Experimental Model of Ischemic Bowel Necrosis

The Role of Platelet-Activating Factor and Endotoxin

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This report deals with the experimental production of ischemic bowel necrosis in rats by the administration of combined bacterial lipopolysaccharide (LPS) and platelet-activating factor (PAF). Neither LPS alone, nor PAF at a low dose, caused ischemic intestinal necrosis when administered intraaortically. With these two compounds in combination, necrotizing lesions of the gastrointestinal tract developed consistently. The lesions showed marked morphologic similarity to human necrotizing enterocolitis (NEC). There were no thrombi in mesenteric arteries or necrotic lesions in other organs to which these bioactive compounds were delivered. These findings suggest a possible synergistic involvement of PAF and LPS in the pathogenesis of NEC and other forms of ischemic bowel necrosis. The authors further suggest that the pathogenesis of experimental NEC in rats is independent of platelet aggregation. (Am J Pathol 1983, 112:127–135)

ISCHEMIC NECROSIS of the bowel is a serious pathologic condition that accounts for high mortality and grave sequelae in patients in all ages. In some instances, its cause may be traced to single, well-defined phenomena, such as thromboembolic sudden occlusion of large mesenteric vessels. In many other cases, however, the cause is probably multifactorial and the pathogenesis is complex. Thus, occlusive vascular lesions may not be demonstrable in adult patients with extensive intestinal infarction,[1] whether the vascular system is normal or already the seat of preexistent disease. In infants, bowel necrosis is considered the single most common surgical emergency,[2] usually presenting under the guise of the clinical complex termed "necrotizing enterocolitis" (NEC). Although the mortality figures attributed to NEC exceed those of all congenital anomalies of the bowel combined,[3] its pathogenesis has remained elusive. Because ischemic bowel necrosis is the defining characteristic of all these patients, there is little difficulty in assenting to hypotheses that propose and to work that documents a central role of bowel hypoperfusion in the development of the lesions. Nevertheless, the specific sequence of events, and their definition by reference to modern concepts of molecular biology, is still to be worked out. In view of this background, our success in reproducing NEC lesions experimentally, and with the use of naturally occurring biologic compounds, is of interest.

Materials and Methods

Platelet-activating factor (PAF), L-α-lecithin, β-acetyl, γ-o-alkyl, lyophilized, was purchased from Calbiochem-Behring (La Jolla, Calif). Stock solution (2 mg/ml ethanol) was kept at -70 C. Before use, an aliquot of stock solution was dried in N_2 and dissolved in saline containing bovine serum albumin (2.5 mg/ml). Lipopolysaccharide-B (LPS) from *Salmonella typhosa* was purchased from Difco (Detroit, Mich, lot 0901). Fresh saline solution was prepared before each use.

Male Sprague–Dawley rats 250 g $\pm$ 20 g in body weight were used in all experiments. The rats were anesthetized with ether, the abdomen was incised along the midline, and the abdominal aorta was exposed. PAF (1 or 2 μg) or LPS (10–40 μg), or a combination of these compounds, was injected into the aorta just beneath the origin of the renal arteries with

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the use of a tuberculin syringe with a 30-gauge needle. The final injection volume was always less than 0.2 ml. Immediately after the needle was withdrawn, a small triangle of absorbant gauze for ocular surgery (Ludwith Fröhnhauser, Munich, Germany) was pressed directly against the injection site for 30 seconds for prevention of bleeding. Bleeding was estimated at less than 1 ml in all rats; in some, only minor hemorrhagic staining of the adventitia was present at postmortem examination. After injection, the abdomen was closed, and the animal was placed back in the cage for recovery. The animals were sacrificed at 5, 15, 30, and 60 minutes, and 3 hours after injection with ether anesthesia, and the blood was collected for a platelet count. A complete postmortem examination, including sampling of abdominal and intrathoracic organs for histologic examination, was performed. Blocks of tissue were immersion-fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 μ, and stained with hematoxylin and eosin (H&E). Segments of the gastrointestinal tract were fixed by intraluminal instillation of the same fixative, and some sections were processed by methacrylate embedding and sectioned at 1 μ thickness with a JB-4 microtome.

To investigate further the role of platelet aggregation in our experimental model, we prepared rat platelet-rich plasma (PRP) from citrated (0.38%) rat blood. Platelet aggregation was measured with the use of a Chrono-log aggregometer (Pennsylvania). Human PRP was used as a positive control substance.

Results

Gross Findings

The results of our observations at the end of 3 hours are summarized in Table 1. The only constant gross lesion detected at postmortem examination was hemorrhagic segmental, often multiple, necrosis of the gastrointestinal tract, which developed as early as 15 minutes. Usually, the jejunum and ileum were involved; the cecum was variably affected, and the colon and rectum were not affected. The esophagus and forestomach were normal, but when advanced gross lesions were seen in other segments of the alimentary tract, the mucosa of the glandular (distal) stomach (though not the serosa) appeared invariably hemorrhagic. As shown in Table 1, LPS alone at all doses failed to produce any pathologic lesions (Figure 1). In contrast, PAF alone at the highest dose (2 μg) was sufficient to cause bowel necrosis, whereas PAF alone at a lower dose (1 μg) did not cause bowel necrosis. When LPS and PAF were combined, however, even low doses (1 μg PAF + 20 μg LPS) invariably resulted in necrotic changes of the intestine. Increasing the dose of either PAF (to 2 μg) or LPS (to 40 μg) did not increase the severity of the lesion.

