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Liver in α_1-Antitrypsin Deficiency: Morphologic Observations and *in Vitro* Synthesis of α_1-Antitrypsin

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Extract

In an effort to characterize the hepatic abnormality in patients with α_1-antitrypsin deficiency, three unrelated children with the disorder (Pi types ZZ and SZ), two heterozygous parents (Pi type MZ), and three normal subjects (Pi type MM) were studied. As expected, the livers of the ZZ- and SZ-deficient subjects showed abnormal accumulation of α_1-antitrypsin in the cisternae of the rough endoplasmic reticulum as judged by immunofluorescent and electron microscopic studies. Their parents (MZ phenotype) demonstrated identical although less extensive hepatic abnormalities. Short term cultures of liver tissue in the presence of radiolabeled amino acids showed both synthesis and release of α_1-antitrypsin in normal control subjects and in the patients with the Z protein. Radiolabeled intracellular α_1-antitrypsin could not be found. These studies demonstrate synthesis of α_1-antitrypsin by the livers of normal and genetically deficient subjects *in vitro*, and suggest several possible mechanisms for α_1-antitrypsin deficiency.

Speculation

Studies *in vitro* of the synthesis and release of α_1-antitrypsin, as shown in the present investigation, may reveal the molecular basis of this genetic disorder.

α_1-Antitrypsin is a 50,000-dalton glycoprotein that accounts for about 90% of the total trypsin inhibitory capacity of normal human serum. It is also a major extracellular inhibitor of other proteolytic enzymes such as plasmin, elastase, collagenase, chymotrypsin, thrombin, and leukocyte proteases (18).

Extensive genetic polymorphism for human α_1-antitrypsin has been demonstrated (11) and over 20 alleles are known for the protease inhibitor (*Pi*) locus which controls α_1-antitrypsin synthesis. The different alleles are expressed in α_1-antitrypsin bands, which, in general, differ in electrophoretic position from each other and from the common Pi M protein. Some of the Pi types are associated with lower serum concentrations than Pi M. Pi Z occurs at a concentration about 10% and Pi S at about 60% of Pi M. Individuals homozygous for Pi^z thus have 5 15% of the normal α_1-antitrypsin level and heterozygotes for Pi^z and Pi^s have about 30% of the normal concentration (10). An allele with no detectable product (Pi^-) has also been described (25) and the heterozygotes for this gene have serum levels that are accordingly reduced.

Individuals homozygous for Pi^z or Pi^- or heterozygous Pi^zPi^- are particularly prone to develop pulmonary emphysema as young adults (8) and liver disease as children (24). Recently, panlobular emphysema alone (26) or with cirrhosis (13) has been reported in children with severe α_1-antitrypsin deficiency. It has also become clear over the past several years that adults with severe deficiency may have distinctive hepatic changes with or without clinical lung disease (3). An increased incidence of lung (16) and liver disease (4, 5) may occur in persons with intermediate levels of α_1-antitrypsin.

Hepatocytes from patients with severe α_1-antitrypsin deficiency contain abundant α_1-antitrypsin protein (23), and it has been postulated that the low serum level in these subjects is associated with a failure of release of the protein from hepatic ribosomes. Although the liver is thought to be the only site of synthesis of this protein (23), no studies *in vitro* of α_1-antitrypsin synthesis have been reported.

The present investigation was undertaken to examine the synthesis of α_1-antitrypsin and its release from the liver in patients with ZZ and SZ deficiency, the MZ phenotype, and in normal subjects (Pi type MM).

MATERIALS AND METHODS

α_1-ANTITRYPSIN QUANTITATION AND TYPING

The concentration of α_1-antitrypsin in serum was determined both by an automated nephelometric method (22) and by electroimmunoassay (17) using monospecific antiserum to α_1-antitrypsin obtained from Atlantic Antibodies (28). Genetic typing was performed by immunofixation with this antiserum after agarose gel electrophoresis at pH 8.6 (15), as well as crossed immunoelectrophoresis after starch gel electrophoresis at pH 4.95 (12).

LIVER BIOPSY

Light Microscopy. A portion of each liver biopsy was fixed in 10% buffered formalin (pH 7.0), and was dehydrated and embedded in paraffin. Sections were stained with hematoxylin-eosin, periodic acid-Schiff (PAS) reagent (with and without prior treatment with diastase), Hale's iron stain, Gomori's trichome and Mallory's aniline blue stains according to standard histologic techniques (19).

