Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 728–732, February 1988
Biochemistry

Platelet-activating factor acetylhydrolase activity in maternal, fetal, and newborn rabbit plasma during pregnancy and lactation

(lipoprotein)

NORIEI MAKI, DENNIS R. HOFFMAN, AND JOHN M. JOHNSTON

Departments of Biochemistry and Obstetrics-Gynecology and the Cecil H. and Ida Green Center for Reproductive Biology Sciences, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235

Communicated by Ronald W. Estabrook, October 5, 1987

ABSTRACT **The inactivation of the biologically active ether-containing glycerophospholipid platelet-activating factor (PAF) is catalyzed by the enzyme PAF acetylhydrolase (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase, EC 3.1.1.48). The specific activity of acetylhydrolase has been assayed in maternal rabbit plasma prior to and throughout pregnancy and after parturition. The specific activity of acetylhydrolase was 131 ± 8 nmol·min^{-1}·ml^{-1} of plasma (mean ± SEM) in the nonpregnant rabbit. Similar specific activities were found throughout the first half of pregnancy; however, on day 15 the acetylhydrolase activity began to decrease and reached a minimum around day 27. Within 24–48 hr following delivery, the specific activity of the enzyme increased to the levels found in the nonpregnant animals. The specific activity of acetylhydrolase in fetuses (gestational ages of 21–30 days) and neonates increased from 22 nmol·min^{-1}·ml^{-1} of plasma (21-day-old) to 328 nmol·min^{-1}·ml^{-1} of plasma (35-day-old rabbits). The decrease in enzymatic activity of the pregnant rabbit plasma cannot be accounted for by the presence of an inhibitor as determined by plasma-mixing experiments with nonpregnant and pregnant (27-day) plasma. The properties of this enzyme in the rabbit were consistent with those reported by others, i.e., substrate specificity, inactivation by heat and various inhibitors, and Ca^{2+} independency. The activity of PAF acetylhydrolase was associated primarily with the high density lipoprotein fraction in rabbit plasma. The decrease of this enzymatic activity during pregnancy may serve to remove the protective effect on myometrium to allow an increased amount of PAF to come in contact with this tissue.**

Platelet-activating factor (PAF) activity was first reported to be released from leukocytes following antigen stimulation by a Ca^{2+}- and temperature-dependent process (1). Subsequently, Benveniste *et al.* (2) described the mechanism by which PAF activity could be obtained from IgE-sensitized rabbit basophils. During this same time period, it had been suggested that a systemic hypotensive factor was produced by the kidney (3). Snyder and colleagues (4) published the structure of the hypotensive lipid, just as Hanahan and colleagues (5) and Benveniste and collaborators (6) published the structure of the platelet-aggregating lipid (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine). Although PAF has been associated with a number of disease states in relation to inflammatory responses (7), it is increasingly apparent that PAF also plays a role in a number of physiological processes, including regulation of glycogenolysis (8), embryo implantation (9, 10), and initiation of parturition (11, 12).

We have detected PAF in amniotic fluid obtained from women at term and in labor (11). This has been confirmed (13). Furthermore, we established that PAF could stimulate prostaglandin E_2 formation in amniotic tissue (14) and that the fetal membranes and decidua vera tissues contain the necessary enzymes for synthesis of PAF (15). No evidence was obtained, however, that fetal membranes had the capacity to secrete PAF. Based on the observation that from 50 to 90% of the PAF, lysoPAF, and alkyl-acyl (long-chain)-glycerophosphocholine were associated with the lamellar body (surfactant-containing)-enriched fraction (11), we proposed that fetal lung was a significant source of the PAF present in amniotic fluid (12). We found that fetal lung has the capacity to synthesize PAF and contained a significant amount of PAF that increased during the latter stages of gestation (16, 17).