The gross lesions of the bowel at the time of postmortem examination were characterized by localized hyperemia, loss of the normal luster of the serosal investment, and a violaceous or purple color suggesting hemorrhage. These changes could 1) be confined to the antimesenteric border of no more than three intestinal loops, measuring less than 0.5 cm each; 2) involve circumferentially the affected bowel loop or large intestine but not exceed 1 cm in extent and three sites of involvement; or 3) form large, confluent hemorrhagic patches that could simultaneously involve multiple segments of the gastrointestinal tract (Figure 2). These patterns of involvement are designated "mild," "moderate," and "massive," respectively, in Table 1.

Microscopic Findings

There was poor correlation between the severity of involvement noted in gross examination and that

Table 1—Experimental Bowel Necrosis in Rats 3 Hours After Intraaortic Platelet-Activating Factor (PAF) and Lipopolysaccharide (LPS) Injection

Compound used	Number of animals	Number of animals with lesions	Percentage of animals with lesions	Severity of lesions		
				Massive	Moderate	Mild
PAF (1 μg)	6	0	–	–	–	–
PAF (2 μg)	4	4	100	0	2	2
LPS (10 μg)	2	0	–	–	–	–
LPS (20 μg)	3	0	–	–	–	–
LPS (40 μg)	3	0	–	–	–	–
PAF (1 μg) + LPS (10 μg)	3	2	67	–	–	2
PAF (1 μg) + LPS (20 μg)	10	9	90	4	5	–
PAF (1 μg) + LPS (40 μg)	3	3	100	–	2	1
PAF (2 μg) + LPS (20 μg)	2	2	100	–	1	1
PAF (2 μg) + LPS (40 μg)	2	2	100	2	–	–

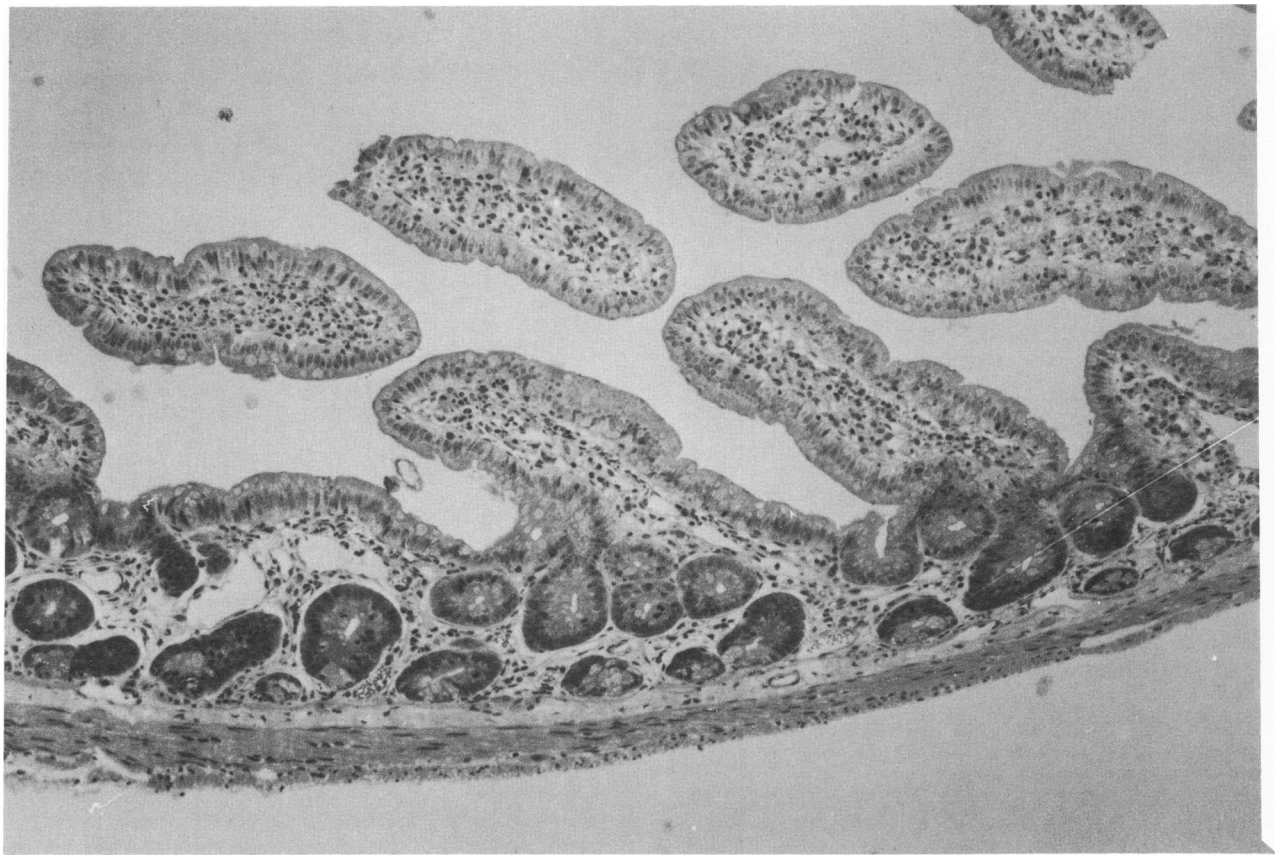

Figure 1 – Microscopic appearance of the small bowel after injection of 40 μg of endotoxin into the abdominal aorta. There is preservation of the normal villous pattern and an absence of necrotic lesions. (H&E, ×120) (With a photographic reduction of 4%)

assessed by histologic examination of the bowel. Mild lesions were commonly seen before 1 hour following PAF injection; such early lesions were characterized by focal necrosis of the mucosa and were often confined to the tips of the intestinal villi (Figure 3). This type of lesion, however, was sometimes noted in sections taken adjacent to grossly massive necrotic segments from areas of bowel presumed normal by naked eye examination. Similarly, some lesions suspected upon gross evaluation of being mild because of their limited extent revealed the transmural pattern of involvement most characteristic of the massive lesions. When the damage was assessed in gross examination as massive, transmural necrosis was usually discovered in histologic examination. Transmural necrosis was reflected by complete loss of normal mucosal architecture, with sloughing of all epithelial cells, and with only the supporting stroma of the lamina propria remaining. The muscular wall showed pale cytoplasmic eosinophilia and loss of nuclear staining. Hemorrhages, despite the gross appearance, were discrete; the purple discoloration could be due to intense capillary congestion seen by translucency from the serosal aspect of the involved bowel. Despite total transmural ischemic necrosis of all layers of the intestine, the lesions were remarkable for the lack of associated inflammatory cellular infiltration, either within the pathologic bowel or at the border between necrotic and normal bowel (Figure 4).