Electron Microscopy. Fresh tissue was immediately fixed in 2.5% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.3, washed with 0.15 M cacodylate buffer, pH 7.3, postfixed with 1.3% osmium tetraoxide, prestained with 1.5% uranyl acetate, dehydrated in graded alcohols and embedded in Epon. Sections were cut with an LKB Ultratome III and examined with a Philips EM 300 electron microscope.

Immunofluorescence Studies. Fresh tissue was quickly frozen in O.C.T. compound (28) and 3 μm thick sections were cut in a cryostat. The sections were washed with phosphate-buffered saline pH 7.4 and incubated with fluoresceinated rabbit antisera to human α_1-antitrypsin (29) at room temperature for 30 min. The sections were then washed with phosphate-buffered saline before being examined with a Leitz fluorescence microscope. The sections were also stained with fluoresceinated rabbit antisera against human IgM, IgG, IgA, C3, fibrin, and albumin (30). Control liver

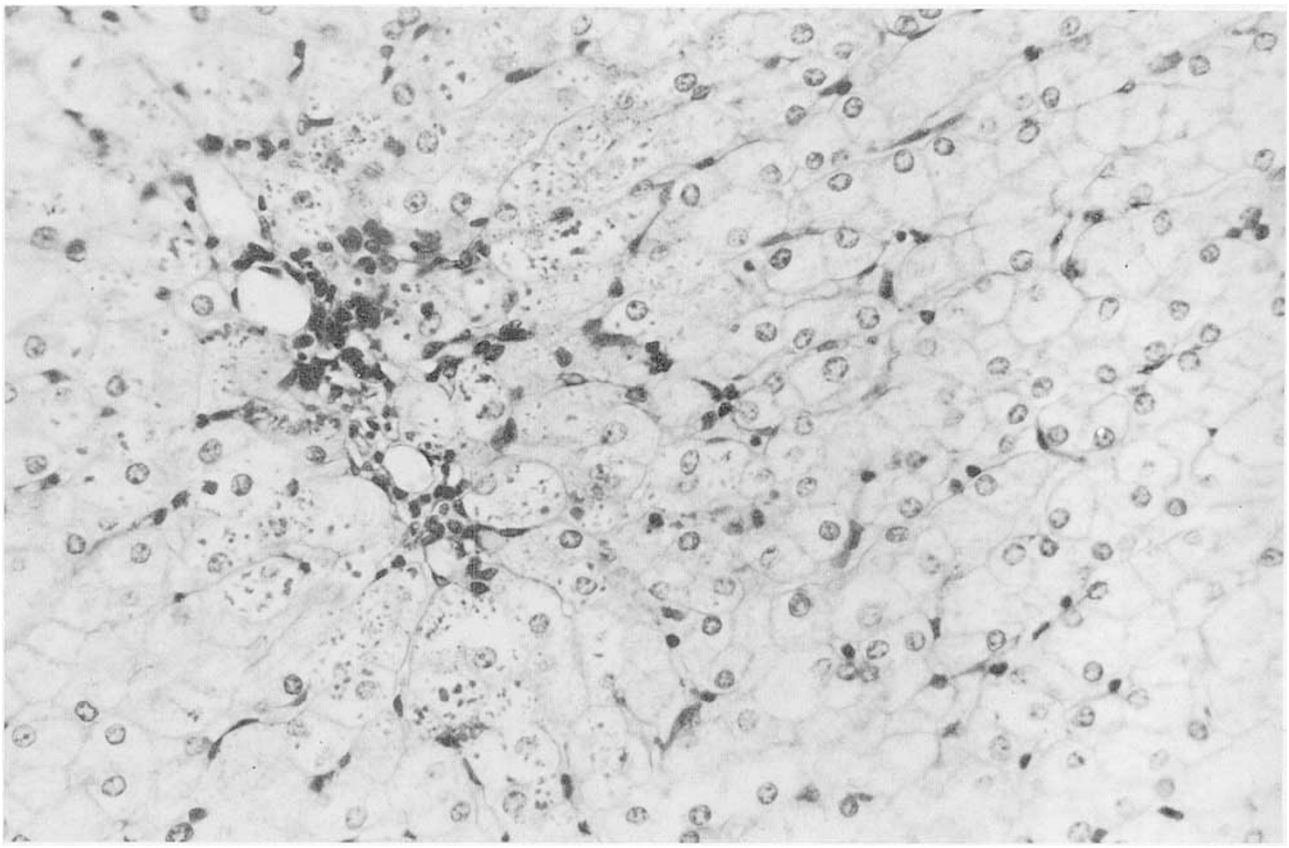

Fig. 1. *Case 2.* Liver biopsy showing periodic acid-Schiff-positive, diastase-resistant cytoplasmic globules predominantly in the periportal hepatocytes. Diastase and periodic acid-Schiff stain; original magnification, × 250.

