PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 495–500 (1983)

Plasma Protease Inhibitors in Premature Infants: Influence of Gestational Age, Postnatal Age, and Health Status[1] (41676)

MAUREEN ANDREW, PATRICIA M. MASSICOTTE-NOLAN,* AND MARGARET KARPATKIN†

Department of Pediatrics, McMaster University Medical Center, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada, and †Department of Pediatrics, New York University Medical School, 550 First Avenue, New York, New York 10016

Abstract. In newborn infants, the influence of gestational age (GA), postnatal age (PA), and health status on the plasma protease inhibitors α_2-macroglobulin (α_2-M), α_1-antitrypsin (α_1-AT), C_1 esterase inhibitor (C_1E-INH), α_2-antiplasmin (α_2-AP), and antithrombin III (AT-III) was investigated. Inhibitor levels were measured by radial-immunodiffusion and expressed as a percentage of pooled plasma from adults (mean ± SEM). In total, 54 premature infants (28–36 weeks gestation) were classified at birth as healthy (N = 22) (IV fluids, antibiotics only) or sick (N = 32) (all other support, but excluding infants with disseminated intravascular coagulation (DIC)) and studied on Days 1 and/or 7 of life. Healthy term infants (N = 18) and infants with DIC (N = 10) were studied on Day 1 only. All inhibitors except C_1E-INH increased with increasing gestational age ($P < 0.01$). In healthy premature infants all inhibitor levels reached the normal adult range by 1 week of age. In contrast, at 1 week of age, sick infants had lower levels of α_2-M and α_2-AP, and higher levels of α_1-AT compared to healthy infants ($P < 0.01$). The presence of DIC depressed all of the inhibitors on Day 1 except α_1-AT when compared to healthy controls ($P < 0.01$). Thus, gestational age, postnatal age, and health status all significantly influenced the levels of these plasma protease inhibitors.

There is considerable evidence to suggest that the coagulation cascade (1), the fibrinolytic system (2), and the kallikrein–kinin system (3–5) are activated in the newborn infant, both during the birth process and in the presence of specific pathophysiologic insults. Since the activation of these proteolytic cascades may lead to several biological effects including thrombosis, hemorrhage, and enhanced vascular permeability, the inhibition of the biologically active products is critical in modulating these potentially deleterious processes. The inhibitors which are important in the regulation of these cascades are: α_2-macroglobulin (α_2-M), α_1-antitrypsin (α_1-AT), C_1 esterase inhibitor (C_1E-INH), α_2-antiplasmin (α_2-AP), and antithrombin III (AT-III) (6). To our knowledge, no information is available for C_1E-INH and α_2-AP in the premature infant. A wide range of values has been published for the other inhibitors in newborn infants; for example levels of AT-III in term infants have been reported to be as low as 35% (7) and as high as 87% (8) of adult levels. This study was designed to determine the effect of gestational age, postnatal age, and health status on these five plasma protease inhibitors.

Methods. Premature infants admitted to Bellevue Hospital between July 1979 and January 1980 were eligible for the study and were included if informed consent was obtained from their parents. Throughout the study, the same investigator determined each infant's gestational age according to the Dubowitz scoring method (9) and classified each infant on Days 1 (the first day of life) and 7 as healthy, sick, or recovered. Healthy infants received normal neonatal care; some required intravenous fluids. Sick infants required a variety of treatments including supplemental oxygen, medications (e.g., antibiotics, theophylline, or digoxin), and respiratory support. "Recovered infants" were sick on Day 1, but became healthy during the first 7 days and were therefore considered separately on Day 7. No infant received fresh-frozen plasma, and all received 1 mg of vitamin K intramuscularly upon delivery. Platelet counts and hematocrits were recorded on Day 1 and fibrinogen concentration (10) on both Days 1 and 7. Infants with platelet counts below 100,000 were examined

[1] Supported by Ontario Ministry of Health Grant No. 215-6050.

