Endoperoxidation, Hyperprostaglandinemia, and Hyperlipidemia in a Case of Erythrophagocytic Lymphohistiocytosis

Reversal With VP-16 and Indomethacin

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Clinicopathologic and histopathologic evidence of both endoperoxidation with hyperprostaglandinemia and hyperlipidemia in a 5-week-old infant with a hemophagocytic syndrome is reported. Institution of histiocytolytic (VP-16) and cyclo-oxygenase inhibitor (indomethacin) therapies returned the prostaglandin levels and lipid profile to a nearly normal state coincidental with clinical recovery. It appears that by reducing the cell mass of histiocytes and controlling the over-production of prostaglandins, some types of hemophagocytic syndrome can be reversed.

Cancer **60:2388–2393, 1987.**

HEMOPHAGOCYTIC lymphohistiocytoses occurring in infants generally have been categorized as either familial erythryophagocytic lymphohistiocytosis (FEL)[1-3] or virus-associated hemophagocytic syndrome (VAHS).[4,5] More recently, the terminology of reactive hemophagocytic syndrome (RHS)[6] has been proposed to supplant the latter and encompass those cases in which other infectious agents or etiologic factors might be involved. Obviously, correct categorization as FEL or RHS is of extreme importance so that appropriate anti-infectious disease therapies, when available, can be instituted and cytotoxic chemotherapies that might further compromise host defenses of an infected patient avoided. In making this distinction, some have utilized the presence of hyperlipidemia as a diagnostic feature of FEL.[7,8]

This report accomplishes the following: (1) presents the clinicopathologic and histopathologic evidence of both endoperoxidation with hyperprostaglandinemia and hyperlipidemia in a 5-week-old infant with a hemophagocytic lymphohistiocytosis; and (2) documents the efficacy of histiocytolytic (VP-16)[9,10] and cyclo-oxygenase inhibitor (indomethacin)[11] therapies in returning the lipid profile and prostaglandin levels to a nearly normal state coincidental with clinical recovery.

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The authors thank Margaret White and Sam Ohirhian for technical help and Karen Hurd for secretarial assistance in preparing the manuscript.

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Accepted for publication March 26, 1987.

Case Report

A 5-week-old boy infant was transferred from a community hospital to Cook-Fort Worth Children's Medical Center (C-FWCMC) Forth Worth, Texas, because of a combination of marked hepatosplenomegaly, anemia, and thrombocytopenia. He was the product of an unremarkable term pregnancy and delivery to a gravida V, para V mother who did not have any previous history of significant personal or family illness.

Physical examination revealed a febrile (38.6°C) infant with tachypnea, mild jaundice, hepatosplenomegaly and oral "thrush." Initial laboratory data revealed the following: leukocytosis (27,700/μl; expected range for age, 5000–19,500/μl)[12] with a predominance of lymphocytes; thrombocytopenia (14,000/μl); conjugated hyperbilirubinemia (7.3 mg/dl total with direct of 6.8 mg/dl); prolongation of the prothrombin and activated partial thromboplastin times (>70 and >180 seconds, respectively); hyperalanine and aspartate aminotransferasemias (361 and 764 IU/L, respectively); and increased serum lactate dehydrogenase total activity (1320 IU/L; expected range for age, 208–473 IU/L).[12] A bone marrow aspiration revealed all hematopoietic elements to be well represented but with a relative increase in histiomonocytic cells, some of which were demonstrating hemophagocytosis. Karyotyping of the bone marrow was read as 46, XY.

On the second hospital day, a Broviac catheter was placed and an open liver biopsy performed in the operating room. Histopathologic studies on this specimen included the follow-

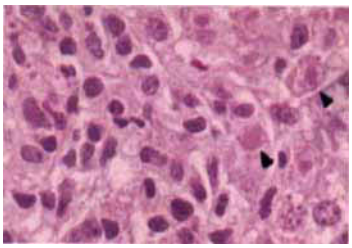

FIG. 1. Medium-power view of infiltrate in the portal region reveals its predominantly lymphohistiocytic nature with extension across the limiting plate and eosinophilic degeneration and/or necrosis (arrowheads) of occasional periportal hepatocytes (H & E, ×460).