in either parent even with special stains. Liver cells contained abundant lipofuscin granules. Immunofluorescence studies revealed isolated clusters of small greenish yellow fluorescent globules in the liver cells after staining with fluoresceinated antisera to human α_1-antitrypsin. These could be differentiated from yellow-orange autofluorescence of lipofuscin. Rough endoplasmic reticulum cisternae filled with abundant fibrillar material were seen in several liver cells on electron microscopic examination. However, the number of cells containing this material was far less than in the biopsies of the ZZ patients.

In Vitro Synthesis of α_1-Antitrypsin. Liver fragments from individuals with Pi type M and from subjects with Pi types ZZ and MZ incorporated ^{14}C-labeled amino acids into α_1-antitrypsin *in vitro* (Fig. 4). In each case, the labeled protein was secreted into the tissue culture medium. No newly synthesized protein was detectable intracellularly. The Pi type of the α_1-antitrypsin synthesized and secreted by each culture corresponded to the type found in the subject's serum.

The incorporation of ^{14}C-labeled amino acids was shown to be the result of net synthesis of α_1-antitrypsin inasmuch as cycloheximide (2 μg/ml) blocked the incorporation of labeled precursors, and there was no evidence for nonspecific absorption of labeled amino acids to preformed protein.

DISCUSSION

Evidence is presented that short term cultures of liver tissue synthesize and release α_1-antitrypsin. In each instance, the newly synthesized protein corresponded in Pi type to the serum α_1-antitrypsin of the donor. Although the techniques used did not permit accurate quantitation, they did allow a semiquantitative estimate of newly synthesized α_1-antitrypsin. Synthesis and secretion of Z protein was less than that of M. No intracellular radiolabeled α_1-antitrypsin was detected in the liver cultures of any subject. There are several possible explanations for these findings. (1) Accumulation of α_1-antitrypsin because of a secretory defect present (23) in α_1-antitrypsin deficiency, may have led to feedback inhibition of synthesis. Hence, accumulation of the abnormal protein in liver cells may be a very slow process. Immunofluorescent methods fail to distinguish newly synthesized from preformed protein. (2) A primary structural gene defect might result in both a decreased rate of synthesis and abnormal accumulation of gene product. In this regard α_1-antitrypsin, type Z, has been shown to be deficient in sialic acid, both in the serum (2, 7) and in the liver (9, 20). (3) Since no newly synthesized intracellular α_1-antitrypsin was detected even in normal liver, it is probable that the newly synthesized normal protein is rapidly secreted, as has been demonstrated for the synthesis and secretion of albumin (21) and complement (6). (4) A less likely possibility is that the Z protein may be heterogeneous, some accumulating and some being secreted. (5) Technical limitations may have prevented detection of newly synthesized intracellular protein.

Previous studies *in vivo* have demonstrated α_1-antitrypsin in hepatocytes by immunofluorescence (23). Such studies do not by themselves prove that synthesis occurs in the liver. However, the finding that the serum level of α_1-antitrypsin rose to normal after liver transplantation in a ZZ-deficient subject (23) suggests strongly that the liver is the primary site of synthesis for this protein. Furthermore, the present studies provide new direct evidence for the hepatic synthesis of α_1-antitrypsin.

The presence of PAS-positive, diastase-resistant, intracytoplasmic globules in the periportal hepatocytes of patients with liver disease due to α_1-antitrypsin deficiency is now well established (23). Electron microscopic studies have revealed dilated cisternae in the rough endoplasmic reticulum containing abundant fibrillar material. Liver from patients without $\alpha_1$antitrypsin deficiency with or without cirrhosis generally do not contain such globules. Recently it has been shown that individuals with α_1-antitrypsin deficiency and pulmonary disease without evidence of liver disease (14), as well as heterozygous deficient individuals without clinical