0037-9727/83 $1.50

for disseminated intravascular coagulation (DIC) and this was diagnosed if they had factor VIII:C levels below 40%, fibrinogen levels below 1.2 g/liter (10), or fibrin-related antigen levels above 6.7 μg/ml (11).

At McMaster University Medical Centre, cord blood samples were obtained from 18 healthy full term infants (37–42 weeks of gestation) between October and December 1981. In addition, 5 full term and 5 premature infants with DIC were studied on Day 1 of life. DIC was diagnosed according to the criteria listed above.

Blood samples (1–2 ml) were obtained from newly placed nonheparinized umbilical lines (when possible) or from antecubital veins, and placed in polypropylene tubes containing 0.13 *M* sodium citrate and 0.1 *M* epsilon-aminocaproic acid (EACA) (one part citrate–EACA to nine parts blood). Cord blood was drawn immediately at birth from the umbilical vein and treated in an identical manner. Platelet-poor plasma was stored in polypropylene tubes at −70°C. Assays on all samples were performed in a blind manner by one technician at McMaster University Health Sciences Centre. The immunologic concentrations of AT-III, α_2-M, α_1-AT, α_2-AP, and C_1E-INH were determined by radial immunodiffusion as previously described (12) using commercially available antibodies (Atlantic Antibodies, Maynard Scientific, Mississauga, Ontario). Not all infants were studied on each day due to death, discharge from hospital, insufficient sample size, or occasional inability to obtain a sample.

The coefficient of variation for each inhibitor (one sample of 10 occasions) was 7% or less. Results were expressed as means ± SEM. Differences between means were analyzed by Student's nonpaired, two-tailed *t* test. The relationships between gestational age and inhibitor level were determined by linear regression analysis. *P* values below 0.05 were considered to be significant.

Results. The study population consisted of 18 fullterm healthy infants, 54 healthy and sick premature infants who did not have DIC, 5 fullterm, and 5 premature infants with DIC (Table I). Birth weight, gestational age, and Apgar score did not differ significantly between the healthy and sick premature infants (Table I), nor did the hematocrit and fibrinogen concentration (data not shown). Data on the inhibitor levels is shown in Table II. When compared to adult values, term infants had depressed levels for C_1E-INH and AT-III only ($P < 0.001$), while the healthy premature infants had depressed levels for C_1E-INH, AT-III, and α_2-AP ($P < 0.001$).

On the first day of life, there was a consistent trend for sick premature infants to have lower levels than healthy infants but the differences were not significant. However, when DIC was present, in either term or premature infants, both α_2-M, and α_2-AP were significantly depressed compared to the healthy age-matched controls ($P < 0.01$). AT-III was also significantly decreased in term infants with DIC ($P < 0.01$) and C_1E-INH in premature infants with DIC ($P < 0.05$) compared to healthy controls.

On Day 7 of life, the premature infants were divided into three groups: healthy, sick (on both Days 1 and 7), or recovered (sick on Day 1 but healthy on Day 7). α_1-AT behaved as an acute phase reactant rising significantly ($P < 0.001$) in recovered and sick infants compared to healthy infants. α_2-AP was depressed in recovered and sick infants compared to healthy infants ($P < 0.001$), while α_2-M was decreased only in sick infants. C_1E-INH was not changed in either group. AT-III tended to be lower in sick infants as well but this did not reach statistical significance.

Because there was no significant difference between healthy and sick premature infants on Day 1 of life, they were grouped to determine the effect of gestational age. All inhibitors except C_1E-INH demonstrated increasing levels with increasing gestational age (Fig. 1) (AT-III: $Y = 2.2x - 32$, $r = 0.59$, $P < 0.001$; α_2-AP: $Y = 2.4x - 5.8$, $r = 0.48$, $P < 0.01$; C_1E-INH: $Y = 0.6x + 42$, $r = 0.18$, $P = 0.5$; α_2-M: $Y = 4.6x - 70$, $r = 0.66$, $P < 0.001$; α_1AT: $Y = 2.8x - 3.3$, $r = 0.46$, $P < 0.01$). Postnatally healthy premature infants had mean values for all inhibitors in the normal adult range (80–130%) on Day 7, while sick infants did not achieve this level for AT-III and α_2-AP (Table II).