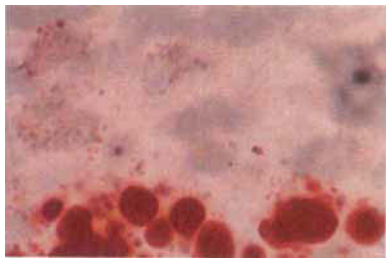

FIG. 2. High-power view of periportal hepatocytes (lower margin) showing numerous, lipid inclusions and, by contrast, only fine droplets, if any, of lipid in cells of the portal infiltrate (oil-red-O, ×1000).

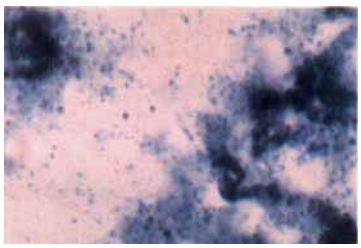

FIG. 3. Portion of portal region showing variable indophenol blue positivity of individual cells (Winkler-Schultze, ×1000).

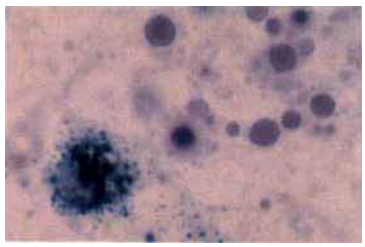

FIG. 4. Periportal region showing indophenol blue positivity in a cell with a reniform nuclear outline, probable histiocyte or macrophage. Notice lipid inclusions in adjacent hepatocytes and absence of indophenol blue positivity (Winkler-Schultze, ×1000).

ing: oil-red-O and Winkler-Schultze histochemical procedures on cryostat sections of fresh tissue for lipids and fatty acid peroxides,[13] respectively (cryostat sections of fresh tissue from a lymph node with a documented reactive B-cell process served as a control in the Winkler-Schultze procedure for a negative reaction by lymphocytic elements); staining of deparaffinized sections with hematoxylin-eosin for routine light microscopic study; and examination, in a transmission electron microscope, of uranyl acetate and lead citrate stained thin-sections (70 nm) prepared from Carson's fixed, osmium postfixed portions of liver. These revealed the following: a (predominantly) lymphohistiocytic infiltrate in the portal triads (Fig. 1) associated with erythrophagoytosis by Kupffer cells and focal intralobular and interlobular cholestasis; panlobular steatosis (Fig. 2); Winkler-Schultze positivity in the portal infiltrate (Figs. 3 and 4); and at the ultrastructural level, morphologic evidence of erythrophagocytosis by histiocytes; an apparent interaction between lymphocytes and histiocytes and among lymphocytes; and finally, peroxidative-type damage[14] to contiguous cells (possibly periportal hepatocytes, Fig. 5). No viral inclusions were appreciated ultrastructurally.

Similarly, he was seronegative for monotypic (IgM) antibodies to Epstein-Barr viral capsid antigen (<1:10), herpes simplex viruses of types 1 and 2 (<1:10), and cytomegalovirus (<1:10).

Subsequent to the histopathologic findings, a fibrinogen level and an 8-hour "fasting" (D_5 solution had to be given during this interval to counter hypoglycemia) lipid profile were obtained. Hypofibrinogenemia (75 mg/dl) and a type IV hyperlipidemic pattern were demonstrated. The latter was characterized by the following: a significant increase in the relative percentage of the pre-β fraction (50.5% by densitometry; expected reference range, 12.0–21.0%)[15] associated with a prominent anodal subfraction displaying a staple-like configuration (Fig. 6); hypertriglyceridemia (314 mg/dl) with eucholesterolemia (109 mg/dl); and marked hyperfattyacidemia (3100 μEq/l; expected range for age, 215–875 μEq/l).[12] Extreme anodal migration of all fractions accompanied the latter [16] (Fig. 6). Additionally, serum prostaglandin E_2 (PG E_2) and F_2 alpha (PG $F_2\alpha$) levels, on an aliquot of this specimen revealed considerable hyperprostaglandinemia (1746 and 2040 pg/ml, respectively; expected ranges, 250–400 and 80–240 pg/ml, respectively).[17]