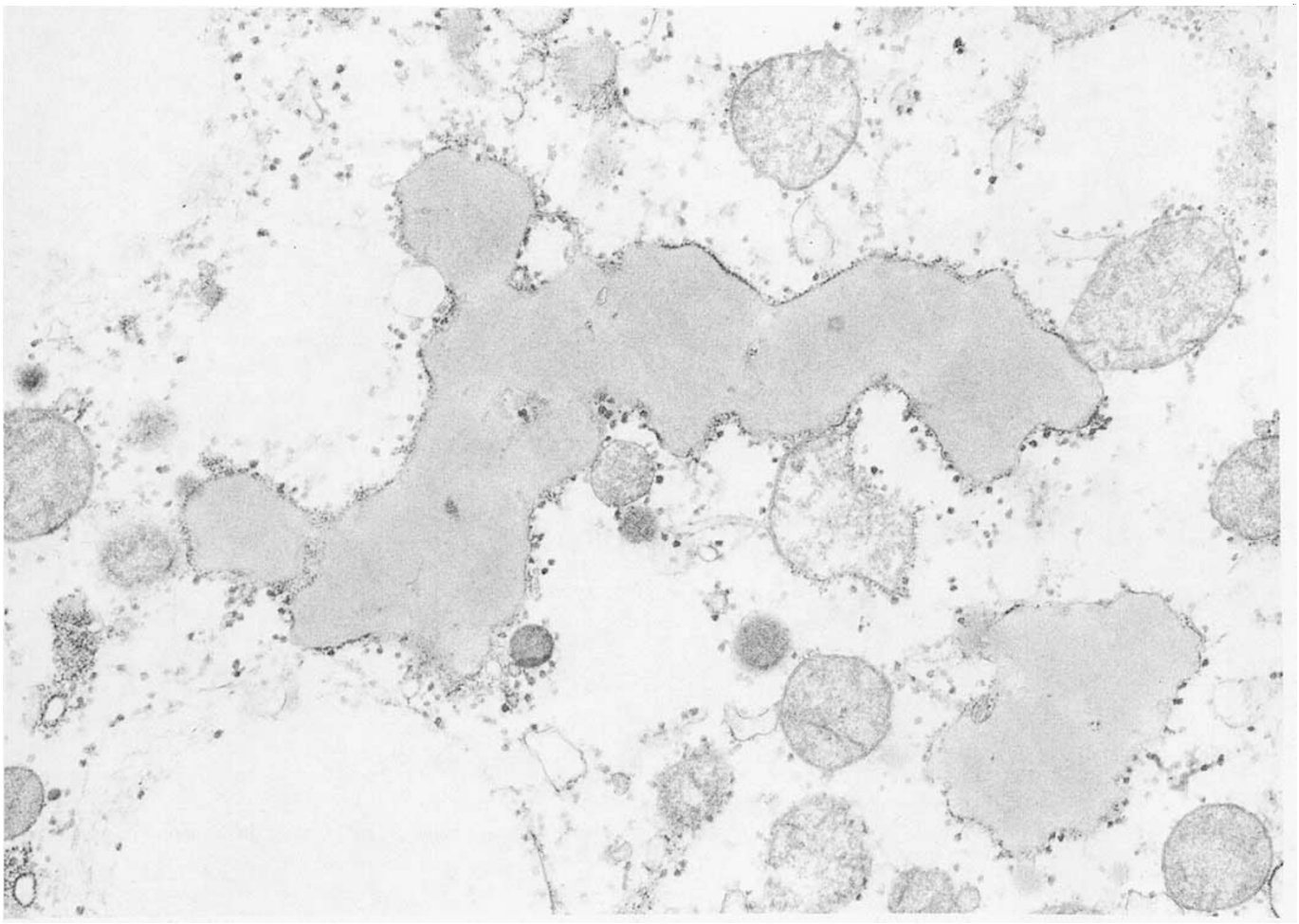

Fig. 2. *Case 2.* A liver cell showing dilated cisternae of endoplasmic reticulum filled with fibrillar material. Electron micrograph stained with uranyl acetate; original magnification, × 28,000.