The mesentery was semiserially sectioned and histologically examined for evidence of thromboembolic alterations in mesenteric vessels. No evidence was found at any of the time intervals examined; the vascular wall was normal histologically. The glandular stomach, when involved, usually showed only mucosal necrosis. A transmural necrosis as in the small bowel was never observed. All rats survived the first 3-hour postoperative period until sacrifice.

All organs, including heart, kidneys, pancreas, spleen, and adrenals, appeared normal in gross and microscopic examination. The only exception was an isolated, microscopic focus of necrosis in the liver of one animal that received the highest dose of PAF-LPS. Sequestration of leukocytes in the lung interstitium and vessels was also present.

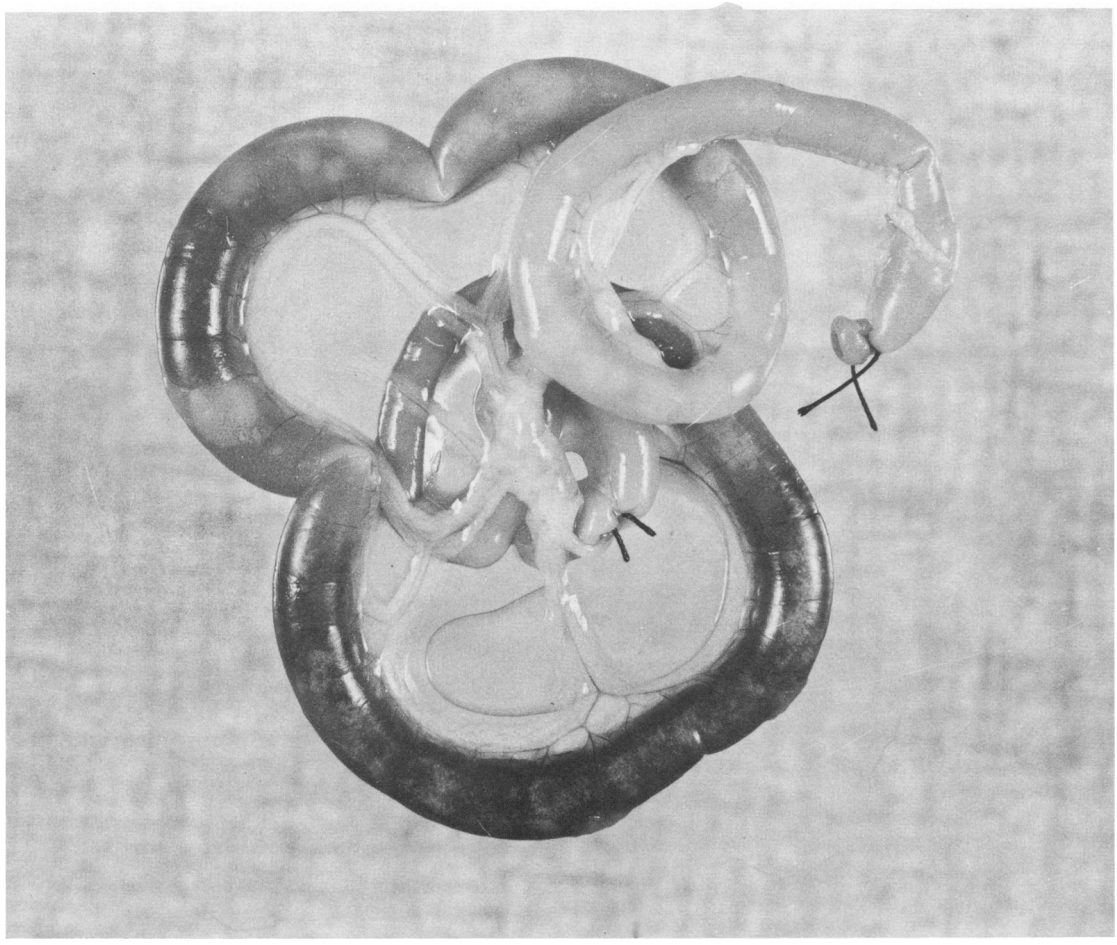

Figure 2—Gross appearance of "massive" lesions induced by the combination of PAF (2 μg) and LPS (40 μg).

Effect of PAF on Platelets

Figure 5 shows that PAF failed to aggregate rat platelets at all concentrations tested (20 ng/ml to 2 μg/ml). In contrast, human platelets aggregate at a very low dose of PAF (20 ng/ml). This phenomenon is in keeping with the observation that the *in vivo* platelet count did not change markedly after the injection of PAF (1 μg) or PAF (1 μg) with LPS (20 μg) (Figure 6).

