α_1-Antitrypsin Deficiency: A Variant with No Detectable α_1-Antitrypsin

Abstract. *No α_1-antitrypsin could be detected in the serum of a 24-year-old man with advanced pulmonary emphysema by agarose electrophoresis, immunoelectrophoresis, double diffusion in agarose gel, or α_1-antitrypsin genetic typing by a combination of starch-gel electrophoresis and crossed antigen-antibody electrophoresis. A circulating α_1-antitrypsin inactivator could not be demonstrated. Evidence was obtained in family members of genetic transmission of this new α_1-antitrypsin variant.*

Multiple types of α_1-antitrypsin, the major protease inhibitor (Pi) in human serum, are inherited by a series of autosomal codominant alleles that determine the concentration and electrophoretic mobility of this protein (*1*). The protein products (Pi types) of the more than 16 different known alleles are demonstrable by a combination of acid starch-gel electrophoresis and crossed antigen-antibody electrophoresis. Serum can be assigned a Pi type by this procedure. Serum from individuals with severe α_1-antitrypsin deficiency usually contains a very slow-moving α_1-antitrypsin (Pi type ZZ) in an amount 10 to 40 percent of that in the commonest serum type (Pi type MM) found in normals (*2*). This severe deficiency state is highly associated with either an early-onset form of familial emphysema (*3*), familial infantile cirrhosis (*4*), or, rarely, a combination of liver and lung disease in childhood (*5*). Early-onset pulmonary disease has been associated in a few instances with Pi types SS and SZ, which are characterized by diminished serum α_1-antitrypsin levels (*6*).

A 24-year-old man with advanced emphysema and normal liver function has been found to have no detectable serum α_1-antitrypsin. There is evidence of inheritance of this new Pi type variant in four generations of his family. No other living family member has emphysema or liver disease, no children died in infancy, and none of the deceased members of the family died of lung or liver disease.

Serum samples from this patient and 11 of his family members in four generations were obtained on two different occasions and studied for their total antitryptic activity by the method of Erlanger *et al.* (*7*). α_1-Antitrypsin concentration was measured by Laurell's electroimmunoassay method (*8*), and Pi typing was performed by the methods of Fagerhol (*9*). Immunoelectrophoresis was performed on the patient's serum and known Pi MM and Pi ZZ serums (*10*) by the method of Scheidegger (*11*), and double diffusion in agarose was by the method of Ouchterlony (*12*). Agarose electrophoresis was performed by the method of Laurell (*8*).

It has not been possible to detect any α_1-antitrypsin by any method in the patient's serum: No discrete α_1-globulin band was present on agarose electrophoresis. Total antitryptic activity, more than 90 percent of which is normally accounted for by the α_1-antitrypsin (*2*), was less than 10 percent of the mean for healthy Pi MM individuals. On immunoelectrophoresis of the patient's serum, no precipitin band was visible with monospecific antiserum against human α_1-antitrypsin (Fig. 1), whereas serum from known Pi MM and Pi ZZ individuals gave discrete bands of differing intensity and mobility. Antiserums from three different suppliers (Behring Diagnostics, Miles Laboratories, and Kallestad Laboratories), as well as antiserums prepared in rabbits and monkeys in our laboratories, gave identical results. No α_1-antitrypsin could be detected in the patient's serum by double diffusion in agarose.

By electroimmunoassay, no α_1-antitrypsin was detected in the patient's serum, undiluted or in dilutions of 1:40 or 1:80. Since this method is capable of detecting as little at 0.7 μg of α_1-antitrypsin per milliliter in dilute serum or other biological fluids, any undetected α_1-antitrypsin in this patient's serum would have to be less than 1/700 of its usual concentration in Pi ZZ serum (0.5 mg/ml) or less than 1/3000 of its usual concentration in Pi MM serum (2.3 mg/ml) (*10*). Typing of the patient's serum by a combination of acid starch-gel electrophoresis and crossed electrophoresis failed to demonstrate any α_1-antitrypsin bands.

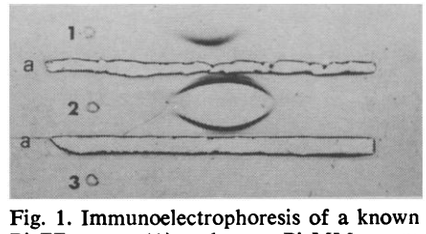

Fig. 1. Immunoelectrophoresis of a known Pi ZZ serum (1), a known Pi MM serum (2), and serum from the proband in this study (3), developed with antiserum to α_1-antitrypsin (a).

Study of serums from 11 members of the patient's family in four generations for α_1-antitrypsin function, concentration, and Pi types revealed that both of his parents, both of his children, two of his three sisters, and his maternal grandmother had α_1-antitrypsin qualitatively similar to that in Pi MM serum, but had intermediate levels of α_1-antitrypsin function and concentration, below the lowest levels usually found in healthy Pi MM individuals (*10*). The other four relatives were Pi MM, with α_1-antitrypsin in the usual Pi MM range (1.4 to 3.0 mg/ml). A study of blood types in the ABO, Rh, MN, and Kell systems verified the assumed relationships in this family (*13*).

Therefore, within the limits of sensitivity of the methods used, the proband can be assumed to have an inherited lack of α_1-antitrypsin (Pi type — —), perhaps secondary to a deletion of both of the alleles that ordinarily determine the production of the α_1-antitrypsin, Both of his parents, both of his children, two of his siblings, and his maternal grandmother apparently possess a single Pi^M allele and a deletion (Pi type M—).