Several therapeutic regimens were designed in an attempt to deal with these biochemical aberrations. Firstly, hypertonic glucose (initially D18) as part of parenteral alimentation was administered in an attempt to mobilize endogenous insulin and thereby block lipolysis and mobilization of free fatty acids.[18] Similarly, an antioxidant dosage[19] (approximately 100 IU/kg/day) of an oral vitamin E preparation was begun to counter any toxic effects of fatty acid peroxides. Although this approach resulted in clinical improvement and stabilization with regression in both liver and spleen size and improvement in biochemical, hematologic, and coagulation parameters (Table 1), he once again became febrile (38.9°C) on the seventeenth hospital day. Three successive routine blood cultures obtained around this episode revealed "no growth." Furthermore, his pyrexia was accompanied by a distinct reenlargement of the liver and spleen.

In light of this, on the nineteenth hospital day, he was begun on a biweekly course of chemotherapy using intravenous epipodophyllotoxin (VP-16), a histiocytolytic agent known to be effective in treating FEL,[9,10] at a dose of 5 mg/kg. Two days later, intravenous indomethacin (1 mg every 12 hours) was added in an attempt to interrupt endoperoxidation and prostaglandin synthesis.[11]

Considerable improvement in his hematologic and clinical state was evident within 1 week after his having been on the latter regimen. He continued to improve and on the fortieth hospital day, the VP-16 was decreased to once weekly doses. His coagulation parameters and lipid profile returned to a more nearly normal state (Table 1, Fig. 7) and he was discharged on the forty-ninth hospital day. The dismissal plan was for him to receive VP-16 at weekly intervals and to remain on indomethacin (5 mg twice daily, orally), nystatin, and vitamin E. At the time of the writing of this report (approximately 4 months postdiagnosis), he has not experienced any relapses