The regulation of PAF concentrations in a variety of cells and tissues has focused on its biosynthesis; however, several investigations have been published suggesting that PAF levels may be regulated by its degradation. PAF is inactivated by a specific PAF acetylhydrolase (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase; EC 3.1.1.48) to form lysoPAF (18). Both an intracellular and plasma form of the enzyme have been reported (19). Farr *et al.* (20, 21) were the first to describe this enzyme in human plasma. The characteristic features were acid lability, Ca^{2+}-independency, inactivation by heating at 65°C for 30 min, and Pronase and trypsin sensitivity but papain resistance. These investigators also reported that the activity of this enzyme was associated with a lipoprotein (20). Ostermann *et al.* (22) reported that the PAF acetylhydrolase activity was associated with very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fractions and was 10- and 100-fold higher than that found in high density lipoprotein (HDL) and serum, respectively. Stafforini *et al.* (23, 24) have purified PAF acetylhydrolase from human plasma. They reported that 30% of the activity was associated with HDL and that 70% was with the LDL fractions and suggested that the activity could be transferred from one lipoprotein to another.

It has been reported that the activity of PAF acetylhydrolase was elevated in eosinophils obtained from patients with eosinophilia and could be stimulated severalfold in neutrophils by ionophore A23187 (25). The acetylhydrolase activity was also elevated in plasma of spontaneously hypertensive rats (19) and in serum of white males who had hypertension (26).

In consideration that the activity of PAF acetylhydrolase in maternal plasma may play a role in the regulation of PAF of fetal origin that reaches the placenta, we have investigated

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PAF, platelet-activating factor; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

the activity of this enzyme in rabbit plasma throughout pregnancy and the postnatal period.

MATERIALS AND METHODS

Chemicals. [^{3}H]PAF [1-hexadecyl-2-[^{3}H]acetyl-*sn*-glycero-3-phosphocholine (12 Ci/mmol; 1 Ci = 37 GBq)] was purchased from New England Nuclear. Nonradiolabeled PAF and 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine were purchased from Avanti Polar Lipids. Bovine serum albumin (fatty acid-poor), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, 1-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine, phenylmethylsulfonyl fluoride, trypsin, and papain were purchased from Sigma. Trichloroacetic acid was purchased from Fisher Scientific (Fair Lawn, NJ). Diisopropyl fluorophosphate was purchased from Fluka (Ronkonkoma, NY). Pronase was purchased from Calbiochem.

Animals. Five adult New Zealand White rabbits (Hickory Hill, Flint, TX) were mated within a 1-hr period. Thirty microliters of plasma were obtained prior to mating and then every other day during pregnancy from the marginal ear veins. Blood was collected in plain tubes or tubes containing heparin, acid-citrate-dextrose, or EDTA as anticoagulants, and the PAF acetylhydrolase activities in serum and in plasma were compared. The activity of acetylhydrolase for the same rabbit blood sample was similar, regardless of the anticoagulant utilized. Heparinized tubes were employed in subsequent experiments. To obtain fetal blood, the pregnant does with gestational ages of 21, 24, 27, and 30 days were anesthetized with metophane, uteri were exposed, the fetuses were removed and decapitated, and fetal blood was collected in heparinized tubes.

Assay for PAF Acetylhydrolase Activity. The activity of PAF acetylhydrolase in plasma was assayed similar to that described by Blank *et al.* (18) as modified (17). The assay mixture contained Tris·HCl (30 mM, pH 7.5), 1-hexadecyl-2-[^{3}H]acetyl-*sn*-glycero-3-phosphocholine (50 μM, 4.0 μCi/μmol) in bovine serum albumin (0.01% final concentration), and 100 μg of plasma protein in a total volume of 0.5 ml. The [^{3}H]acetate released from PAF was determined by liquid scintillation. The assay was linear with protein concentration to 200 μg and with incubation time to 60 min. Protein was quantitated by the method of Lowry *et al.* (27).

Characterization of PAF Acetylhydrolase Activity in Rabbit Plasma. *Substrate specificity.* The substrate specificity of PAF acetylhydrolase was ascertained by addition of various nonradioactive analogs to the reaction mixture. These analogs, complexed to albumin, were added at a concentration equimolar to [^{3}H]PAF.