Discussion. This study demonstrates the effect of health status, gestational age, and postnatal age on five plasma protease inhibitors in newborn infants. C_1E-INH and AT-III are significantly lower in term infants than in

TABLE I. CHARACTERISTICS OF INFANT POPULATION ON DAY 1 OF LIFE

	N	Weight (kg)	Gestational age (weeks)	Agar score 1 min	Agar score 5 min
Term					
Healthy	18	3.5 ± 0.1	40.0 ± 0.3	8.1 ± 0.2	9.7 ± 0.1
DIC	5	3.5 ± 1.6	41.2 ± 1.2	3.8 ± 1.5	3.5 ± 1.0
Premature[a]					
Healthy	22	1.9 ± 0.1	33.9 ± 0.5	6.6 ± 0.5	8.1 ± 0.3
Sick[b]	32	1.5 ± 0.1	31.6 ± 0.6	6.0 ± 0.4	7.6 ± 0.3
DIC	5	1.0 ± 0.1	26.8 ± 1.5	2.2 ± 1.5	4.3 ± 1.0

Note. Values are given as mean ± SEM.

[a] Fifty-four premature infants were classified as healthy or sick on Day 1 of life. Eleven of the sick infants recovered by Day 7. Five infants with DIC were considered separately.

[b] Twenty-four of the 32 sick infants had the respiratory distress syndrome (respiratory distress, hypoxemia, and characteristic X-ray findings; 18 were dependent on respirators, 5 had an associated pneumothorax), and 10 had a patent ductus arteriosus requiring medical or surgical intervention.

adults, and α_2-AP is decreased in premature infants as well ($P < 0.001$). In general, levels of these inhibitors rise with increasing gestational and postnatal age. The presence of DIC results in depressed levels of all inhibitors except α_1-AT in term or premature infants on the first day of life. When DIC is not present, the inhibitor levels of sick premature infants who primarily have RDS are like those of healthy infants on the first day of life. However, on the seventh day of life α_2-M and α_2-AP are significantly depressed in sick premature infants compared to healthy premature infants ($P < 0.01$) while α_1-AT behaves as an acute phase reactant ($P < 0.001$).

Previously, minimal data has been available on the influence of the above parameters on these inhibitor levels in premature infants. In healthy term infants C_1E-INH and α_2-AP levels are reported to be 50% of normal adult levels (13). To our knowledge, there is no information on these two inhibitors in prema-

TABLE II. PLASMA PROTEASE INHIBITORS IN TERM AND PREMATURE INFANTS DURING THE FIRST WEEK OF LIFE[a]

	N	α_2-M (%)	α_1-AT (%)	C_1E-INH (%)	α_2-AP (%)	AT-III (%)
Adults	18	105 ± 5	96 ± 4	100 ± 4	108 ± 3	100 ± 3
Infants						
Day 1						
Term						
Healthy	18	109 ± 4	105 ± 4	65 ± 3*	92 ± 4	54 ± 2*
DIC	5	60 ± 8**	113 ± 12	54 ± 6	57 ± 3**	35 ± 4**
Premature						
Healthy	15	92 ± 9	99 ± 6	64 ± 3*	77 ± 4*	41 ± 3*
Sick	16	74 ± 8	81 ± 9	59 ± 4	68 ± 8	39 ± 5
DIC	5	46 ± 8**	103 ± 16	46 ± 8**	56 ± 5**	31 ± 6
Day 7						
Premature						
Healthy	10	118 ± 11	100 ± 8	106 ± 11***	112 ± 2***	77 ± 10***
Recovered	11	107 ± 11	125 ± 10**	103 ± 5	79 ± 6**	72 ± 8
Sick	11	88 ± 7**	128 ± 9**	103 ± 6	73 ± 6**	55 ± 5

[a] Percentage of adult pooled plasma, mean ± SEM.

