in a Beckman microzone cell, with the use of a Duostat® power supply and the Beckman B_2 buffer, μ 0.075, *p*H 8.6 Proteins were stained by the Ponceau red technique.

Immunoelectrophoresis on 1 per cent agarose gel was carried out for 1 hour at 7 volts per centimeter at zero degrees in Michaelis diethyl barbiturate buffer, μ 0.1, *p*H 8.2. Rabbit antihuman serum and rabbit antialpha$_1$-antitrypsin serum were allowed to diffuse at room temperature into the agarose, which was placed in a moist, closed chamber for 24 hours prior to washing, fixing, and staining of the precipitin arcs. Alpha$_2$-macroglobulin concentrations

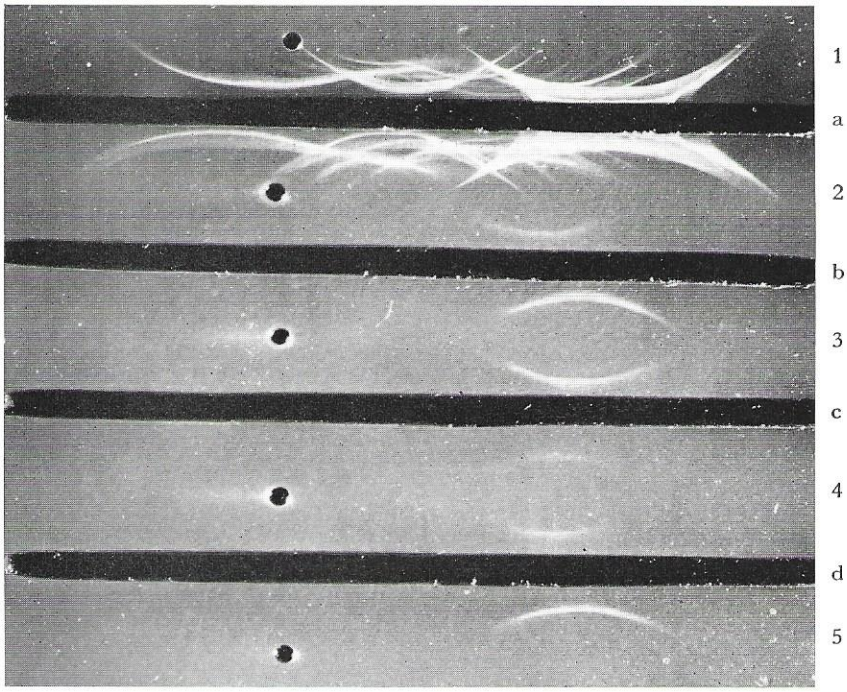


FIG. 3. Immunoelectrophoresis of normal human serum, 1, 3, and 5, and serum from two patients with alpha$_1$-antitrypsin deficiency, 2 and 4, against rabbit antihuman serum, slot d-a, and rabbit antihuman alpha$_1$-antitrypsin, slot b-c and d.

were determined by a quantitative immunoelectrophoresis technique previously described by Laurell (11).

Alpha$_1$-antitrypsin was prepared from bovine plasma by ammonium sulphate precipitation techniques and electrophoresis on acrylamide gel and a cellulose column. Details of this procedure are to be reported elsewhere (20).

RESULTS

Study of plasma proteins in the patients. Microzone electrophoresis of these plasma samples on cellulose acetate strips clearly reveals the absence of the alpha$_1$-globulin band. In contrast, the alpha$_2$-globulin fraction appears normal (Fig. 2). Immunoelectrophoresis of these plasma samples against rabbit antihuman alpha$_1$-antitrypsin produces a faint precipitin line, in contrast to the pronounced precipitant band obtained with normal plasma (Fig. 3). A distinct band was present on immunoelectrophoresis against rabbit antihuman alpha$_2$-macroglobulin. Quantitative immunoelectrophoresis performed according to the technique described by Laurell (11) revealed that the alpha$_2$-macroglobulin concentrations in the plasma of these patients were similar to those of normal human plasma.