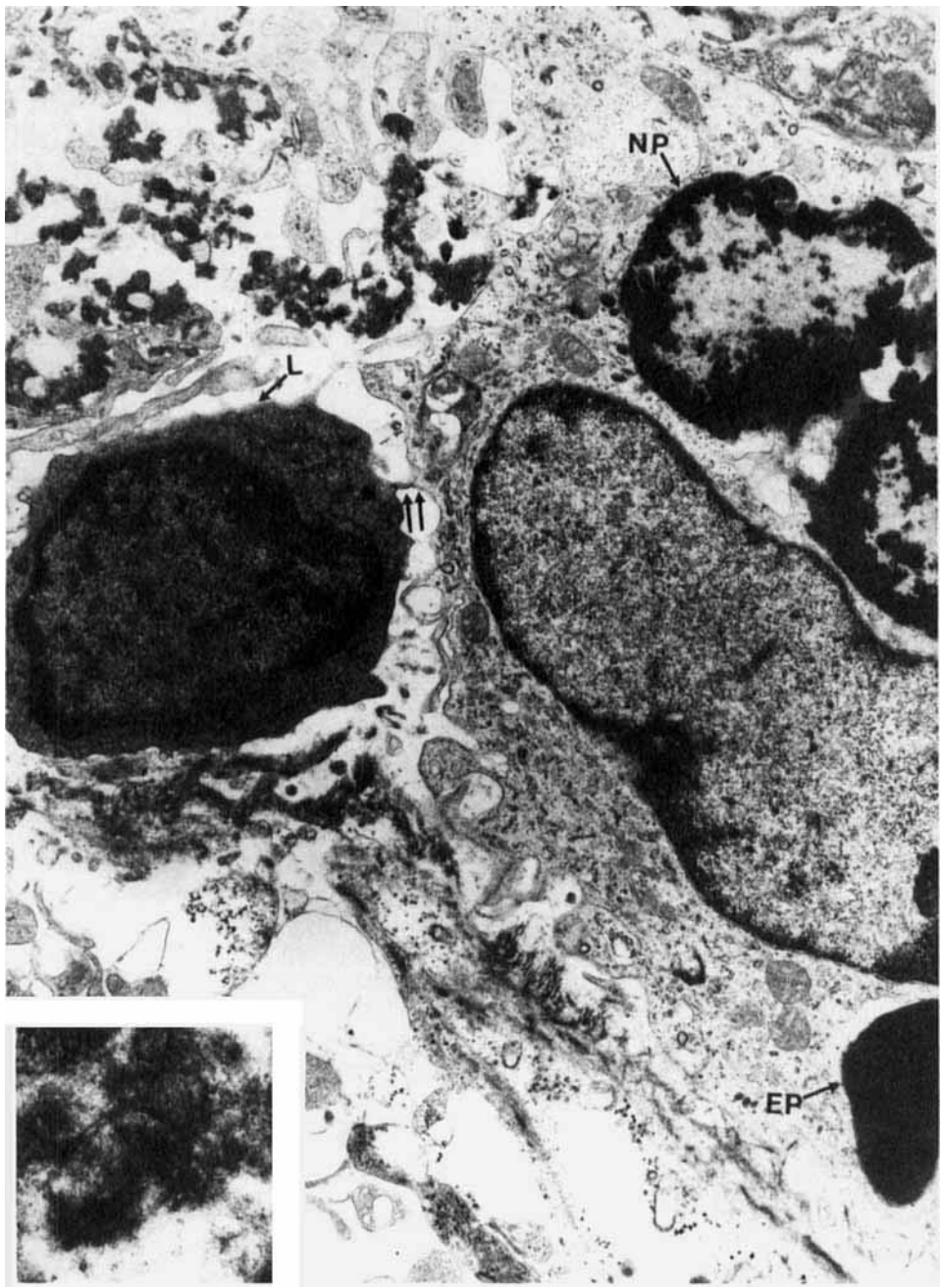

FIG. 5. Ultrastructural overview of portion of portal triad with a histiocyte showing erythrophagocytosis (arrow EP) and nucleophagocytosis (arrow NP); intimate association (double arrows) with a lymphocyte (arrow L); and close proximity to amorphous aggregates of osmiophilic material (upper left). Inset: Amorphous aggregates consist of granular, electron-dense mate rial with entrapped membranous structures (×10,000; Inset: ×48,000).

and continues to grow and develop in a normal fashion, although his liver and spleen are still easily palpable.

Discussion

In the absence of serologic, microbiologic or histopathologic evidence of an infectious cause, it appears that this infant's hemophagocytic lymphohistiocytosis probably falls into the subcategory of FEL. Therefore, the histochemical finding of a positive Winkler-Schultze reaction for fatty acid peroxides[13] in his (portal) lymphohistiocytic infiltrate when coupled with hyperprostaglandinemia suggests to us that excessive endoperoxidation is occurring in FEL. Furthermore, our interpretation is consistent with the studies of Devictor and co-workers suggesting a hyperactivation of the reticuloendothelial system in FEL with secretion by monocytes of large quantities of PG E_2.[20]

Moreover, this phenomenon could account, at least theoretically, for many of the clinical, histopathologic

and clinicopathologic findings in FEL to include the following: fever; inhibition of lymphocyte transformation *in vitro;* progressive visceral involvement by lymphocytes and histiocytes; hypofibrinogenemia; thrombocytopenia; erythrophagocytosis; and hyperfattyacidemia with both panlobular steatosis in the liver and type IV hyperlipidemia. By way of elaboration, pyrogen-induced fever is associated with a rise in hypothalamic PG $F_{2}\alpha$ (which initially was quite elevated in the serum of our patient; *vide supra*) and such fevers can be inhibited by indomethacin which blocks the synthesis of same.[11] Similarly, the plasma factor from patients with FEL that inhibits the proliferative response of lymphocytes from normal donors[21] could be linked, at least in part, to elevated PG E_2 levels (again as documented in the serum of our patient) given the fact that phytohemagglutinin-induced lymphocyte transformation is inhibited by PG E_2.[11] In addition, the immunosuppressive effects of PG E_2, *in vivo,* could hamper immunologically mediated modulation of the usual barrage of postnatal antigenic challenges resulting in multifocal stimulation of the lymphoreticular system and thereby progressive visceral involvement by lymphohistiocytic proliferations in such infants. Furthermore, proliferating histiocytes engaged in antigen processing and hemophagocytosis could be responsible for hypofibrinogenemia through the release of plasminogen-activator[22,23] with resultant plasmin-mediated fibrinogenolysis.[24] The concurrent rise in our patient's plasminogen and fibrinogen levels, as previously documented in Table 1, subsequent to the institution of histiocytolytic (VP-16) therapy is consistent with this thesis.

