PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 145–150 (1975)

Serum Antitrypsin Synthesis by the Isolated Perfused Rat Liver (38493)

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Alpha$_1$-antitrypsin in man is a serum alpha$_1$-globulin which inhibits many proteases (1); it is believed to be genetically determined by a single locus with at least 11 alleles (2). The uncommon ZZ genotype has only about 10% of normal serum alpha$_1$-antitrypsin activity and is associated with pulmonary emphysema in adult life (3) and with cirrhosis in childhood (4).

The pulmonary emphysema in the ZZ homozygote is presumably the result of deficient serum antitryptic activity although the mechanism of production is only speculative. Since Z type antitrypsin has the same specific activity as that of the M type (5, 6), and since there is no evidence for accelerated removal of the Z type *in vivo* (7, 8), the cause of the deficient serum tryptic activity of the ZZ homozygote appears to be deficient synthesis and/or secretion into the circulation. To understand the pathogenesis of the lung disease it is necessary to establish firmly the site of synthesis of serum antitryptic activity.

An individual of the ZZ genotype is also predisposed to cirrhosis (4, 9, 10). Whether or not cirrhosis occurs, such an individual has amorphous material in the lumen of the rough endoplasmic reticulum of the periportal hepatocytes identifiable as alpha$_1$-antitrypsin by fluorescent antibody (9, 11–14). This material has not yet been shown to have antitryptic activity (15). Therefore, it is not established whether it represents incompletely synthesized alpha$_1$-antitrypsin yet to be secreted, or degraded alpha$_1$-antitrypsin which has been removed from the circulation and which perhaps had been synthesized extrahepatically.

Thus, to clarify the mechanism of both liver and lung injury in alpha$_1$-antitrypsin deficiency, it is necessary to firmly establish the site of synthesis. Asofsky and Thorbecke (16) reported that minced monkey liver in culture made alpha$_1$-acid glycoprotein identified by immunoelectrophoresis. In man, it has been briefly reported that when a ZZ homozygote with liver disease underwent liver transplantation, the serum trypsin inhibitory capacity increased to normal within 2 days and remained at that level until the patient's death from respiratory complications (9). Although these observations are consistent with the liver as the chief site of synthesis of serum trypsin inhibitory capacity, there has been no direct demonstration of its total synthesis by the liver.

To demonstrate total synthesis of serum antitrypsin activity in the liver, we have studied the isolated perfused liver of the rat. The isolated perfused rat liver has been a useful model for establishing that the liver synthesizes many plasma proteins (17, 18). Our results show that in the rat the liver synthesizes material with properties similar to those of human alpha$_1$-antitrypsin at a rate compatible with the liver as the principal site of synthesis.

Methods. Details of the isolated rat liver perfusion method have been previously described (18). The perfusate consisted of a suspension of bovine red cells in Krebs–Ringer bicarbonate buffer containing 3% bovine serum albumin (Fraction V, Miles Laboratories). During each 12 h of perfusion there was a continuous infusion of 16 ml of Ringer's solution containing 320 mg of a complete mixture of amino acids (18) plus 500 mg glucose neutralized to pH 7.40 with 1 *N* NaOH. When used, cortisol (Solucortef, Upjohn), 5 mg, and insulin 6.8 units (glucagon-free) (generously supplied by the Eli Lilly Co.) were added to the continuous infusion; in addition, 5 mg of cortisol and 5.1 units of insulin were added to the perfusate reservoir at the start of the perfusions.

In the assay for antitrypsin activity, adapted from Eriksson (3), each test contained 10 μg trypsin, 3 μl serum, and 0.482 μmole BAPNA (benzoyl-DL-arginine-*p*-nitro-

anilide HCl) in 1.0 ml of 10 m*M* $CaCl_2$-50 m*M* Tris–Cl, pH 8.2, at 25°. The buffer, enzyme and serum were preincubated 5 min before the addition of substrate (19). Instead of determination at 10 min the formation of product was in each case continuously monitored at 410 nm by the Gilford spectrophotometer (Model 200) and recorder (Model 6040). This method permitted determination of the initial reaction rate which was often not linear after 5 min.

The values given for antitrypsin activity are relative rather than absolute values. To obtain absolute values the concentration of active trypsin in the commercial preparation, usually 40–60%, must be determined. This was not done in the present case because our interest was not in the absolute values but in relative values, e.g., between rat serum and human serum.