* Indicates statistical significance of at least $P < 0.05$ when compared to adult values.

** Indicates statistical significance of at least $P < 0.05$ when compared to the healthy age-matched controls.

*** Indicates statistical significance of at least $P < 0.05$ when compared to Day 1 healthy prematures.

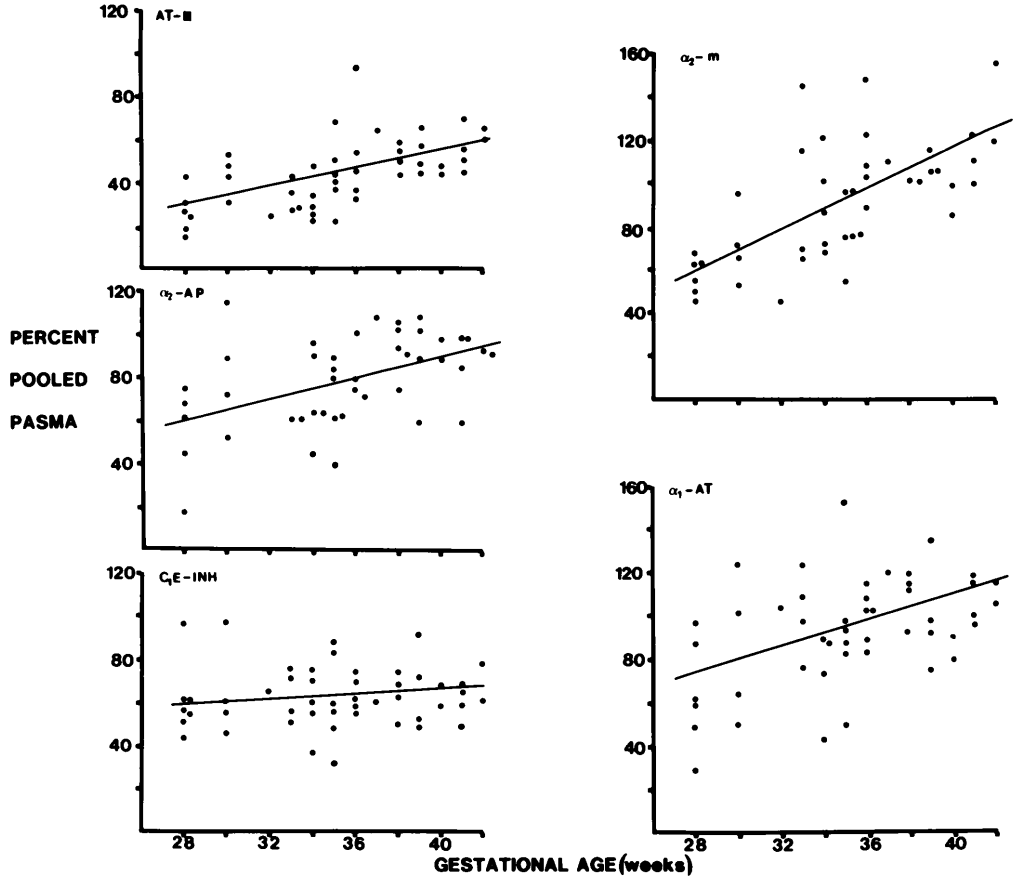

FIG. 1. Influence of gestational age on the immunologic level of the plasma protease inhibitors AT III, α_2AP, C_1E-INH, α_2-M, and α_1AT on the first day of life. Each symbol represents data from one infant. There is a significant correlation for all inhibitors except C_1E-INH between immunologic level and gestational age.