Discussion

The work we have described establishes that endotoxin delivered to the bowel via the systemic circulation is not sufficient by itself and at the concentrations used to provoke bowel necrosis. In contrast, PAF alone is sufficient to provoke lesions of this nature that are confined to the bowel despite simultaneous infusion to other parenchymal organs, but only at doses that approach the lethal dose for this potent mediator. Last, a combination of LPS and PAF was found most effective in inducing necrotizing bowel lesions, suggesting that a mutually enhancing effect may develop when these two compounds are administered together. The relevance of these findings for human pathology, and for NEC in particular, is best brought into focus by a brief recapitulation of current concepts about the pathogenesis of this disease.

The numerous theories to explain the genesis of NEC and other, presumably related, conditions[4,5] have been the subject of repeated reviews.[2,3,6,7] In recent years, neonatal asphyxia or hypoxia has been thought to play a central role in the development of NEC lesions. Circumstantial evidence from clinical observations[8,9] and direct evidence from the experimental work of Touloukian et al[10] supported the concept that hypoxemic episodes may lower gastrointestinal blood flow to levels incompatible with maintenance of normal tissue integrity. Touloukian et al[10] demonstrated that neonatal piglets asphyxiated

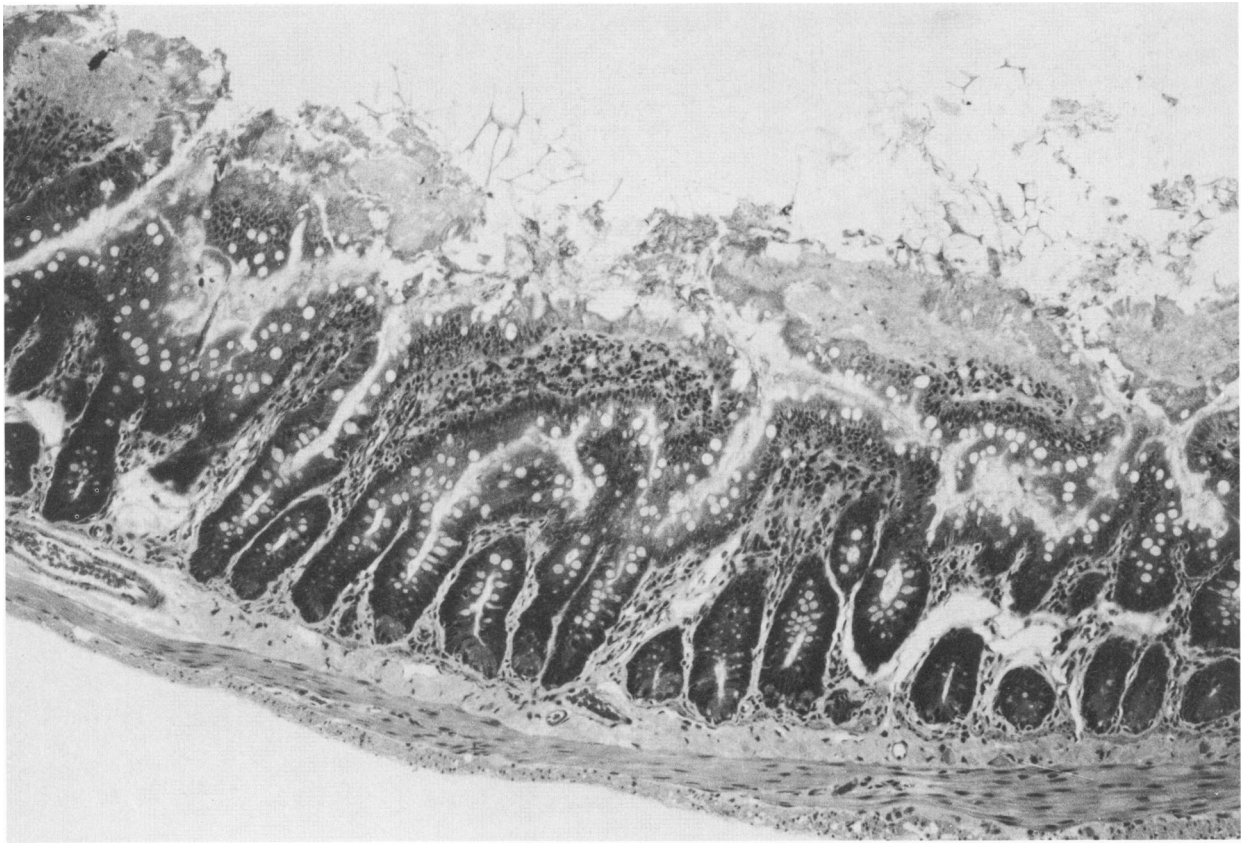

Figure 3—Intestinal necrosis confined to the tips of the mucosal villi, characteristic of lesions of mild severity. (H&E, × 120) (With a photographic reduction of 6%)

by forced rebreathing of their own expired air suffered decrements in perfusion of 35% in the proximal colon and 50% in the distal jejunum; this important information correlates with the topographic selectivity displayed by NEC lesions, thereby underscoring the relevance of local factors in enhancing the risk of bowel necrosis. It should be noted, however, that the characteristic transmural necrosis of NEC was not documented in the mentioned experiments; only mucosal hemorrhages were demonstrated. It was speculated that the short duration of the experiments explained the absence of necrosis, which, however, *may* have been present if the animals had been sacrificed later. Moreover, it had been previously remarked that outside of descriptive studies of patients with NEC, perinatal hypoxia seemed no more frequent in patients who ultimately developed NEC than in those who did not.[11] Recent epidemiologic investigations using age- and time-matched control subjects confirm the general inability to identify specific individual risk factors preceding the onset of NEC.[12] This work seems to indicate that perinatal anoxia is not etiologic for NEC. Accordingly, attention has shifted from strictly circulatory redistribution of flow, as occurs in perinatal anoxia, to other factors that may be fundamental in initiating, promoting, suppressing, or perpetuating ischemic bowel necrosis. Among these are bacteria and their toxins[12-14] and endogenously or exogenously administered chemical compounds, such as various feeding formulas[15,16] and bile salts.[17]