Study of the antiproteolytic activity of plasma in the patients. Determination of the antitrypsin content of the plasma of these patients showed that it contained less than 40 per cent of normal control plasma (18). This finding indicates that the alpha$_1$-antitrypsin concentration of their plasma was in the range previously observed in patients with homozygous alpha$_1$-antitrypsin deficiency.

The capacity of defibrinated alpha$_1$-antitrypsin-deficient human plasma to neutralize thrombin-clotting activity was less than the inhibition obtained with defibrinated, pooled, normal human plasma. This reduction was considerable. After one-half hour incubation with thrombin, it amounted

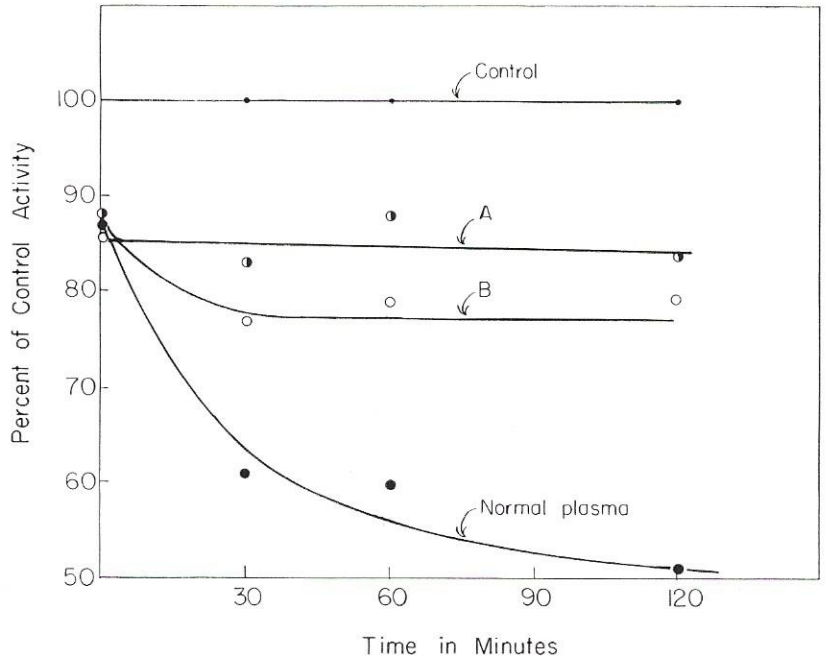


FIG. 4. Determination of antiplasmin activity in two patients with homozygous alpha$_1$-antitrypsin deficiency. Fast antiplasmin activity represents the difference in residual plasmin activity between plasmin buffer mixture—control—and plasmin plasma mixture at zero time. Slow antiplasmin activity represents the difference in residual plasmin activity between these two mixtures at 30, 60, and 120 minute incubation periods. Note that the fast antiplasmin content of the plasma of the patients with alpha$_1$-antitrypsin deficiency, samples *A* and *B*, is the same as that of the control plasma sample. In contrast, there is no or minimal slow antiplasmin activity in the plasma samples of the patients as compared to that of the control plasma sample.