ture infants. α_2-M, α_1-AT, and AT-III have been studied more extensively in both term and premature infants (2, 7, 8, 13–29). As in our study, α_2-M and α_1-AT levels in term infants are in the normal adult range (21, 27). Published AT-III levels in term infants have ranged between 37 to 87% of adult values (7, 8, 13, 14, 19, 20). This large variation probably results from the study design, as frequently healthy and sick infants were not considered separately and postnatal age and the presence or absence of DIC were not considered. Our study found healthy term infants to have a mean value of 54 ± 2% for AT-III.

Two observations in our study contradict previously reported data for α_1-AT and AT-III levels in premature infants. The first is that in our study α_1-AT behaves as an acute phase reactant in sick and recovered infants. Previous investigators reported that infants with RDS have lower levels of this inhibitor than do controls (22, 23, 25, 28). The most likely reason for this discrepancy is that we measured the immunologic levels whereas the previous reports were based primarily upon functional assays. This suggests the very interesting possibility that α_1-AT and perhaps other inhibitors as well are bound to enzymes and may be measured immunologically but not functionally. Bruce *et al.* recently reported that this is true for α_1-AT in infants with bronchopulmonary dysplasia (30).

The second conflicting observation is that we were unable to demonstrate that infants with RDS had significantly lower levels of AT-III when DIC was excluded and appropriate

controls were used (15, 20, 29). A likely explanation is that our control group was carefully matched for gestational and postnatal age which has been demonstrated to significantly influence the level of other coagulation factors (31). Based on these previous reports, investigators (29) have advocated the infusion of AT-III to premature infants in hopes of preventing conditions associated with fibrin deposition (thrombosis, respiratory distress syndrome (RDS)). In view of the results of our present study and the lack of information available on the biologic activity of these inhibitors, it would appear to be premature to undertake this kind of trial now.

The five plasma protease inhibitors reported in this study have important roles in regulating the coagulation cascade, the fibrinolytic system, and the kallikrein–kinin pathway. In adults, abnormally low levels for all of these inhibitors have been reported, with resultant serious pathologic consequences. For example, AT-III is an important control protein for the coagulation system and inhibits factors XIIa, XIa, IXa, Xa, and thrombin. In adults, levels of AT-III in the 50% range have been associated with recurrent thromboses (32, 33). Deficiencies of α_2-AP, the major inhibitor of the fibrinolytic pathway, are thought to be responsible for recurrent bleeding episodes (34, 35). Deficiencies of C_1E-INH, the major inhibitor of the kallikrein–kinin system, result in the disorder hereditary angioneurotic edema which is characterized by recurrent, often life-threatening mucocutaneous angioedema and abdominal pain (36, 37). α_2-M, an inhibitor capable of neutralizing serine proteases generated during either the coagulation or fibrinolytic process, has not been associated with serious defects (38, 39). Finally, deficiencies of α_1-AT, an important inhibitor for many enzymes, are well documented and lead to serious pulmonary and hepatic dysfunction (40). Compared to normal adults, C_1E-INH and AT-III were depressed in the healthy term infant while C_1E-INH, AT-III, and α_2-AP were depressed in the healthy premature infants on Day 1. The levels for these inhibitors were in the range associated with the pathologic disorders described in adults. It is not unreasonable to speculate that added insults such as hypoxia and acidosis may activate the coagulation and fibrinolytic system leading to further depletion of these inhibitors in the newborn infant. This would place these infants at risk for hypotensive, thrombotic, and hemorrhagic complications. All of these are frequently observed in sick infants.