That bacteria or their toxins play a role in the etiology of NEC is suggested by several epidemic outbreaks of this disease, in some cases associated with a specific microorganism, and ultimately suppressed by infection-control measures,[18,19] even though recovery of either the bacteria or the toxins has been possible in some instances[13,19] and impossible in others.[20] That bacterial endotoxins do not account, by themselves, for the damage observed may be inferred from the observation that endotoxin may be recovered from the stools of subjects who are not sufferers of NEC[21,22] and from a considerable body of experimental evidence establishing that several biologic actions of endotoxin are mediated by exudate cells.[23-25] The effect of endotoxin injected into

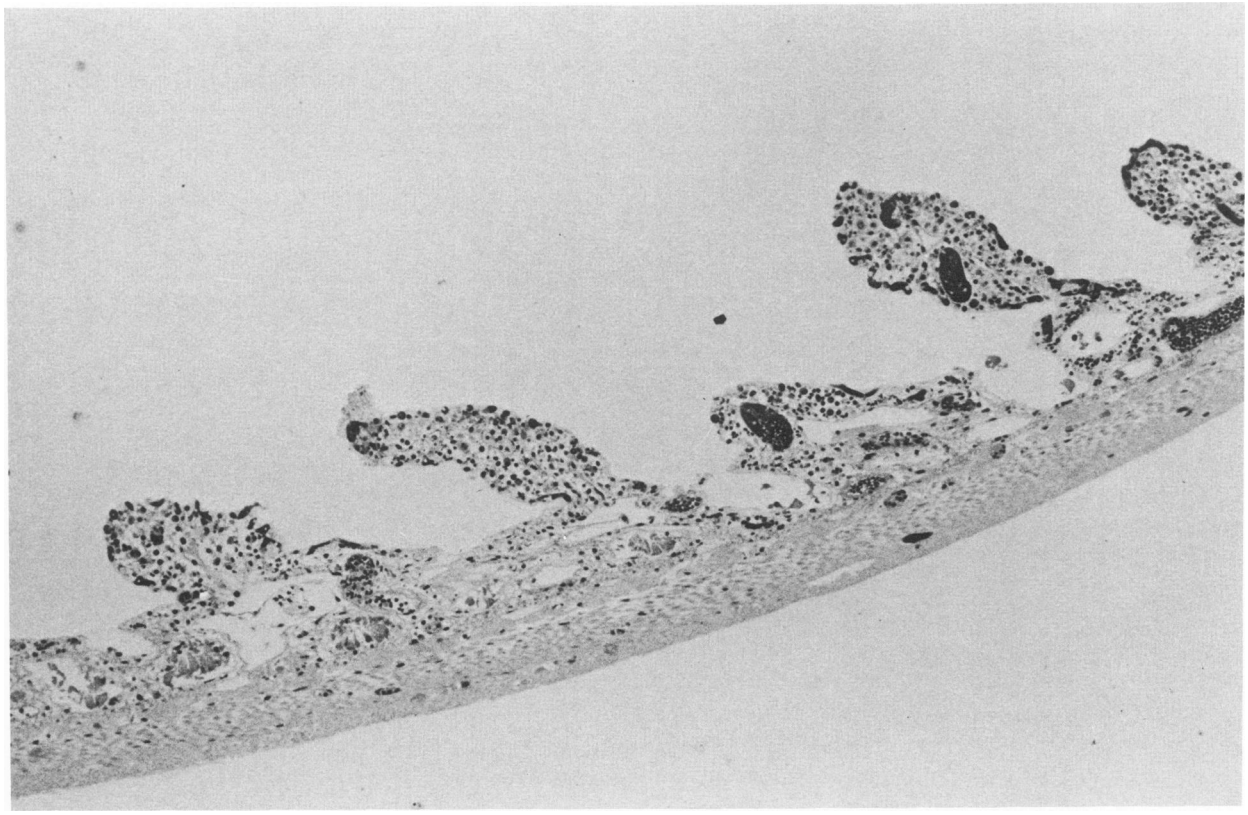

Figure 4—Transmural intestinal necrosis with complete loss of epithelial cells and poor nuclear staining in the muscular coat but no inflammatory cells. (H&E, ×120)

mesenteric arteries of rabbits has been studied[26] and shown to produce severe endothelial damage, but the dose used (LD_{50}) was many times the order of magnitude of that employed in our model. Intravenous administration of *Escherichia coli* endotoxin to piglets is followed by endothelial cell damage in the coronary arteries, but again at very high doses (4 to 8 mg/kg), or with continuous infusion.[27] A single injection of a large dose of endotoxin (10 mg/kg) to rhesus monkeys produced extensive damage to Kupffer cells, but the endothelium of heart, lung, kidney, and jejunum was intact 4 hours after injection.[28] None of these studies could discern between direct endotoxin effects and effects exerted by mediators triggered by endotoxin administration. It is not likely that under the conditions of our experiments, severe endotoxin-induced vascular damage could have accounted for the observations. This statement is supported by the low dose used and by the absence of demonstrable structural lesions in the mesenteric vasculature and is in keeping with current views that attribute the effects of endotoxins largely to the ability of these substances to interact with a variety of cells and tissues of the host.[29,30]