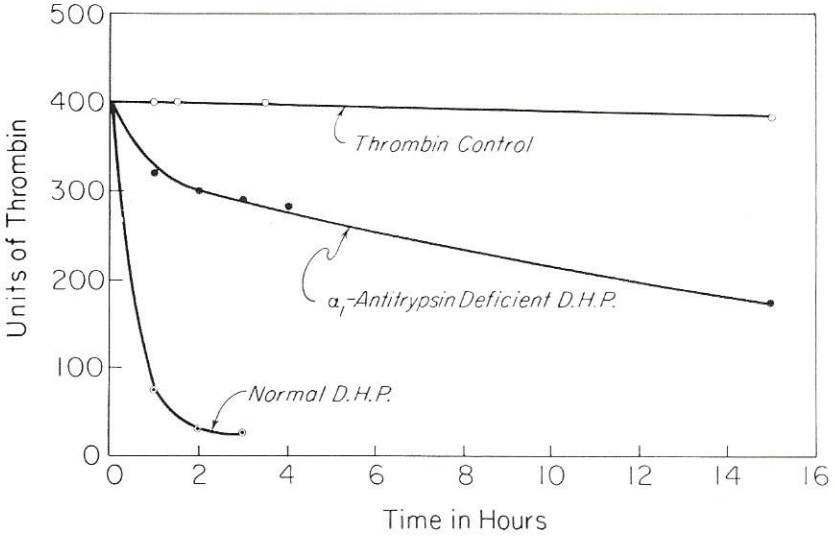


FIG. 5. Antithrombin activity in normal and alpha$_1$-antitrypsin-deficient defibrinated human plasma, *D.H.P.*, as determined by thrombin-clotting assay.

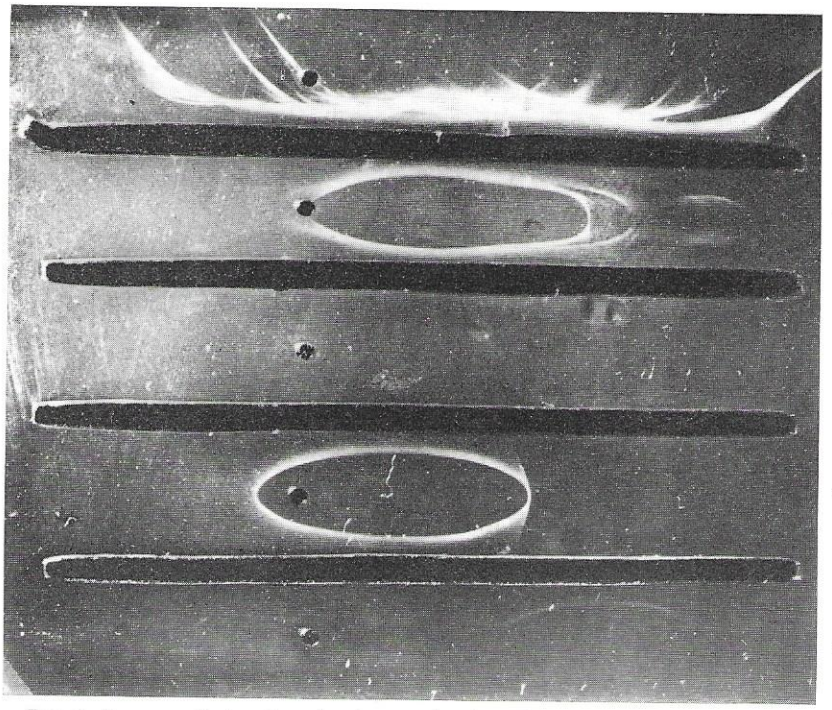


FIG. 6. Immunoelectrophoretic characterization of bovine plasma, a, partially purified alpha$_1$-globulin, b, purified alpha$_2$-macroglobulin, c, and alpha$_1$-antitrypsin, d, against rabbit antibovine serum.

to more than 75 per cent of the activity observed in normal, defibrinated human plasma. There was a striking absence of the initial, rapid decline in thrombin-clotting activity so commonly observed with the dilutions used in the experiments during the first ten to 15 minutes after the addition of thrombin to defibrinated plasma as shown in Figure 4.

In addition, changes in antiplasmin were observed in the alpha$_1$-antitrypsin-deficient plasma. In one patient, the slow antiplasmin activity was absent; in the other, it was markedly decreased (Fig. 5).

Alpha$_1$-antitrypsin-deficient plasma completely inhibited the activity of human leukoprotease when tested with bovine fibrin as a substrate (Table I).

Antiproteolytic activity of purified bovine alpha$_1$-antitrypsin. The purified bovine alpha$_1$-antitrypsin preparation obtained showed a single precipitant band on immunoelectrophoresis against rabbit antibovine serum (Fig. 6). On ultracentrifugation, the protein fraction moved as a single symmetrical peak. It had a sedimentation velocity of 3.5 and a molecular weight of approximately 45,000. This material showed antithrombin, antitrypsin, slow antiplasmin (Figs. 7 and 8), as well as antileukoprotease activity (Table I).