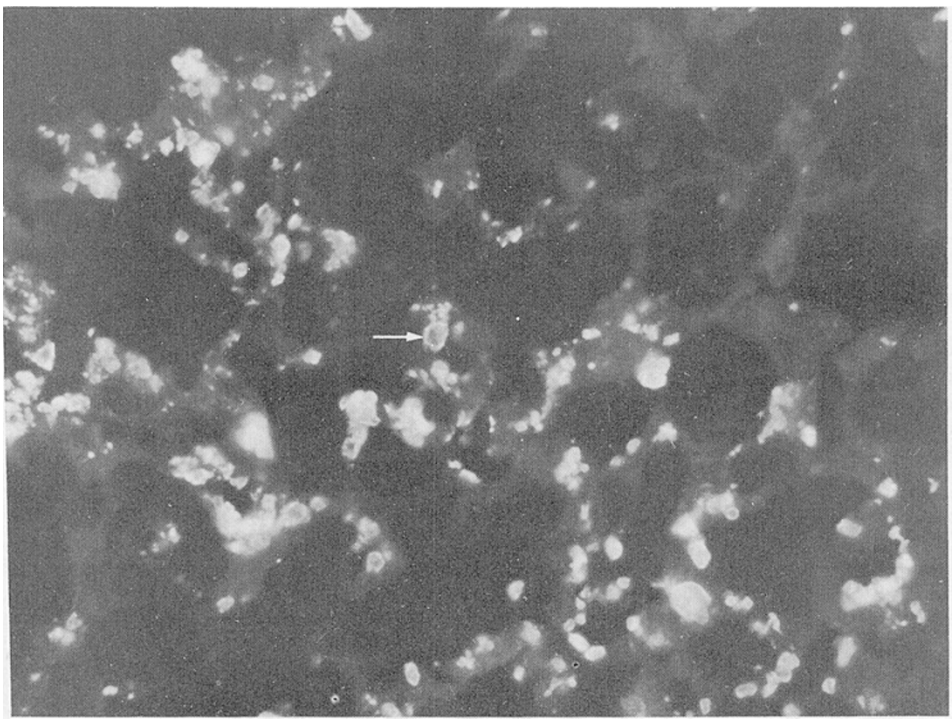

Fig. 3. *Case 2.* Liver biopsy stained with fluorescein-labeled antisera to α_1-antitrypsin. Fluorescent globules are seen in the cytoplasm of hepatocytes. Larger globules (*arrow*) show intense fluorescence at the periphery of the globules. Original magnification, × 400.