The pathobiologic effects of PAF are incompletely elucidated. Its chemical structure was reported in 1979 as 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphoryl choline by two independent groups (chemical identification reviewed by Cusack),[31] but investigation of its functional role had to await the availability of the synthetic molecule in sufficiently large quantities for *in vivo* studies.[32] Impetus for using this substance in our studies derived from current knowledge that PAF represents an extremely potent bioactive substance: it is the most potent platelet-aggregating agent yet discovered with as little as 10^{-9} M triggering aggregation of washed rabbit platelets.[32,33] It induces profound thrombocytopenia in guinea pigs at doses as low as 20 ng/kg; and it is secreted by a number of cells, including monocytes, peritoneal and alveolar macrophages, basophils, neutrophils, mast cells, and platelets themselves, in experimental animals as well as in man.[32] There is little question that this compound is an important mediator of the inflammatory reaction as well as a mediator of IgE anaphylaxis in rabbits.[34] It is thus noteworthy that delivery of low doses of PAF alone into the arterial mesenteric circulation failed to consistently produce lesions of

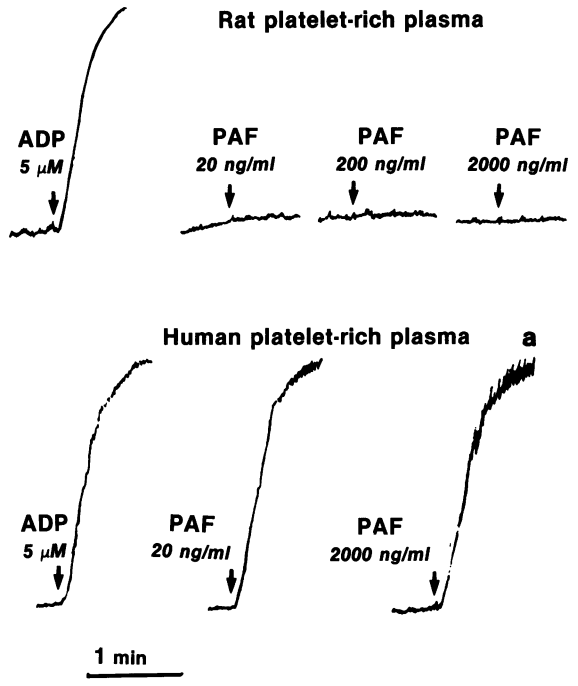

Figure 5—Platelet aggregation *in vitro* induced by ADP (5 μM) or PAF (20 ng/ml–2 μg/ml). Platelet-rich plasma was prepared as described in Materials and Methods.

bowel necrosis until the dose was increased to levels comparable to the lethal dose for rabbits.[32]

Our data suggest that the development of NEC in our model is independent of platelet aggregation and subsequent thrombus formation. This hypothesis is based on the following observations: 1) thrombi cannot be demonstrated at any time in mesenteric arteries or in the small vessels in the affected bowel; 2) the decrease of platelet counts following injection of PAF is very mild, contrary to what has been reported in rabbits;[32] and 3) *in vitro* studies using rat PRP show no aggregation of rat platelets in response to a dose of PAF several times the order of magnitude of that sufficient to aggregate rabbit platelets.[32,33] This finding corresponds well with that of Sanchez-Crespo et al,[35] who reported that rat platelets did not release serotonin *in vitro* in response to synthetic PAF.

The reproducibility of NEC-like lesions when the combination of low doses of PAF-endotoxin was infused, but not when either low doses of PAF or endotoxin were used alone, suggests several hypothetical considerations concerning the pathogenesis of ischemic bowel necrosis. It may be proposed that the development of NEC is a carefully monitored process that requires the intervention of inflammatory mediators, and our results implicate PAF in its pathogenesis. LPS could secondarily contribute to magnify, perpetuate, or aggravate the effects of this mediator. This proposal stands in contrast to theories that would attribute most of the damage to direct attack on the enteric mucosa by toxic or bacterial agents, or by ischemia. Bacterial products could gain entry into the mucosa, either by virtue of the natural ability of the neonatal ileal mucosa to absorb macromolecules, as recently proposed by Lawrence et al,[7] or in consequence of stress, hypoxia, and immaturity, as required by those who believe that macromolecules are entirely degraded intracellularly within the absorptive cells.[36] Once within the mucosa, endo- or exotoxins would come in contact with a number of cells that normally reside in the lamina propria of the gut; and a result of this interaction could be the production of inflammatory mediators, including PAF. PAF is suspected of being capable of inducing tissue breakdown, because some of its most pronounced effects, such as bronchoconstriction or thrombocytopenia, appear to be exerted directly, and not mediated by metabolites of arachidonic acid.[37] Whether directly or indirectly produced, once intestinal tissue damage is established, other mediators could be called into play, thus resulting in self-perpetuating and probably mutually amplifying pathologic effects. The work reported here demonstrates that PAF, enhanced by endotoxin, suffices to produce necrotic bowel lesions grossly and microscopically indistinguishable from NEC in humans.[38]

The precise manner in which inflammatory mediators come into play in the production of NEC has not been explored. In an undefined number of human neonatal NEC cases, an immune complex vasculitis can be demonstrated by immunofluorescence methods in areas of damaged intestine.[39] Although our

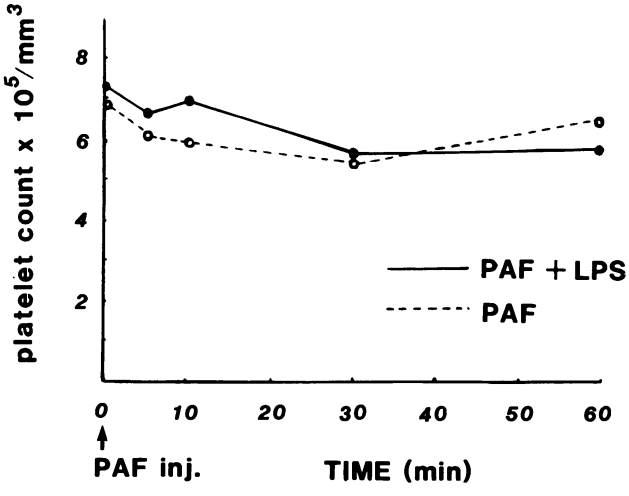

Figure 6—Change of platelet count *in vivo* following injection of PAF (1 μg) or PAF (1 μg) with LPS (20 μg). Four experiments were performed; the results of a typical experiment are shown.