DISCUSSION

A number of inherited metabolic defects are associated with hepatic cirrhosis. Stanbury and his associates and Iber and Maddrey reviewed those that appear in childhood. These are galactosemia, cystic fibrosis, tyrosinemia, glycogen storage disease fructose, intolerance, porphyria, and Wilson's disease. Occasionally, the first evidence of disease occurs at a later age, as in hemochromatosis. The pathogenesis of hepatic cirrhosis in patients with metabolic defects remains to be elucidated.

The possibility that the two children described herein suffered from hepatitis or cirrhosis secondary to biliary atresia, cystic

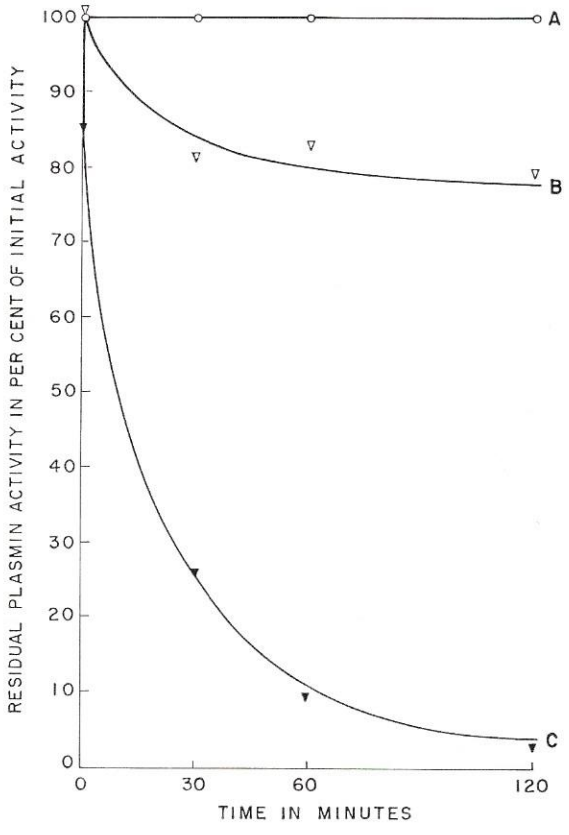


FIG. 7. Determination of residual plasmin activity of different incubation mixtures. *A*, Plasmin-buffer mixture; *B*, plasmin-alpha$_2$-globulin mixture, 6 milligrams per milliliter incubation mixture; and *C*, plasmin-alpha$_1$-globulin mixture, 6 milligrams per milliliter incubation mixture.

fibrosis, tyrosinemia, or drugs with secondary abnormal protein formation should be considered. However, Sharp and his associates found that the serum antitrypsin levels in infants with these forms of cirrhosis, rather than low or near absent, are normal or elevated. Also, the patients described herein failed to show evidence of biliary atresia or one of the other aforementioned defects.

Both children had absent alpha$_1$-globulin bands on serum electrophoresis and a weak alpha$_1$-antitrypsin precipitant arc on immunoelectrophoresis. Their serum antitrypsin levels were less than 40 per cent of normal. Older siblings with equally low plasma alpha$_1$-antitrypsin levels presented with pulmonary emphysema, thus confirming a syndrome previously described by Ericksson. In addition, alpha$_1$-antitrypsin levels in several kindreds were found to range from 45 to 75 per cent of normal. These asymptomatic siblings clearly fall into the category previously defined as heterozygous alpha$_1$-antitrypsin deficiency, as reported by Laurell and Ericksson (13). These findings, in addition to the history of four related children who died from liver disease, in all of whom a distinct cause for the cirrhosis was absent, suggests that the tendency for liver cirrhosis to develop in infants with homozygous alpha$_1$-antitrypsin deficiency at an early age is greatly increased.