Inhibition study, heat inactivation, protease treatment, and mixing experiments. Diisopropyl fluorophosphate (10 mM) (18), 2 mM phenylmethylsulfonyl fluoride (28, 29), and 10% (wt/vol) trichloroacetic acid (30) were used as inhibitors. Experiments were carried out as described with the exception that the reaction mixtures were preincubated with various inhibitors for 10 min at 37°C prior to addition of [^{3}H]PAF. Heat inactivation studies were performed as described (21). To determine the effects of trypsin, Pronase (19), and papain (31) on PAF acetylhydrolase, the reaction mixtures in the presence or absence of the proteases were preincubated at 30°C for 15 min. The presence of inhibitors in pregnant rabbit plasma or activators in nonpregnant rabbit plasma of acetylhydrolase activity was determined after mixing pregnant (27-day) plasma with nonpregnant plasma at various ratios.

Separation of VLDL plus LDL and HDL fractions. Blood was collected from ear arteries of unanesthetized rabbits, mixed with 1 mg of EDTA per ml, and placed on ice. VLDL plus LDL fractions were precipitated by treatment of nonpregnant and pregnant (21-day) rabbit plasma with sodium phosphotungstate and $MgCl_2$ as described (32). The precipitated VLDL plus LDL fractions were redissolved in Tris buffer and assayed for acetylhydrolase activity. The total recoveries of acetylhydrolase in both nonpregnant and pregnant rabbits ranged between 76 and 81%, respectively. VLDL plus LYL and HDL fractions were separated from plasma obtained from 24-day pregnant rabbits by ultracentrifugation as described (33). The recovery of acetylhydrolase activity was >95%.

RESULTS

Plasma PAF Acetylhydrolase Activity During Pregnancy. The specific activity of acetylhydrolase in plasma of the five rabbits before mating was 131 ± 8 nmol·min^{-1}·ml^{-1} of plasma or 1.6 ± 0.1 nmol·min^{-1}·mg^{-1} of protein (mean ± SEM) (Fig. 1). Similar findings were obtained regardless of expression of the data on a ml of plasma or mg of protein basis.

The activity of PAF acetylhydrolase in plasma of rabbits that had uncomplicated pregnancies increased slightly during the first 13 days to ≈160 nmol·min^{-1}·ml^{-1} of plasma and then decreased to a minimum between 23 and 29 days. In postpartum rabbits, the activity of the enzyme increased rapidly to the nonpregnant level. The fourth rabbit proved not to be pregnant, and rabbit 5 delivered two dead offspring.

Characteristics of PAF Acetylhydrolase Activity in Rabbit Plasma. *Substrate specificity.* Substrate competition studies were utilized to establish the specificity of the rabbit plasma acetylhydrolase (Table 1). The potential substrates contained long chain fatty acid moieties (i.e., oleoyl or arachidonoyl) in the *sn*-2 position and alkyl or acyl groups at the *sn*-1 position. A dilution effect was evident when unlabeled PAF was added to the assay mixture, whereas the analogs did not dilute the radioactive substrate. Similar substrate specificities were evident in both nonpregnant and 24-day pregnant rabbit plasma.

Inhibition study, heat inactivation, and protease treatment. The enzyme was inhibited by diisopropyl fluorophosphate (77%) and trichloroacetic acid (83%). Phenylmethylsulfonyl fluoride (2 mM) also inhibited the enzyme to a limited extent (51%). The plasma acetylhydrolase activity was not affected by the addition of Ca^{2+} or EDTA to the reaction mixture or by blood collection in the presence of EDTA or acid-citrate dextrose, thus indicating the Ca^{2+} independency of the enzyme. When plasma was heated at 65°C for 30 min, 75% of the total acetylhydrolase activity was lost. Pretreatment of nonpregnant rabbit plasma with trypsin or Pronase resulted in an 86 and 100% loss of acetylhydrolase activity, respectively, whereas papain treatment was without effect. Similar findings were observed when plasma from pregnant rabbits (24-day) was employed.