The human serum was $alpha_1$-antitrypsin type MM (20). The rabbit antiserum to human $alpha_1$-antitrypsin and to human albumin was obtained from Behring Diagnostics, Woodbury, NY; the human $alpha_1$-antitrypsin antigen had been purified by the method of Heide and Haupt (21) and the antiserum shown to be specific by electroimmunodiffusion. The antiserum to rat albumin was produced by us in rabbits.

Results and Discussion. Antitrypsin activity of normal rat serum. The antitrypsin activity of normal rat serum based on samples from seven rats was 1.28 ± 0.15 SD mg of trypsin inhibited per ml of serum. By the same method, the antitrypsin activity of normal human serum based on samples from 20 subjects of $alpha_1$-antitrypsin type MM was 2.04 ± 0.05 SD mg of trypsin inhibited per ml of serum.

Starch block electrophoresis of normal rat serum. Figure 1 shows the pattern of antitrypsin activity associated with the fractions resulting from electrophoresis of normal rat serum on starch block. The antitrypsin activity is found in a broad peak extending from the beta-globulin through the alpha-globulin and albumin fractions. The pattern differs from that of human serum in which most of the antitrypsin activity is found in the alpha-globulin region. However, as can be inferred from the figure, the antitrypsin activity per mg protein is greatest in the alpha-globulin fraction.

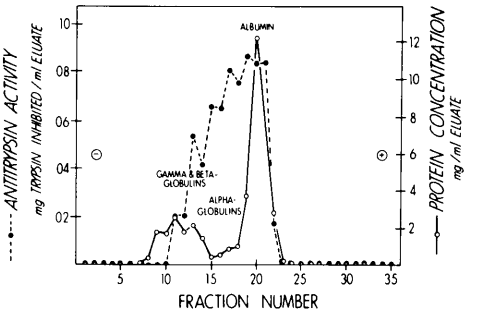

FIG. 1. Starch block electrophoresis of normal rat serum. The method of Kunkel and Slater (23) was used with sodium veronal buffer, ionic strength 0.05, at pH 8.5.

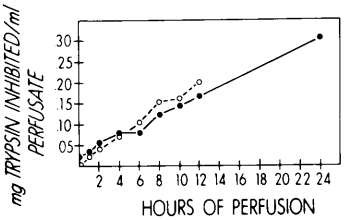

FIG. 2. Time course of production of antitrypsin activity in the isolated rat liver perfusate. Open circles: without cortisol and insulin in the perfusing medium. Solid circles: with cortisol and insulin in the perfusing medium.

Synthesis by the isolated perfused rat liver. A series of experiments were performed on the isolated perfused rat liver. Figure 2 shows results of two representative experiments. The total antitrypsin activity of the perfusate is shown as a function of time. When a liver from a normal animal was perfused for 12 hr with a medium containing glucose and amino acids, the antitrypsin activity rose steadily over a 12 hr period. Because the combination of the hormones cortisol plus insulin has been demonstrated (18) to induce increased synthesis of the acute phase proteins fibrinogen, $alpha_1$-acid glycoprotein, haptoglobin, and $alpha_2$-(acute phase) globulin, effects of the hormones on synthesis of antitrypsin activity were investigated. However, over a 24-hr course no increase in the rate of synthesis was observed (Fig. 2). This is in distinct contrast to the two- to fivefold increase in rate of synthesis of other acute phase proteins

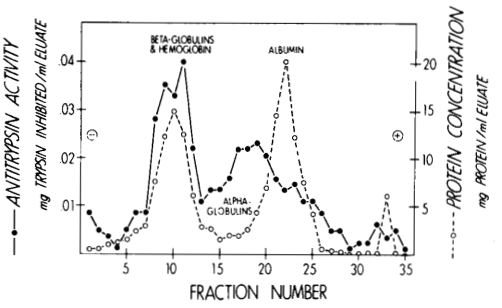

FIG. 3. Starch block electrophoresis of isolated rat liver perfusate. For details of the method, see legend of Fig. 1.

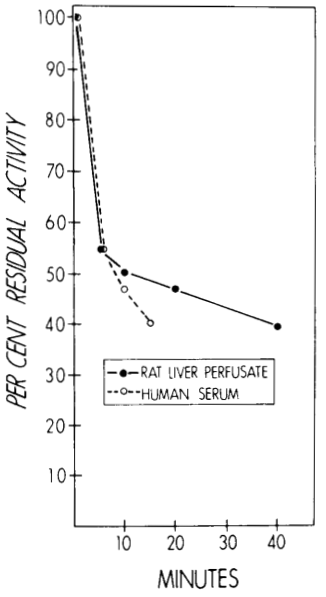

FIG. 4. Heat lability of antitrypsin activity of isolated rat liver perfusate and of human serum. The rat perfusate was exposed to 56° for various times and then assayed at the standard temperature, 25°. Residual antitrypsin activity of the rat perfusate is compared to that of normal human serum which had been similarly treated.