The possibility that the absence of α_1-antitrypsin in the patient's serum could result from an increased catabolic rate was excluded by the observation (*14*) that the α_1-antitrypsin, supplied to this individual in fresh normal plasma, disappeared from his serum with a half-life of 6 days; two individuals with Pi ZZ α_1-antitrypsin deficiency had similar rates of elimination (5 and 6 days). This half-life was similar to that observed by Kueppers and Fallat after injection of ^{125}I-labeled α_1-antitrypsin (*15*). To exclude the possibility of a circulating "autoantibody" to α_1-antitrypsin or an excess of an enzyme that might complex with α_1-antitrypsin, thus preventing detection of the protein immunologically or functionally, the patient's serum was incubated with an equal amount of known Pi MM serum for

18 hours at 37°C. The resulting mixture had α_1-antitrypsin concentration and function of approximately half the original levels in the Pi MM sample; the Pi type of the mixture was MM.

The other major protein fractions in the patient's serum were normal, as determined by agarose electrophoresis; immunoglobulins G, A, and M were found to be normal on immunoelectrophoresis.

While the Pi ZZ variant of α_1-antitrypsin deficiency is relatively common, with an incidence of approximately 1 out of 1400 individuals in one large study (*16*), the Pi$^-$ variant described here has not been previously encountered; other investigators have proposed its existence, on the basis of finding Pi MM individuals with unusually low levels of α_1-antitrypsin.

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Nonhistone Chromosomal Protein Synthesis: Utilization of Preexisting and Newly Transcribed Messenger RNA's

Abstract. *Treatment of HeLa S_3 cells with actinomycin D during mitosis suppresses the synthesis of several major classes of nonhistone chromosomal proteins during the subsequent period before DNA replication, but allows the synthesis of other species of these proteins. Such results are consistent with the utilization of preexisting, as well as newly transcribed, messenger RNA's for nonhistone chromosomal protein synthesis during the prereplicative phase of the cell cycle.*

Evidence is rapidly accumulating which suggests that nonhistone chromosomal proteins are responsible for the regulation of gene expression in eukaryotic cells (*1*) and, specifically, in the control of transcription during the cell cycle (*2*). A regulatory function for nonhistone chromosomal proteins in continuously dividing cells and in quiescent cells that are stimulated to proliferate is supported by significant variations in the rates of synthesis (*3–6*), turnover (*7*), and phosphorylation (*8*) of these proteins during defined periods of the cell cycle. The synthesis and phosphorylation of specific molecular-weight classes of nonhistone chromosal proteins has also been observed during defined periods of the cell cycle (*3–6, 8*). Furthermore, unlike the histones, whose synthesis is restricted to S phase (the period of DNA synthesis in the cell cycle) and is tightly coupled to DNA replication (*9*), synthesis of nonhistone chromosomal proteins is independent of concomitant DNA synthesis (*5, 6*). More direct evidence that nonhistone chromosomal proteins are involved in the regulation of DNA-dependent RNA synthesis during the cell cycle comes from studies showing that chromatin reconstituted with cell cycle stage–specific nonhistone chromosomal proteins exhibits a template activity characteristic of the native chromatin (*2*), and recent results suggest that nonhistone chromosomal proteins regulate transcription during the cell cycle by mediating the binding of histones to DNA (*10*). The aim of the present study was to determine whether the nonhistone chromosomal protein synthesis that occurs during the prereplicative phase of the cell cycle in continuously dividing HeLa S_3 cells requires the synthesis of new messenger RNA, or whether preexisting messenger RNA templates are utilized.

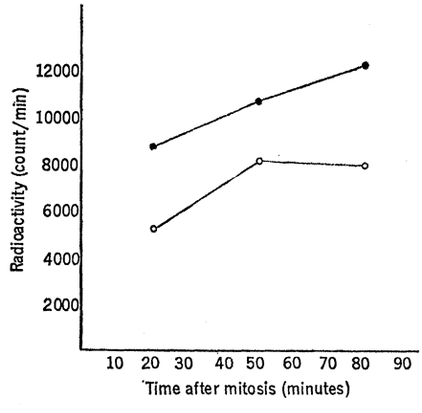

Fig. 1. Incorporation of L-[^{3}H]tryptophan into chromatin 20, 50, and 80 minutes after selective detachment of mitotic cells. The cells were pulse-labeled for 30 minutes, and chromatin was isolated as described in the text. The data are expressed as counts per minute per 50 μg of DNA. Control, solid circles; actinomycin D–treated, open circles.

Mitotic HeLa S_3 cells were detached selectively from semiconfluent monolayers, and actinomycin D (*11*) was added immediately to a final concentration of 2 μg/ml. Controls were not treated with the antimetabolite. Procedures for growth and synchronization of the cells have been described (*5*); in the present experiments, 97 percent of the detached cells were in mitosis, as determined by phase-contrast microscopy. At 20, 50, and 80 minutes following mitosis, as the cells progressed through early G_1 (the phase of the cell cycle preceding DNA replication), 5×10^7 control cells and cells treated with actinomycin D were pulse-labeled for 30 minutes at a concentration of 5×10^6 cells per milliliter in tryptophan-free Eagle's minimal essential spinner medium containing 2 percent fetal calf serum and L-[^{3}H]tryptophan (10 μc/ml) (2.18 c/mmole, New England Nuclear). The actinomycin D–treated cells were labeled in the presence of the inhibitor (2 μg/ml). Cells were harvested by centrifugation at 600*g* for 3 minutes and washed four times in 80 volumes of Earle's balanced salt solution at 4°C. Nuclei and chromatin were then prepared as described (*5*), the entire procedure at 4°C. The cells were lysed in a solution contain-