To assess the possible presence of an activator or an inhibitor in plasma obtained from nonpregnant or pregnant (27-day) rabbits, respectively, we carried out a mixing experiment. The activity of PAF acetylhydrolase was assayed following incubation of the two plasma samples combined in various proportions. The specific activities of the acetylhydrolase present in nonpregnant and pregnant (27-day) rabbit plasma was 2.1 and 0.1 nmol·min^{-1}·mg^{-1} of protein, respectively. When 50% of each sample was mixed and assayed, the specific activity found was 1.1 nmol·min^{-1}·mg^{-1} of protein; the theoretical value being 1.1 nmol·min^{-1}·mg^{-1} of protein. Similarly, when the mixture was composed of 75% nonpregnant plasma and 25% pregnant plasma, the specific activity was 1.7 nmol·min^{-1}·mg^{-1} of protein; in agreement with the theoretical value. Likewise, reversing the plasma ratios resulted in an activity of 0.6 nmol·min^{-1}·mg^{-1} of

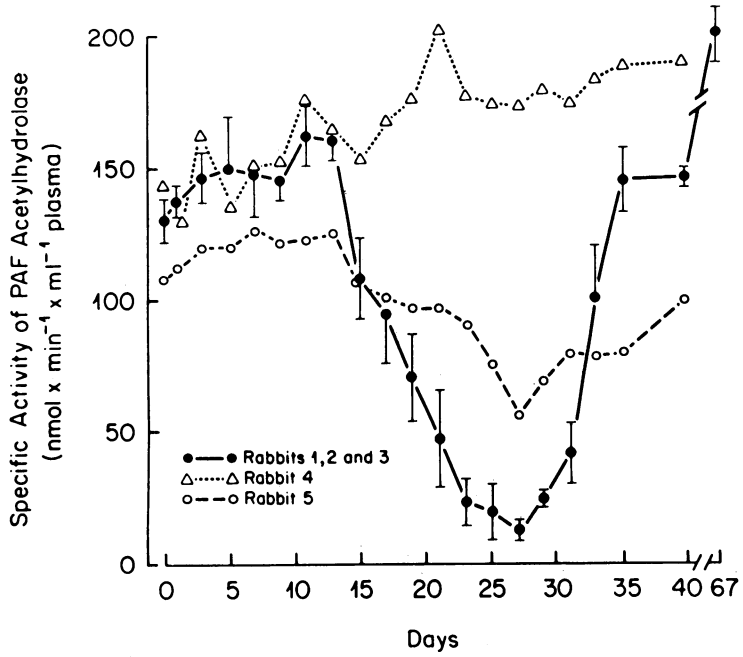

FIG. 1. Specific activities of plasma PAF acetylhydrolase in five rabbits during pregnancy and lactation. The day-0 value was obtained before impregnation. Values are mean ± SEM (nmol·min^{-1}·ml^{-1} of plasma). Three rabbits with uncomplicated pregnancies (●) delivered a total of 20 live and 3 dead offspring on an average of 31 days of gestation. The fourth rabbit (△) showed no signs of pregnancy during the study period and subsequently was examined by x-ray on day 34; no fetuses were found. The fifth rabbit (○) delivered 2 dead offspring on day 34 [weight similar to 28-day fetuses (17)].

protein. We have, therefore, concluded that the presence of various positive and negative effectors cannot explain the change in observed activity.

Lipoprotein fractionation. PAF acetylhydrolase activity was assayed in the isolated lipoprotein fractions from nonpregnant and pregnant (21-day) rabbit plasma following sodium phosphotungstate and $MgCl_2$ precipitation (Table 2). Almost all enzymatic activities in both plasma samples were associated with the HDL fractions (>98%). When a KBr density gradient procedure was utilized to separate and purify the various lipoprotein fractions, >99% of the acetylhydrolase activity was associated with the HDL fraction.