DIC, a process in which proteases are generated and inhibitors consumed caused further depression of the levels of α_2-M, α_2-AP, and C_1E-INH ($P < 0.01$) in premature infants compared to healthy age-matched controls. On Day 7, sick premature infants with RDS also had lower levels of α_2-M and α_2-AP compared to healthy age-matched controls. This suggests that these inhibitors are being consumed in RDS further impairing the infant's ability to inhibit proteases. The same infants may develop several complications thought to be at least partially mediated by active proteases e.g., bronchopulmonary dysplasia.

1. Markarian M, Githens JH, Rosenblut E, Fernandez, Jackson JJ, Bannon AE, Lindley A, Lubchenco LO, Martorell R. Hypercoagulability in premature infants with special reference to the respiratory distress syndrome and haemorrhage. Biol Neonate **17**:84–97, 1971.
2. Ekelund H, Finnstrom O. Fibronolysis in pre-term infants and in infants small for gestational age. Acta Paediatr Scand **61**:185–196, 1972.
3. Andrew M, Karpatkin M. A kallikrein-like protease in unactivated plasma of premature infants. Blood **58**:211, abstr, 1981.
4. Melmon KL, Cline MJ, Hughes T, Nies AS. Kinins: Possible mediators of neonatal circulatory changes in man. J Clin Invest **47**:1295–1302, 1968.
5. Mellins RB, Davidson D, Stalcup SA. Non-respiratory functions of the lung in the perinatal period. Ciba Found Symp **78**:313–331, 1980.
6. Schreiber AD. Plasma inhibitors of the Hageman factor dependent pathways. Sem Thromb Hemostas **3**:43–51, 1976.
7. Schettini F, DeMattia D, Montagna O, Altomare M. S viluppo post-natale dell-antithrombina III, del plasminogeno e dell' α_2-macroglobulina nell'uomo. Riv Ital Ped **2**:315–322, 1976.
8. Biland L, Duckert F. Coagulation factors of the newborn and his mother. Thromb Diath Haemorrh **29**:644–651, 1973.
9. Dubowitz LMS, Dubowitz V, Goldberg C. Clinical assessment of gestational age in the newborn infant. J Pediatr **77**:1–10, 1970.
10. Searcy RL, Simms NM, Low EMY. A simple method for rapid detection of hypofibrinogenemia. Amer J Med Technol **33**:326–329, 1967.
11. Merskey C, Lalezari P, Johnson AJ. A rapid, simple, sensitive method for measuring fibrinlytic split prod-