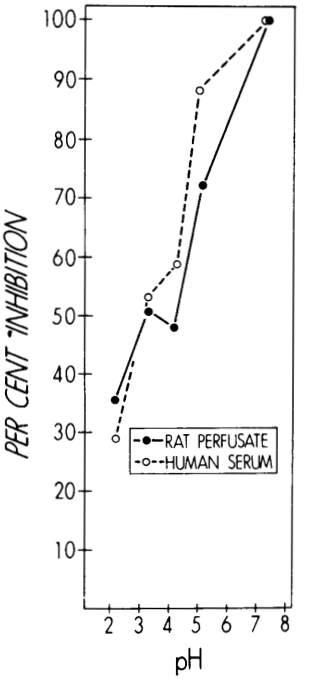

FIG. 5. Acid lability of antitrypsin activity of isolated rat liver perfusate and of human serum. The rat perfusate was exposed for 30 min at 25° to a given pH between 7.4 and 2.3. Following this the pH was returned to the original pH for assay of antitrypsin activity. The residual antitrypsin activity of the rat perfusate is compared to that of normal human serum which had been similarly treated.

measured for the same perfusion (not shown).

It has been asserted that alpha$_1$-antitrypsin in the human behaves like an acute phase protein in response to some clinical conditions. However, the kinetics of such induction in the human have not been described. In man, diethylstilbesterol given orally requires two days to raise serum antitrypsin activity and 5 to 10 days for a maximal response (22). It may be that failure of the rate of antitrypsin synthesis to increase during 24-hr perfusion may reflect the need for a longer induction period than is needed for fibrinogen. In any event the quantitatively large capacity of the isolated perfused rat liver to elaborate antitrypsin activity as estimated below may be interpreted as an indication that the amount synthesized may be adequate even for needs associated with the response to injury.

If one assumes a half-life of rat serum antitrypsin activity similar to that of human serum alpha$_1$-antitrypsin (about 4 days) (7, 8), and that the liver is the chief site of synthesis in the rat, one can compare the synthetic rate in the isolated perfused rat liver with that of the rat liver *in vivo*. The isolated perfused liver achieved a level (0.3 mg trypsin inhibited per ml) one-quarter that of normal rat serum (1.28 mg trypsin inhibited