Fetal and neonatal acetylhydrolase activity. Plasma samples from anesthetized pregnant does and their fetuses were assayed for enzymatic activity. Acetylhydrolase activity was significantly lower in fetal plasma at 21 days of gestation compared to the mothers (Table 3); however, by day 30 it was nearly twice that of the pregnant doe. Similarly, the enzyme activities in the offspring were significantly greater than that of the mother.

Table 1. Substrate specificity of rabbit plasma acetylhydrolase

Addition	% acetylhydrolase activity	
	Nonpregnant	24-day pregnancy
None	100.0	100.0
1-Palmitoyl-2-oleoyl-GPC	98.6*	104.1*
1-Palmitoyl-2-arachidonoyl-GPC	102.4	90.1
1-Hexadecyl-2-oleoyl-GPC	97.5	98.2
PAF	54.0	60.0

PAF or its analogs (25 nmol) were added as an albumin complex to the assay mixture containing 25 nmol of [^{3}H]PAF. GPC, glycero-3-phosphocholine. 100.0% values equal 160.9 and 39.2 nmol·min^{-1}·ml^{-1} of plasma from nonpregnant and pregnant rabbits, respectively.
*Values are averages of duplicate experiments.

DISCUSSION

The concentration of PAF that is present in various biological samples may be regulated by the rate of its biosynthesis or degradation. In most of the systems thus examined, it has been suggested that biosynthesis of PAF is altered due to an increase in the amount or activity of biosynthetic enzymes (for review see ref. 34). We found no change in the activity of intracellular PAF acetylhydrolase during development in fetal rabbit lung (17) or in human fetal lung organ cultures (16). In contrast, a regulatory enzyme involved in the remodeling pathway of PAF biosynthesis, namely lysoPAF: acetyl-CoA acetyltransferase, and the dithiothreitol-insensitive cholinephosphotransferase, which catalyzes the final step in the synthesis of PAF by the *de novo* pathway, increase in activity severalfold during fetal rabbit lung development. The activity of PAF acetylhydrolase was first described by Pinckard *et al.* (35) as the acid-labile factor in rabbit plasma that could inactivate PAF. Snyder and colleagues (19, 26) have found an increase in this activity in genetically spontaneous hypertensive rats and also in white male populations suffering from hypertension. These investigators have suggested that the increase in acetylhydrolase activity may be important in the pathogenesis of hypertension by increasing the rate of breakdown of the hypotensive

Table 2. Association of PAF acetylhydrolase with lipoproteins of rabbit plasma

Method	Plasma source	% of activity in fraction	
		HDL	VLDL+LDL
Precipitation	Nonpregnant	99.7	0.3
Precipitation	Pregnant (21 days)	98.6	1.4
KBr gradient	Pregnant (24 days)	99.7	0.3

Lipoproteins were separated by precipitation with sodium phosphotungstate and $MgCl_2$ or by ultracentrifugation on a KBr density gradient. Results are from duplicate experiments.

Table 3. PAF acetylhydrolase activity in fetal, postnatal, and maternal plasma

Age, days	PAF acetylhydrolase specific activity in plasma, nmol·min^{-1}·ml^{-1} of plasma	
	Fetus or offspring	Mother
Gestational		
21	21.7 ± 1.3 (8)	98.1 (1)
24	28.7 ± 2.3 (8)	16.2 (2)
27	94.5 ± 3.3 (8)	30.1 (1)
30	98.2 ± 2.2 (8)	45.3 (1)
Postnatal (postpartum)		
1	187.0 ± 13.9 (6)	101.5 ± 20.5 (3)*
8	323.6 ± 14.2 (4)	147.0 ± 2.6 (3)*
35	327.7 ± 20.5 (6)	202.4 ± 10.9 (3)*

Timed pregnant rabbits with gestational ages of 21, 24, 27, and 30 days were the source of fetal and maternal plasma. Specific activity values are mean ± SEM (*n*).
*Values are significantly different from corresponding offspring values at $P < 0.001$.

lipid PAF. Pritchard *et al.* (36) observed higher acetylhydrolase activity in plasma of a patient with Tangier disease that was characterized by HDL deficiency and triacylglycerol-rich LDL. Thus, prior to the present investigation, the change in acetylhydrolase activities has been associated only with pathological conditions. As reported, we have demonstrated that the acetylhydrolase activity decreased by 90% during the latter half of pregnancy.

