Summary. Serum specimens of three unrelated black males had an unusual alpha-1-antitrypsin phenotype, designated Pi Ecincinnati because of its electrophoretic mobility. Family studies indicated that the new phenotype was the expression of an alpha-1-antitrypsin allele, labeled $\text{Pi}_{\text{Ecincinnati}}$.

Introduction

Deficiency of alpha-1-antitrypsin (aAT) involves an increased risk of the development of early-onset pulmonary emphysema (Eriksson, 1965). In the affected individual the deficiency is associated with Pi type Z (phenotype ZZ). The question as to whether patients with other respiratory diseases have a different frequency of usual aAT phenotypes from that observed in a control population has repeatedly been asked (Fagerhol and Hauge, 1969; Schwartz et al., 1977). While pursuing this question, we found a previously unrecorded aAT phenotype in three unrelated black patients [two of whom have been mentioned in an abstract (Hug et al., 1978)] and in members of their respective families.

Patients and Methods

The first patient (Family B) was a 2½-year-old black boy who had been admitted to hospital seven times with asthma and pneumonia. The second patient (Family L) was a 2-year-old black boy with four hospital admissions for asthma and pneumonia. The third patient (Family J) was a 53-year-old black man who had pancreatitis.

After identification of the unusual phenotype in the initial three serum specimens, blood was drawn from the antecubital vein of the patients and their relatives. Transaminases, bilirubin, and alkaline phosphatase were determined in the serum, which was stored at $-20^\circ\text{C}$ after the addition of sodium azide (0.02%) for prevention of bacterial growth.

aAT concentration was determined by radial immunodiffusion and expressed as mg aAT per ml serum (Manecini et al., 1965). Trypsin-inhibitory capacity (TIC) was assayed according to Eriksson (1965) and expressed as mg trypsin inhibited per ml serum.

Pi Type was determined in three ways: The first method was horizontal discontinuous starch gel electrophoresis at pH 4.95. A more acidic gel was also prepared by adding 1.5-2.0 ml gel buffer A (citric acid), which slowed protein migration and enhanced differentiation of faster-moving bands (Fagerhol, 1968). The second method of Pi typing used was agarose gel electrophoresis at pH 8.6 according to Axelsson and Laurell (1965), the LKB plastic mask being used instead of a slot former for sample application. The third method was polyacrylamide gel-isoelectric focusing electrophoresis (PAG-IEF) according to Allen et al. (1974), modified so that the gel had a final concentration of 12% sucrose, 1.1% Ampholyte pH 3.5-5.0 (LKB), 1.1% Ampholyte pH 4-6 (Brinkmann), and 0.1% Ampholyte pH 2-4 (Brinkmann). The gels were made in advance in a Brinkmann 1-mm-thick polymerization chamber. They were covered with Parafilm and stored for up to 2 weeks in a moisture chamber at 4°C. The anode buffer was 1 M phosphoric acid. The cathode buffer was 0.1 M glycine. Gels were prefocused on the Brinkmann Desage double-chamber IEF apparatus at 12°C and 10 mA per gel by means of the LKB constant power supply 2103 for 30-60 min. Sample pads were then applied at the cathode end of the gel. The power limit was set at 5 W per gel. After 45 min the pads were removed. The power setting was increased to 20 W and/or 1500 V per gel. IEF was completed in 3-4 h. The longer the gels were allowed to focus, the greater was the separation of the bands. The gels were fixed for 10 min in 12% trichloroacetic acid, 5% sulfosalicylic acid in 30:65:5 for ethanol: water: acetic acid, washed three times in distilled water, and stained with 0.2% Coomassie brilliant blue R-250 in 45:45:10 of ethanol: water: acetic acid for 10 min at 60°C in the Dubonoff shaking water.
bath. After destaining of the gels for 30–60 min with 25:65:10 of ethanol:water:acetic acid, most Pi types could be distinguished. In the event of an unusual Pi type, the serum was electrophoresed again after the addition of dithiothreitol (30 mM). The gels were further destained overnight and preserved between two layers of DuPont nonmoistureproof cellophane that had been soaked in destaining solution and glycerin (13%).

Crossed immunoelectrophoresis (Fagerhol and Laurell, 1967) was used after PAG-IEF and starch gel electrophoresis; and immunofixation (Arnaud et al., 1977) after PAG-IEF to verify the α1AT bands.

**Results**

Transaminases, bilirubin, and alkaline phosphatase were normal in all specimens of serum. In the serum of individuals with Pi Ecincinnati, the mean α1AT concentration was 2.62 mg/ml ± 0.5 SD and the mean TIC was 0.82 mg/ml ± 0.2; these values were normal, the respective control values being 2.74 ± 0.5 and 0.77 ± 0.2.

In Fig. 1, the PAG-IEF pattern of the new Pi type is compared with that of Pi EM2, FM, and the recently discovered Etokyo M (Miyake et al., 1979). Etokyo and the new Pi type were not separable. The new Pi type migrated faster than F but more slowly than E. Thus, E was the next phenotype on the anodal side of the new Pi Type.

Fig. 2 indicates that on acid starch gel electrophoresis the new Pi type migrated more slowly than E, F, or Etokyo. F was the next phenotype on the anodal side of the new Pi type.

On agarose electrophoresis at pH 8.6, the new Pi type moved as a single band, like M.

Immunofixation (Fig. 3) and cross electrophoresis into antibody-containing agarose after PAG-IEF established the α1AT nature of the protein bands.

The new Pi type appeared unchanged after treatment of the serum with dithiothreitol or in different blood specimens obtained from the same individual. Serum of the new Pi type was submitted to three outside laboratories, in which the electrophoretic findings were confirmed.

In the three families we found nine males and two females with Pi Ecincinnati (Fig. 4).

**Discussion**

Pi E migrated next to the anodal side of the new Pi type on PAG-IEF; therefore, following the rules of nomenclature established by the International Pi Committee (Cox, 1978) the new Pi type is designated Pi Ecincinnati. The committee decided that as of July 1979, new Pi types are to be classified according to how they migrate on PAG-IEF and not on acid starch gel electrophor-