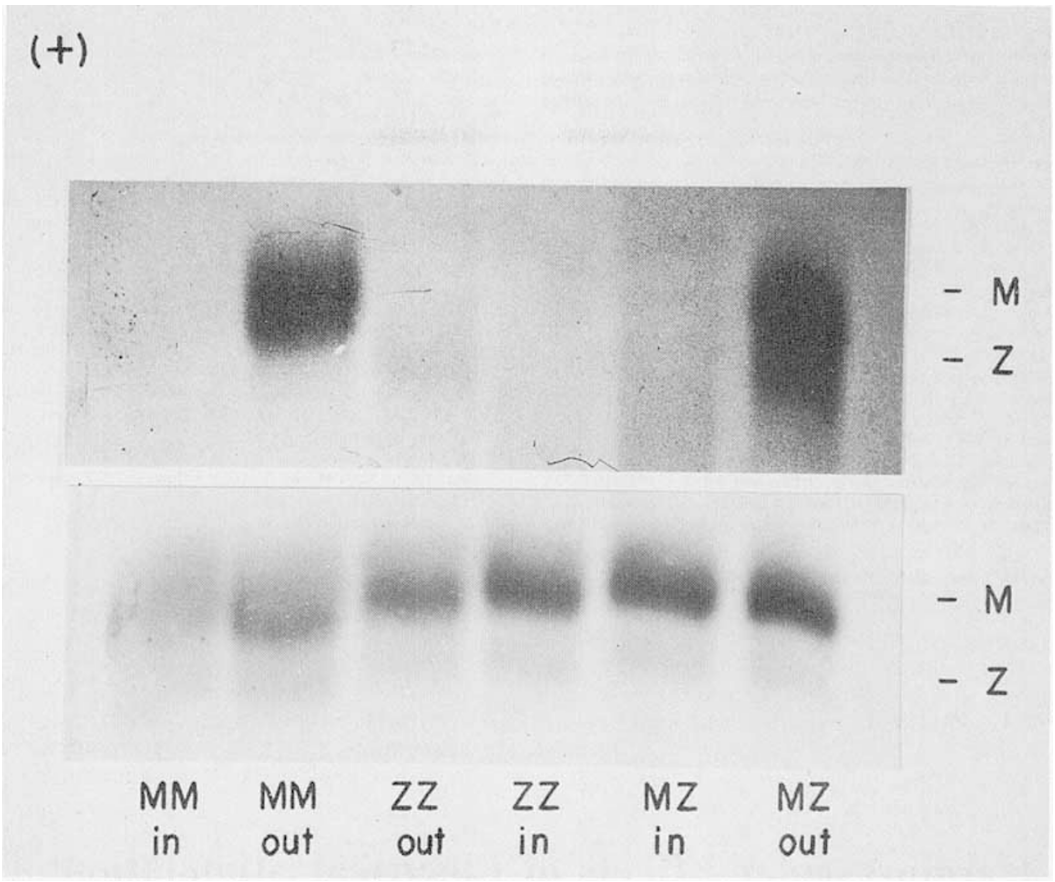

Fig. 4. Agarose electrophoresis of tissue culture media and liver cell extracts in the presence of carrier serum of Pi type MZ. Short term liver cell cultures were studied as described under *Materials and Methods*. Radioautograph (*top panel*) and corresponding plate stained for protein (*lower panel*). Dialyzed concentrated tissue culture media of liver fragments from patients with Pi types MM, MZ, and ZZ are identified as "out." Intracellular contents from the same cultures are shown as "in."

symptoms (4) may show similar intracellular globules in the hepatocytes.

The ZZ and SZ patients in the present study had increased accumulation of α_1-antitrypsin in the hepatocytes by light, electron, and/or fluorescence microscopy. However, in the MZ subjects, the globules were not seen in the liver by light microscopy; they were demonstrable by immunofluorescence technique. Electron microscopic examination confirmed the presence of rough endoplasmic cisternae filled with fibrillar material, but both this examination and the immunofluorescence studies showed considerably less intracellular α_1-antitrypsin than in the ZZ-deficient liver.

Diastase-resistant, PAS-positive bodies are not specific for α_1-antitrypsin deficiency, but may be seen in a variety of other liver diseases. We have identified such material in the liver of patients with cystic fibrosis, biliary atresia and neonatal hepatitis with giant cell transformation (without deficiency of α_1-antitrypsin in the serum). In such patients, however, the globules do not show staining with fluoresceinated antibody to α_1-antitrypsin, and they do not appear to be lipofuscin or bilirubin pigment. Considerable further work is needed to characterize the globules found in these hepatic disorders, and to confirm the biochemical defect which produces the trapping of α_1-antitrypsin in liver cells of patients with α_1-antitrypsin deficiency.

SUMMARY

These studies demonstrate synthesis and release of α_1-antitrypsin *in vitro* in short term liver cell cultures. Synthesis and secretion of both Z and M proteins have been detected; however, no intracellular trapping of newly synthesized protein was found.

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Arterial blood pressure
heart rate
hypothalamus
sheep
sympathetic nervous system
vagus nerve

Cardiovascular Effects of Electrical Stimulation of the Forebrain in the Fetal Lamb

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Extract

Modified stereotaxic techniques were applied to fetal lambs during the latter third of gestation. Electrical stimulation in the region of the hypothalamus in 10 acute experiments was associated with three patterns of arterial blood pressure and heart rate changes: a pressor-tachycardia response; a pure tachycardia response (abolished by propranolol); and a pure bradycardia response (abolished by atropine). The pressor-tachycardia response was examined in detail in 13 chronic preparations (115–135 days of gestation at operation). The systolic arterial blood pressure increase was never greater than 35 mm Hg and was probably blunted by the large noninnervated placental circulation. This pressure increase was abolished by phentolamine and was thus mediated by stimulation of α-adrenergic receptors. The initial tachycardia was prevented by propranolol and was due to β-adrenergic stimulation. The tachycardia was followed in a few seconds by a bradycardia, abolished by atropine and possibly a vagal baroreflex. The pressor-tachycardia response was accentuated in two lambs who were delivered spontaneously and were studied after birth. These studies indicate that a suprabulbar neural framework exists in the fetal lamb for influencing the cardiovascular system from as early as 90 days of gestation.

Speculation

Since electrical stimulation in the region of the hypothalamus is associated with various changes in blood pressure and heart rate, it is possible that the upper brain stem in the late gestation fetus has a role in the central nervous regulation of the circulation. Complex cardiovascular patterns may be organized in this region in response to fetal stress.

Although most experiments on central nervous regulation of the circulation have been performed in mature animals, there is evidence that the fetal and neonatal cardiovascular systems are also under central autonomic control. Resting sympathetic and parasympathetic influences on the heart and blood vessels of the fetal lamb are present by 0.55 gestation (20). Baroreflex (4, 17) and chemoreflex (7) responses can be elicited after 0.60 gestation and the nervous pathways almost certainly involve the lower brainstem. Gootman *et al.* (11) observed blood pressure and heart rate changes with electrical stimulation of the brainstem in anesthetized newborn piglets.

The developmental pattern of central nervous influences on the fetal circulation has not been studied although the above examples