We have determined that the properties of PAF acetylhydrolase in the plasma of pregnant and nonpregnant rabbits were similar to those described (19–21) and are different from those of a typical phospholipase A_2 (37). The enzyme was not inhibited by PAF analogs, did not require Ca^{2+}, was inactivated by heating and treatment with diisopropyl fluorophosphate, phenylmethylsulfonyl fluoride, and trichloroacetic acid. In addition, the lipoprotein distribution and protease inhibition patterns of acetylhydrolase from pregnant and nonpregnant rabbits are nearly identical.

Blank *et al.* (18, 19) reported the activity of acetylhydrolase in a wide variety of rat tissues and demonstrated some differences between that of plasma and kidney regarding treatment with various proteases. They suggested that these differences were due to various degrees of glycosylation of the plasma enzyme (38). Similarly, Wardlow *et al.* (31) reported that human serum acetylhydrolase was sensitive to trypsin and to Pronase and resistant to papain. The rabbit plasma enzyme has characteristics similar to that of human serum.

It has been reported (20, 22) that the activity of this enzyme was closely associated with the LDL fraction of human plasma. Stafforini *et al.* (23) have purified to near homogeneity the plasma enzyme from a human LDL fraction and have also suggested that the biologically active acetylhydrolase was associated with the LDL fraction, although significant activity was also present in the HDL fraction. The enzyme activity could also be transferred from LDL to HDL fractions and vice versa (24).

We found that >95% of the PAF acetylhydrolase activity was associated with the HDL fraction utilizing both precipitation and density gradient procedures (Table 2). The association of this activity with the HDL in rabbit compared to the LDL fraction in humans is not surprising since it is well documented that in human and some other primates, the LDL fraction is the major lipoprotein involved in various metabolic reactions; whereas in other vertebrates, the HDL fraction is the more metabolically active (for review see ref. 39). Depressed concentrations of lipid in rabbit plasma during pregnancy have been reported (40). Subsequently, Zilversmit *et al.* (41) reported that the total amounts of cholesterol, phospholipid, and all lipoproteins decreased in the latter half of pregnancy. These investigators also reported that the lipid concentration in plasma rapidly returned to that found in the nonpregnant state during the immediate postpartum period. The activity of acetylhydrolase also returned to the level of the nonpregnant animal within 2 days after delivery (Fig. 1).

The multifaceted reactions of antigen-induced anaphylaxis have been correlated with intravascular accumulations of PAF in the rabbit (42). However, a failure to respond to appropriate antigen challenge has been noted in neonate and young animals (43–45). Furthermore, young rabbits failed to manifest many of the physiological responses associated with PAF infusion into adult rabbits (46). In 21-day fetal plasma, the mean specific activity of PAF acetylhydrolase was 22 nmol·min^{-1}·ml^{-1} of plasma, and during the perinatal period it increased rapidly. In the neonate, acetylhydrolase activity was significantly higher than that of the mother (Table 3). Thus, the higher activity of PAF acetylhydrolase in the young animals may contribute to their resistance to PAF.