Presently, nothing is known about the incidence of this syndrome. However, the disease process is distinctly progressive. A splenorenal shunting procedure, which prevented recurrence of gastrointestinal hemorrhage in one patient, failed to check the progress of the disease, as evidenced by the subsequent development of ascites and further deterioration of liver function.

Whereas changes in alpha$_2$-macroglobulin levels have previously been found by us (7) to be associated with changes in plasma antiproteolytic activity, its values in these patients were normal. The observation that the serum of these two patients is also deficient for other antiproteases would suggest, therefore, that alpha$_1$-antitrypsin inhibits more than trypsin alone, an assumption amendable to verification only with a purified alpha$_1$-antitrypsin fraction. Such a fraction prepared from bovine plasma exerted marked antithrombin and antiplasmin-activity. The antiplasmin is of the slow-acting type. Little is presently known concerning its function.

Donaldson and Evans and Rosen and his associates previously demonstrated that deficiency of plasma inhibitors for proteolytic enzymes can indeed cause disease in patients with hereditary angioneurotic edema who lack an alpha$_2$-globulin fraction required to inhibit the esterase activity of the first

component of complement. In its absence, a disease characterized by recurrent, acute circumscribed and transient subepithelial edema of the skin, gastrointestinal mucosa, and mucosa of the upper portion of the respiratory tract develops. Similarly, the association of alpha$_1$-antitrypsin deficiency and pulmonary emphysema, as already mentioned previously, has been clearly established.

Deutsch has indicated that antithrombin and antiplasmin are produced by the liver. Alpha$_1$-antitrypsin, according to Schultze (17), also derives from this organ. The reason that the failure to manufacture adequate amounts of this proteolytic enzyme inhibitor would result in progressive destruction of the liver remains to be explained.

In the liver, although its tissues may be exposed to large numbers of noxious stimuli derived from the intestine, mechanisms that provide protection reside in the Kupffer cells. It is conceivable that phagocytosis is associated with damage of these cells or the release of their lysosomal enzymes. Presently, however, there is no evidence to support such a concept. A second mechanism of liver injury is the possible release of trypsin by the pancreas. Normally, only trypsinogen is formed and released into the pancreatic duct system. If active enzymes are released into the blood, it would be expected that the pancreas itself would be the first organ to be affected. However, the pancreatic function in these patients was normal.

Results of a preliminary experiment indicate the ability of the alpha$_1$-trypsin inhibitor to completely inhibit the proteolytic activity of leukocytes (Table I). Hence, alpha$_1$-antitrypsin has a much wider range of antiprotease activity than its name would suggest. Kueppers and Bearn attempted to relate the alpha$_1$-antitrypsin deficiency to pulmonary emphysema by demonstrating that the plasma in these patients failed to adequately neutralize leukoprotease activity. We were unable to confirm this, since we

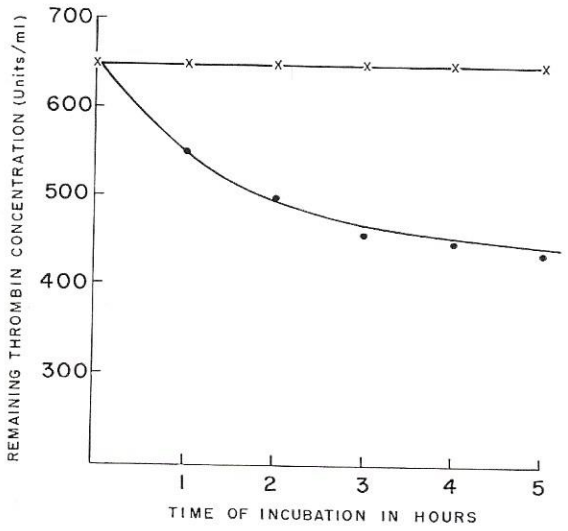


FIG. 8. Antithrombin activity of purified bovine alpha$_1$-antitrypsin preparation. x—x, thrombin incubated with buffered, normal saline solutions, *p*H 7.4; •—• thrombin incubated with 3 milligrams of purified, bovine alpha$_1$-antitrypsin per milliliter incubation mixture.