ucts in human serum. Proc Soc Exp Biol Med **131**:871–875, 1969.
12. Johnston M, Zipursky A. Microtechnology for the study of the blood coagulation system of newborn infants. Canad J Med Technol **42**:159–164, 1980.
13. Mullen AD, Van Doorm JM, Hemker HC. Heparin-like inhibitor of blood coagulation in the normal newborn. Nature (London) **267**:616–617, 1977.
14. Foley ME, Clayton JK, McNicol GP. Haemostatic mechanisms in maternal, umbilical vein and umbilical artery blood at the time of delivery. Brit J Obstet Gynaecol **84**:81–87, 1977.
15. Barnard DR, Simmons MA, Hathaway WE. Coagulation studies in extremely premature infants. Pediatr Res **13**:1330–1335, 1979.
16. McDonald MM, Jacobson LJ, Hay WW, Hathaway WE. Heparin clearance in the newborn. Pediatr Res **15**:1015–1018, 1981.
17. Zipursky A, deSa D, Hsu E, Johnston M, Milner R. Clinical and laboratory diagnosis of hemostatic disorders in newborn infants. Amer J Pediatr Hematol Oncol **1**:217–226, 1979.
18. Teger-Nilsson AC. Antithrombin in infancy and childhood. Acta Paediatr Scand **64**:624–628, 1975.
19. Weissback G, Domula M, Lenk H, Schneider P. The progressive antithrombin activity and its relations to other factors of the coagulation system in newborns. Acta Paediatr Scand **67**:555–561, 1974.
20. Mahasandana C, Hathaway WE. Circulating anticoagulants in the newborn: Relation to hypercoagulability and the idiopathic respiratory distress syndrome. Pediatr Res 7:670–673, 1973.
21. Ganrot PO, Schersten B. Serum α_2-macroglubin concentration and its variation with age and sex. Clin Chim Acta **15**:113–120, 1967.
22. El-Bardessy MW, Johnson AM. Serum proteinase inhibitors in infants with hyaline membrane disease. J Pediatr **81**:579–587, 1972.
23. Evans HE, Keller S, Mandl I. Serum trypsin inhibitory capacity and the idiopathic respiratory distress syndrome. J Pediatr **81**:588–592, 1972.
24. Omene JA, Glew RH, Thongbe JC. Trypsin inhibitory capacity in umbilical cord serum of nigerian newborn babies. East Afr Med J **56**:203–207, 1979.
25. Jasso-Gutierrez L, Contreras G, Puente-Banuelos E, Dominguez C, Romero G. Inhibitory activity of α_1-antitrypsin in infants with idiopathic respiratory distress syndrome. Arch Invest Med **10**:1–6, 1979.
26. Jasso-Gutierrez L, Vargas-Origel A, Puente-Banuelos E, Romero-Villasenor G. Effects of pH on α_1-antitrypsin activity in premature infants with idiopathic respiratory distress syndrome. Arch Invest Med **10**:7–14, 1979.
27. Kueppers F, Offord K. Alpha-1 antritrypsin elevation in healthy neonates. J Lab Clin Med **94**:475–489, 1979.
28. Kotas RV, Fazen LE, Moore TE. Umbilical cord serum trypsin inhibitor capacity and the idiopathic respiratory distress syndrome. J Pediatr **81**:593–599, 1972.
29. Hendricksson P, Wesstrom G, Hedner U. Umbilical artery catheterization in newborns. Acta Paediatr Scand **68**:719–723, 1979.
30. Bruce MC, Boat TF, Martin RJ, Dearborn DG, Fanaroff A. Inactivation of alpha proteinase inhibitor in infants exposed to high concentrations of oxygen. Amer Rev Respir **123**:166, abstr, 1981.
31. Andrew M, Bhogal M, Karpatkin M. Factors XI and XII and prekallikrein in sick and healthy premature infants. N Engl J Med **305**:1130–1133, 1981.
32. Egeberg O. Inherited antithrombin deficiency causing thrombophilia. Thromb Diath Haemorrh **13**:516–520, 1965.
33. Marciniak E, Farley CH, DeSimone PA. Familial thrombosis due to antithrombin III deficiency. Blood **43**:219–231, 1974.
34. Miles LA, Plow EF, Donnelly KJ, Hougie C, Griffin JH. A bleeding disorder due to deficiency of α_2-antiplasmin. Blood **59**:1246–1251, 1982.
35. Kluft C, Vellenga E, Brommer EJP, Wijngaards G. A familial hemorrhagic diathesis in a dutch family: An inherited deficiency of α_2-antiplasmin. Blood **59**:1169–1180, 1982.
36. Donaldson VH, Evans RR. A biochemical abnormality in hereditary angioneurotic edema. Absence of serum inhibitor of C'1-esterase. Amer J Med **35**:37–44, 1963.
37. Frank M, Gelfaud JA, Atkinson JP. Hereditary angioedema. The clinical syndrome and its management. Ann Intern Med **84**:580–593, 1976.
38. Bergqvist D, Nilsson IM. Hereditary alpha$_2$-macroglobulin deficiency. Scand J Haematol **23**:433–436, 1979.
39. Stenbjerg S. Inherited alpha$_2$ macroglobulin deficiency. Thromb Res **22**:491–495, 1981.
40. Talamo, RC. Basic and clinical aspects of the alpha$_1$-antitrypsin. Pediatrics **56**:91–99, 1975.

Received December 13, 1982. P.S.E.B.M. 1983, Vol. 173.