We have suggested that the increase in PAF found in amniotic fluid of women in labor and found associated with lamellar body fractions (11) may have its origin in fetal lung and was due to an increase in PAF biosynthesis (15). Based on the reported results, we suggest that during early pregnancy, when PAF acetylhydrolase activity is relatively high, this enzyme might prevent PAF from reaching the myometrium by hydrolyzing the small amounts of PAF formed in the fetal compartment (14). We have suggested that the PAF acetylhydrolase in amniotic fluid may play a similar protective role (11). Since PAF is known to be a potent stimulus to myometrial contraction in rat (13), guinea pig (47), and human (48), the high activity of plasma acetylhydrolase during early pregnancy may maintain the uterus in a quiescent state. However, during the latter stage of gestation when biosynthesis of PAF is dramatically increased in the fetus (17), the capacity of maternal plasma to inactivate PAF is significantly reduced due to the decrease of PAF acetylhydrolase activity reported herein. Thus, we speculate that the increased PAF produced in the fetal compartment (e.g., fetal lung, membranes, etc., for review see ref. 12) may reach the myometrium in late gestation to initiate the rhythmical contraction of this tissue and thus initiate parturition.

The authors are indebted to Ms. Dolly Tutton for editorial assistance and to Dr. Marlane J. Angle for helpful discussions. This work was supported, in part, by Grant HD11149 from the National Institutes of Health and the Robert A. Welch Foundation, Houston, TX. D.R.H. is the recipient of a Chilton Fellowship.

1. Henson, P. M. (1970) *J. Exp. Med.* **131,** 287–304.
2. Benveniste, J., Henson, P. M. & Cochrane, C. G. (1972) *J. Exp. Med.* **136,** 1356–1377.
3. Muirhead, E. E. (1974) *Adv. Int. Med.* **19,** 81–107.
4. Blank, M. L., Snyder, F., Byers, L. W., Brooks, B. & Muirhead, E. E. (1979) *Biochem. Biophys. Res. Commun.* **90,** 1194–1200.
5. Demopoulos, C. A., Pinckard, R. N. & Hanahan, D. J. (1979) *J. Biol. Chem.* **254,** 9355–9358.
6. Benveniste, J., Tence, M., Varenne, P., Bidault, J., Boullet, C. & Polonsky, J. (1979) *C.R. Hebd. Seances Acad. Sci. Ser. D* **289,** 1037–1040.
7. Benveniste, J. & Pretolani, M. (1985) *Adv. Inflam. Res.* **10,** 7–19.
8. Shukla, S. D., Buxton, D. B., Olson, M. S. & Hanahan, D. J. (1983) *J. Biol. Chem.* **258,** 10212–10214.
9. O'Neill, C. (1985) *J. Reprod. Fertil.* **73,** 567–577.
10. Spinks, N. R. & O'Neill, C. (1987) *Lancet* **i,** 106–107.
11. Billah, M. M. & Johnston, J. M. (1983) *Biochem. Biophys. Res. Commun.* **113,** 51–58.