noted that plasma deficient in alpha$_1$-antitrypsin completely neutralized the proteolytic activity of leukocytes. Moreover, there is no reason to assume that the mechanism of disease as previously postulated by Kueppers and Bearn for the lung is also operative in the liver. One might speculate, however, that if thrombin is generated, it might be inadequately neutralized in the absence of sufficient alpha$_1$-antitrypsin. The possible presence of hyaline thrombi in the

TABLE I.—EFFECT OF NORMAL AND ALPHA$_1$-ANTITRYPSIN DEFICIENT PLASMA AND BOVINE ALPHA$_1$-ANTITRYPSIN ON THE PROTEASE ACTIVITY OF HUMAN LEUKOCYTES DETERMINED BY THE BOVINE FIBRIN PLATE TECHNIQUE

Experiment	*Area of lysis of bovine fibrin plate (mm.^{2}) 24 hrs. incubation at 37°C.*
A	225
B	0
C	0
D	0

A=0.5 ml. of a suspension of fragmented leukocytes, 1.4x10^8 cells/ml., was incubated at 37°C. for one-half hour, with an equal volume of 0.05 M. phosphate buffer of *p*H 7.5, and three 20 μl. aliquots of this solution were applied to a fibrin plate.

B=same as A, but incubated with an equal volume of a solution of alpha$_1$-antitrypsin, 6 mgm./ml.

C=same as B, but incubated with an equal volume of normal plasma.

D=same as A, but incubated with an equal volume of alpha$_1$-antitrypsin-deficient plasma.

liver tissue for biopsy of the second patient suggests that activation of the clotting mechanism may have played a role in the genesis of this disease.

Whatever the cause of the cirrhosis, the ultimate fate of these patients is dismal. Complications of liver cirrhosis, particularly those related to hemorrhage in the upper gastrointestinal tract secondary to portal hypertension, have been treated for the last 20 odd years with portacaval shunts. Recently, it was established by Callow and his associates and by Conn and Lindemuth that, although treatment with portacaval shunt virtually eliminates the incidence of hemorrhage in the upper gastrointestinal tract, it does not significantly alter the life expectancy of patients with liver cirrhosis. Consequently, splenorenal shunts are currently favored on the assumption that, if they remain patent, they adequately reduce portal vein pressure and are less liable to result in brain and spinal cord lesions, while the function of the liver is not adversely affected.

In the one patient treated with splenorenal shunt for upper gastrointestinal tract hemorrhage, recurrence of hemorrhage was not observed. Yet, the disease continued to progress. Presently, it is not clear if this progress resulted from the shunting procedure or if it is a typical feature for this syndrome. It is obvious, however, that the available modes of therapy fail to correct or halt the disease process. It is conceivable that these patients might eventually benefit from specific transfusion therapy or the administration of synthetic protease inhibitors in an attempt to prevent the development of pulmonary emphysema or liver cirrhosis. If anyone is to profit from liver transplantation, however, these patients would, for the removal of the diseased liver and its replacement by an organ from a donor without this inborn error of metabolism would provide these children with a source that produces normal alpha$_1$-antitrypsin and thus potentially with a normal life expectancy. One might extend the analogy even further by also considering the patient with homozygous alpha$_1$-antitrypsin deficiency who suffers from emphysema at an early stage as a future candidate for this curative surgical procedure.

SUMMARY

The history, laboratory and histologic findings, and special chemical determinations of two children with homozygous alpha$_1$-antitrypsin deficiency and liver cirrhosis were investigated. This represents a new syndrome. Although its cause is far from clear, the liver cirrhosis appears to be related to the serum antiprotease deficiency of the patient.

This clinical entity is of particular significance to the surgeon because the disease progresses relentlessly even after adequate reduction of portal hypertension by splenorenal shunt and because of the potential curability of the antiprotease deficiency and the liver cirrhosis by liver transplantation.

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