work did not address this problem, it is nonetheless important to note that PAF has been implicated in immune complex deposition in rabbits[40] and in the local Arthus reaction.[41] Whether the etiopathogenesis of NEC be immunologic or nonimmunologic, the multiple biologic actions and vasoactive properties of PAF strongly suggest that it may play a role in the development of NEC lesions. PAF induces contraction of guinea pig ileal smooth muscle[42] and has been reported to induce vasoconstriction in this animal with 100–1000 times greater potency than histamine on a molar basis[43] and at least 10 times greater potency than leukotriene C-1.[44] Although in rats and rabbits vasoconstrictive potency was difficult to assess with a skin-blueing model,[43] it is possible that different patterns of reactivity exist in various anatomic circulatory territories, just as they do in different animal species.

Local vascular factors are often invoked to explain otherwise puzzling disparities in the severity of endotoxin-induced injury of similarly treated tissues.[45,46] We feel compelled to resort to the same explanation in order to account for the absence of necrotizing lesions in anatomic sites other than the gastrointestinal tract after injection of a PAF–endotoxin mixture into the abdominal aorta. Equally problematic is the lack of inflammatory cell infiltration at lesional sites, despite the known chemotactic ability of PAF[47]; but this may be related to the capacity of both endotoxin[29] and PAF[32,37] to induce neutropenia and sequestration of leukocytes in other vascular territories. The paucity of inflammatory cell infiltration has been one of the characteristic histopathologic features of NEC in humans.[38]

References

1. Britt LG, Cheek RC: Non-occlusive mesenteric vascular disease: Clinical and experimental observations. Ann Surg 1969, 169:704–711
2. Touloukian RJ: Neonatal necrotizing enterocolitis an update on etiology, diagnosis and treatment. Surg Clin North Am 1976, 56:281–298
3. Koloske AM: Necrotizing enterocolitis in the newborn. Surg Gynecol Obstet 1979, 148:259–269
4. Dunn PM: Intestinal obstruction in the newborn, with special reference to transient functional ileus associated with respiratory distress syndrome. Arch Dis Child 1963, 38:459–467
5. Firor HV, Myers HAP: Perforating appendicitis in premature infants. Surgery 1964, 56:581–583
6. Santulli TV, Schullinger JN, Heird WC, Gongaware RD, Wigger J, Barlow B, Blanc WA, Berdon WE: Acute necrotizing enterocolitis in infancy: A review of 64 cases. Pediatrics 1975, 55:376–387
7. Lawrence G, Bates J, Gaul A: Pathogenesis of neonatal necrotizing enterocolitis. Lancet 1982, 1:137–139
8. Lloyd JR: The etiology of gastric perforations in the newborn. J Pediatr Surg 1969, 4:77–84
9. Livaditis A, Wallgren G, Faxelius G: Necrotizing enterocolitis after catheterization of the umbilical vessels. Acta Paediatr Scand 1974, 63:277–282
10. Touloukian RJ, Posch JN, Spencer R: The pathogenesis of ischemic gastroenterocolitis of the neonate: Selective gut mucosal ischemia in asphyxiated neonatal piglets. J Pediatr Surg 1972, 7:194–205
11. Frantz ID, L'Heureuz P, Engel RR, Hunt CE: Necrotizing enterocolitis. J Pediatr 1975, 86:259–263
12. Kliegman RM, Hack M, Jones P, Fanaroff AA: Epidemiologic study of necrotizing enterocolitis among low-birth-weight infants: Absence of identifiable risk factors. J Pediatr 1982, 100:440–444
13. Cashore WJ, Peter G, Lauermann M, Stonestreet BS, Oh W: Clostridia colonization and clostridial toxin in neonatal necrotizing enterocolitis. J Pediatr 1981, 98:308–311
14. Kliegman RM: Neonatal necrotizing enterocolitis: Implications for an infectious disease. Pediatr Clin North Am 1979, 26:327–344
15. Book LS, Herbst JJ, Jung AL: Necrotizing enterocolitis in infants fed an elemental formula. Pediatr Res 1974, 8:379; 105
16. Barlow B, Santulli TV, Heird WC, Pitt J, Blanc WA, Schullinger JN: An experimental study of acute necrotizing enterocolitis—the importance of breast milk. J Pediatr Surg 1974, 9:587–595
17. Diaz J, Samson H, Kessler D, Stamper C, Moore E, Robiseh E, Hodson A: Experimental necrotizing enterocolitis: The possible role of bile salts in its etiology and treatment. Pediatr Res 1980, 14:595
18. Book LS, Overall JC Jr, Herbst JJ, Britt MR, Epstein B, Jurg AL: Clustering of necrotizing enterocolitis: Interruption by infection-control measures. N Engl J Med 1977, 297:984–986
19. Hill RR, Hunt CE, Matsen JM: Nosocomial colonization with Klebsiella, type 26, in neonatal intensive care unit associated with an outbreak of sepsis, meningitis and necrotizing enterocolitis. J Pediatr 1974, 85: 415–419
20. Chang TW, Areson P: Neonatal necrotizing enterocolitis: Absence of enteric bacterial toxins (Letter). N Engl J Med 1978, 299:424
21. Donta ST, Myers MG: Clostridium difficile toxin in asymptomatic neonates. J Pediatr 1982, 100:431–434
22. Sherertz RJ, Sarubbi FA: The prevalence of Clostridium difficile and toxin in a nursery population: A comparison between patients with necrotizing enterocolitis and an asymptomatic group. J Pediatr 1982, 100:435–439
23. Kampschmidt RF, Pulliam LA, Upchurch HF: The activity of partially purified leucocytic endogenous mediator in endotoxin-resistant C3H/HeJ mice. J Lab Clin Med 1980, 95:616–623
24. Sipe JD, Vogel SN, Ryan JL, MacAdam KPW, Rosenstreich DL: Detection of a mediator derived from endotoxin-stimulated macrophages that induces the acute phase serum amyloid A response in mice. J Exp Med 1979, 150:597–606
25. Kawakami M and Cerami A: Studies of endotoxin-induced decrease in lipoprotein lipase activity. J Exp Med 1981, 154:631–639
26. Stewart GJ, Anderson MJ: An ultrastructural study of endotoxin-induced damage in rabbit mesenteric arteries. Brit J Exp Pathol 1971, 52:75–80
27. Pesonen E, Kaprio E, Rapola J, Soveri T, Oksanen H: Endothelial cell damage in piglet coronary artery after intravenous administration of E. coli endotoxin. Atherosclerosis 1981, 40:65–73
28. McKay DG, Margaretten W, Csavossy I: An electron microscope study of endotoxin shock in Rhesus monkeys. Surg Gynecol Obstet 1967, 125:825–832
29. Wilson ME, Munckenbeck P, Morrison DC: Influence