12. Johnston, J. M., Bleasdale, J. E. & Hoffman, D. R. (1987) in *Platelet-Activating Factor and Related Lipid Mediators*, ed. Snyder, F. (Plenum, New York), pp. 375–402.
13. Nishihira, J., Ishibashi, T., Imai, Y. & Muramatsu, T. (1984) *Lipids* **19**, 907–910.
14. Billah, M. M., Di Renzo, G. C., Ban, C., Truong, C. T., Hoffman, D. R., Anceschi, M. M., Bleasdale, J. E. & Johnston, J. M. (1985) *Prostaglandins* **30**, 841–850.
15. Ban, C., Billah, M. M., Truong, C. T. & Johnston, J. M. (1986) *Arch. Biochem. Biophys.* **246**, 9–18.
16. Hoffman, D. R., Truong, C. T. & Johnston, J. M. (1986) *Am. J. Obstet. Gynecol.* **155**, 70–75.
17. Hoffman, D. R., Truong, C. T. & Johnston, J. M. (1986) *Biochim. Biophys. Acta* **879**, 88–96.
18. Blank, M. L., Lee, T.-c., Fitzgerald, V. & Snyder, F (1981) *J. Biol. Chem.* **256**, 175–178.
19. Blank, M. L., Hall, M. N., Cress, E. A. & Snyder, F. (1983) *Biochem. Biophys. Res. Commun.* **113**, 666–671.
20. Farr, R. S., Cox, C. P., Wardlow, M. L. & Jorgensen, R. (1980) *Clin. Immunol. Immunopathol.* **15**, 318–330.
21. Farr, R. S., Wardlow, M. L., Cox, C. P., Meng, K. E. & Greene, D. E. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 3120–3122.
22. Ostermann, G., Kertscher, H.-P., Winkler, L., Schlag, B., Ruhling, K. & Till, U. (1986) *Thromb. Res.* **44**, 303–314.
23. Stafforini, D. M., Prescott, S. M. & McIntyre, T. M. (1987) *J. Biol. Chem.* **262**, 4223–4230.
24. Stafforini, D. M., McIntyre, T. M., Carter, M. E. & Prescott, S. M. (1987) *J. Biol. Chem.* **262**, 4215–4222.
25. Lee, T.-c., Malone, B., Wasserman, S. I., Fitzgerald, V. & Snyder, F. (1982) *Biochem. Biophys. Res. Commun.* **105**, 1303–1308.
26. Crook, J. E., Mroczkowski, P. J., Cress, E. E., Blank, M. L. & Snyder, F. (1986) *Circulation* **74**, 329 (abstr.).
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
28. Touqui, L., Jacquemin, C., Dumarey, C. & Vargaftig, B. B. (1985) *Biochim. Biophys. Acta* **833**, 111–118.
29. Vivier, E., Bulioust, A., Meslier, N., Benveniste, J. & Roubin, R. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 855 (abstr.).
30. Pinckard, R. N. & Ludwig, J. C. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 486 (abstr.).
31. Wardlow, M. L., Cox, C. P., Meng, K. E., Greene, D. E. & Farr, R. S. (1986) *J. Immunol.* **136**, 3441–3446.
32. Burnstein, M. & Scholmick, H. R. (1973) *Adv. Lipid Res.* **11**, 67–108.
33. Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955) *J. Clin. Invest.* **34**, 1345–1353.
34. Lee, T.-c. & Snyder, F. (1985) in *Phospholipids and Cellular Regulation*, ed. Kuo, J. F. (CRC, Boca Raton, FL), Vol. 2, pp. 1–39.
35. Pinckard, R. N., Farr, R. S. & Hanahan, D. J. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1667 (abstr.).
36. Pritchard, H., Chonn, A. & Yeung, C. C. H. (1985) *Blood* **66**, 1476–1478.
37. Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604**, 191–246.
38. Snyder, F. (1985) *Med. Res. Rev.* **5**, 107–140.
39. Calvert, G. D (1976) in *Low Density Lipoproteins*, eds. Day, C. E. & Levy, R. S. (Plenum, New York), pp. 281–319.
40. Baumann, E. J. & Holly, O. M. (1925) *Am. J. Physiol.* **75**, 618–632.
41. Zilversmit, D. B., Hughes, L. B. & Remington, M. (1972) *J. Lipid Res.* **13**, 750–756.
42. Pinckard, R. N., Farr, R. S. & Hanahan, D. J. (1979) *J. Immunol.* **123**, 1847–1857.
43. Bihaghi, R. A., Oettgen, H. F. & Benacerraf, B. (1966) *Int. Arch. Allergy Appl. Immunol.* **29**, 105–111.
44. Hargis, B. J. & Malkiel, S. (1970) *J. Immunol.* **104**, 942–949.
45. Meng, A. L., Pinckard, R. N. & Halonen, M. (1973) *J. Immunol.* **110**, 1554–1561.
46. Angle, M. J., Pinckard, R. N., Showell, H. J. & McManus, L. M. (1987) *Lab. Invest.* **57**, 321-328.
47. Montrucchio, G., Alloatti, G., Tetta, C., Roffinello, C., Emanuelli, G. & Camussi, G. (1986) *Prostaglandins* **32**, 539–554.
48. Tetta, G., Montrucchio, G., Alloatti, G., Roffinello, C., Emanuelli, G., Benedetto, C., Camussi, G. & Massobrio, M. (1986) *Proc. Soc. Exp. Biol. Med.* **183**, 376–381.