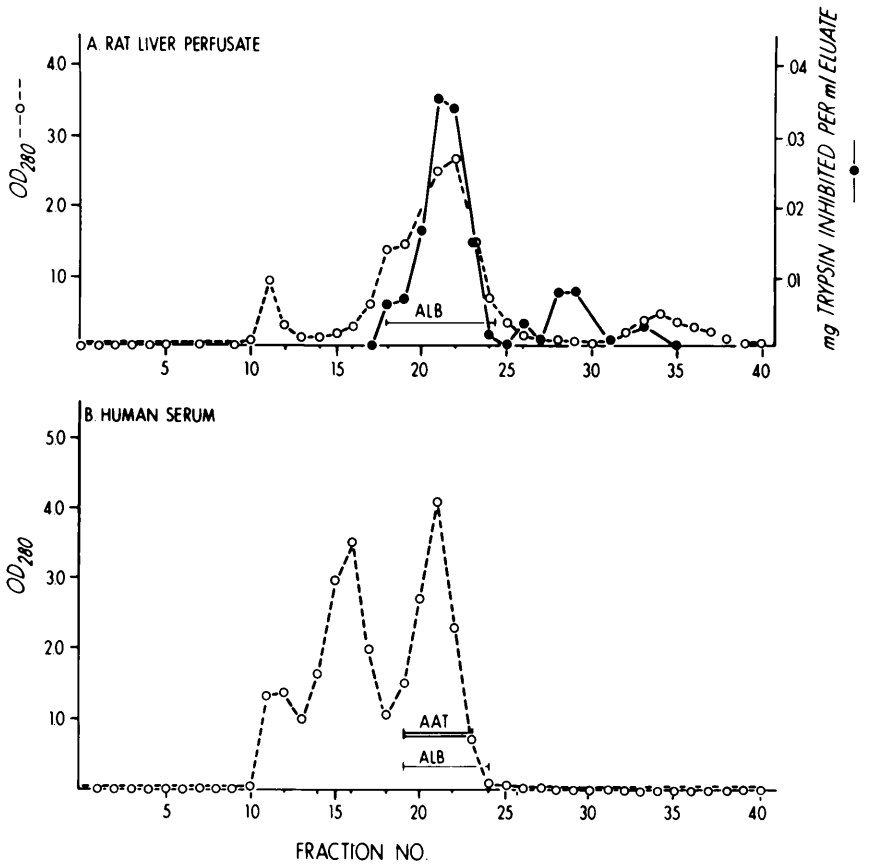

FIG. 6. Sephadex G-200 chromatography of isolated rat liver perfusate and of human serum. Sephadex G-200 chromatography was performed on an 80 × 1.5 cm column in 0.1 *M* NaCl, 0.05 *M* Tris–Cl, pH 8.2, at 4°. The rat perfusate sample at 12 hr contained 62 OD_{280} units in 1.3 ml. The human serum sample contained 111 OD_{280} units in 1.5 ml. Fractions (4 ml) were tested for rat or human albumin and for human alpha$_1$-antitrypsin by radial immunodiffusion (24). Antitrypsin activity in the rat perfusate was estimated by the rate that residual trypsin hydrolyzed BAPNA. The fact that there is a single predominant protein peak in the rat liver perfusate is not surprising since bovine serum comprises 95% of the perfusate protein.

per ml) in a perfusate plasma volume of 60 ml which is five to six times the normal rat plasma volume. It can thus be calculated that the isolated perfused rat liver may be synthesizing antitrypsin at 10 times its *in vivo* rate.

Characterization of perfusate antitryptic activity. The perfusate of the isolated rat liver at the end of 12 hr was characterized in several ways.

1. *Electrophoresis.* Starch block electrophoresis revealed two peaks of protein (Fig. 3), the faster-moving is in the alpha-golbulin and albumin region, and the slower-moving is in the region of beta-globulins and contains some hemoglobin from hemolysis; rat serum alpha$_1$-globulins and albumin are not resolved by electrophoresis. There are two peaks of antitryptic activity, a broader peak at the trailing edge of the alpha$_1$-globulin region and a sharper peak in the beta-globulin region.

2. *Heat lability.* The rat perfusate was exposed to 56° for various times and then assayed at the standard temperature, 25°. Figure 4 shows the residual activity of the rat perfusate compared to that of normal human serum which had been similarly treated. Like that of human serum, the antitrypsin activity of the rat perfusate is quite labile at 56°, losing 45 % of its activity in 5 min.

3. *Acid lability.* The rat perfusate was ex-

posed for 30 min at 25° to a given pH between 7.4 and 2.3. Following this the pH was returned to the original pH for assay of activity. Figure 5 shows the residual activity of the rat perfusate compared to that of normal human serum which had been similarly treated. It can be seen that the antitrypsin activity of the rat perfusate and that of human serum have a similar acid lability.

4. *Apparent molecular size.* Figure 6 shows the results of gel filtration of rat perfusate and of human serum separately analyzed on the same column of Sephadex G-200. In the case of the rat perfusate, several peaks of antitryptic activity are seen. By far the largest peak of antitryptic activity coeluted with the albumin fraction and was detected by measuring antitryptic activity with the standard assay. In the case of gel filtration of human serum also, the alpha$_1$-antitrypsin activity corresponded to the albumin peak.

The important result of the gel filtration studies is that the antitryptic activity of the rat liver perfusate corresponds to that of human serum in gel elution position and hence is similar to it in apparent molecular size.

Taken together, our findings suggest that in the rat, the liver synthesizes material with properties similar to those of human alpha$_1$-antitrypsin at a rate compatible with the liver as the principal site of synthesis. Determining the exact relationship between the serum antitrypsin activity of the rat and the serum alpha$_1$-antitrypsin activity of man will require isolation and structural analysis.

Summary. The isolated perfused rat liver synthesizes antitrypsin activity in a linear fashion as long as 24 hr. The rate of synthesis may be ten times the rate necessary for physiologic levels *in vivo*. These data are compatible with the liver as the principal site of synthesis of serum antitryptic activity in the rat. The antitrypsin activity of the rat perfusate resembles that of normal human plasma in lability to heat (56°) and to acid (below pH 6) and in apparent molecular size (elution from Sephadex G-200).

We thank Maria Luz Sevilla for expert technical assistance and Dr. Robert H. Schwartz for antitrypsin typing of the human serum.

This paper is based on work partially performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, and partially by Grant No. 1-R01-AM110 MET and Contract No. NHLI 71-2221 from the National Institutes of Health, United States Public Health Service. It has been assigned Report No. UR-3490-519.

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Received April 19, 1974. P.S.E.B.M. 1974, Vol.148.