of bacterial endotoxins on neutrophilic leukocytes: Lack of correlation between in vivo and in vitro response, Pathophysiological Effects of Endotoxins at the Cellular Level. Edited by JA Majde, RJ Person. New York, Alan R. Liss, 1981, pp 173-185
30. Morrison DC, Ulevitch RJ: The effects of bacterial endotoxins on host mediation systems. Am J Pathol 1978, 93:527-616
31. Cusack NJ: Platelet-activating factor. Nature 1980, 285:193
32. McManus LM, Hanahan DJ, Demopoulos CA, Pinckard RN: Pathobiology of the intravenous infusion of acetyl glyceryl ether phosphorylcholine (AGEPC), a synthetic platelet-activating factor (PAF), in the rabbit. J Immunol 1980, 124:2919-2924
33. Chignard M, LeCouedic JP, Tance M, Vargaftig BB, Benveniste J: The role of platelet activating factor in platelet aggregation. Nature 1979, 279:799-800
34. Pinckard RN, Farr RS, Hanahan DJ: Physicochemical and functional identity of platelet-activating factor (PAF) released *in vivo* during IgE anaphylaxis with PAF released *in vitro* from IgE sensitized basophils. J Immunol 1979, 123:1847-1857
35. Sánchez-Crespo M, Alonso F, Iñarrea P, Egido J: Non platelet-mediated vascular actions of 1-O-alkyl-2-acetyl-sn-3-glyceryl-phosphorylcholine (a synthetic PAF), Pharmacologie de l'inflammation et l'allergie: Lipids et cellules. Edited by F Russo-Marie, B Vargaftig, J Benveniste. Paris, Editions INSERM, 1981, pp 473-478
36. Williams RAM: Pathogenesis of neonatal necrotizing enterocolitis (Letter). Lancet 1982, 1:451
37. Vargaftig BB, Chignard M, Lefort J, Benveniste J: Platelet-tissue interaction: Role of platelet-activating factor (PAF-acether). Agents Actions 1980, 10:502-506
38. DeSa D: The spectrum of ischemic bowel disease in the newborn. Perspect Pediatr Pathol 1976, 3:273-309
39. Gray ES, Lloyd DJ, Miller SS, Davidson AI, Balch NJ, Horne CHW: Evidence for an immune complex vasculitis in neonatal necrotizing enterocolitis. J Clin Pathol 1981, 34:759-763
40. Henson PM, Cochrane CG: Acute immune complex disease in rabbits: The role of complement and of a leucocyte-dependent release of vasoactive amines from platelets. J Exp Med 1971, 133:554-571
41. Kravis TC, Henson PM: Accumulation of platelets at sites of antigen-antibody-mediated injury: A possible role for IgE antibody and mast cells. J Immunol 1977, 118:1569-1573
42. Findlay SR, Lichtenstein LM, Hanahan DJ, Pinckard RN: The contraction of guinea pig ileal smooth muscle by acetyl glyceryl ether phosphorylcholine. Am J Physiol 1981, 241:C130-C133
43. Humphrey DM, McManus LM, Satouchi K, Hanahan DJ, Pinckard RN: Vasoactive properties of acetyl glyceryl ether phosphorylcholine and analogues. Lab Invest 1982, 46:422-427
44. Drazan JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, Corey EJ: Comparative airway and vascular activities of leukotrienes Cl and D *in vivo* and *in vitro*. Proc Natl Acad Sci USA 1980, 77:4354-4358
45. McKay DG, Margaretten W, Csavossy I: An electron microscope study of the effects of bacterial endotoxin in the blood vascular system. Lab Invest 1966, 15: 1815-1829
46. Richman AV, Gerber LI, Balis JU: Peritubular capillaries: A major target site of endotoxin-induced vascular injury in the primate kidney. Lab Invest 1980, 43:327-332
47. Humphrey DM, Hanahan DJ, Pinckard RN: Induction of leucocytic infiltrates in rabbit skin by acetyl glyceryl ether phosphorylcholine. Lab Invest 1982, 47:227-234