Thrombocytopenia and erythrophagocytosis in FEL might be secondary to the effects of several products of endoperoxidation. Platelet trapping as a result of both the proaggregatory action of PG E_2 and thromboxane A_2 and the promotion of platelet adhesion to vessel walls by the latter could account in part for the thrombocytopenia.[11,23,25] Modification of the erythrocyte membrane

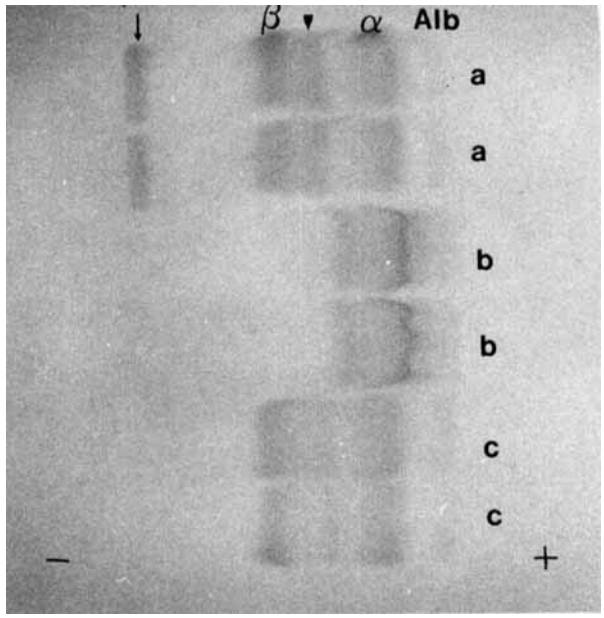

FIG. 6. Photographic reproduction of the oil red O-stained electrophoregram comprising six electrophoretic "strips." a: Replicate applications of the normal control (lipotrol) are represented; and reading from cathode (–) to anode (+) reveals the application point (↓); β, pre-β (▼), and α lipoprotein bands; and albumin (Alb). c: Replicate applications from an essentially normal patient are represented showing bands in the β, pre-β, and α regions. b: Replicate applications of the patient's specimen are represented showing distinct and marked anodal migration of all fractions (particularly β); some reduced staining intensity of the β and α fractions; and a relatively broad pre-β band with an intensely stained anodal subfraction that has a staple-like configuration.

TABLE 1. Biochemical, Hematologic, and Coagulation Parameters, *Versus* Therapeutic Regimens

	Parameters						
	Biochemical (serum)				Hematologic	Coagulation	
Therapeutic regimens	Triglyceride (mg/dl)	FFA (μEq/l)	PG E_2 (pg/ml)	PG $F_{2\alpha}$ (pg/ml)	Platelets/μl	Fibrinogen (mg/dl)	Plasminogen (% NHP)
*	314 (2)	3100 (2)	1746 (2)	2040 (2)	14000 (2)	75 (2)	31.8 (2)
†	285 (5)	650 (5)	—	—	47000 (12)	210 (12)	68.9 (12)
Exacerbation	143 (19)	—	—	—	36000 (19)	165 (19)	—
‡	—	—	285 (21)	310 (21)	28000 (20)	125 (20)	47.6 (20)
§	—	—	190 (37)	82 (37)	243000 (35)	215 (35)	57.9 (35)
‖	134 (47)	650 (47)	—	—	206000 (47)	340 (47)	59.0 (47)

FFA: free fatty acids; PG E_2: prostaglandin E_2; PG $F_{2\alpha}$: prostaglandin $F_{2\alpha}$; % NHP: percentage normal human plasma.

* Supportive: ± D5 · 1/3 NS ± ceftazidime ± nystatin ± platelets ± fresh frozen plasma ± cryoprecipitate ± erythrocytes.

† Supportive + vitamin E (approximately 100 IU/kg/d) + hypertonic glucose (≥ d 18).

‡ Supportive + vitamin E + hypertonic glucose + VP-16 (5 mg/kg, twice weekly).

§ Supportive + vitamin E + hypertonic glucose + VP-16 + indomethacin (1 mg every 12 hours).

‖ Nystatin + vitamin E + VP-16 (5 mg/kg, weekly) + indomethacin (5 mg twice daily, orally).

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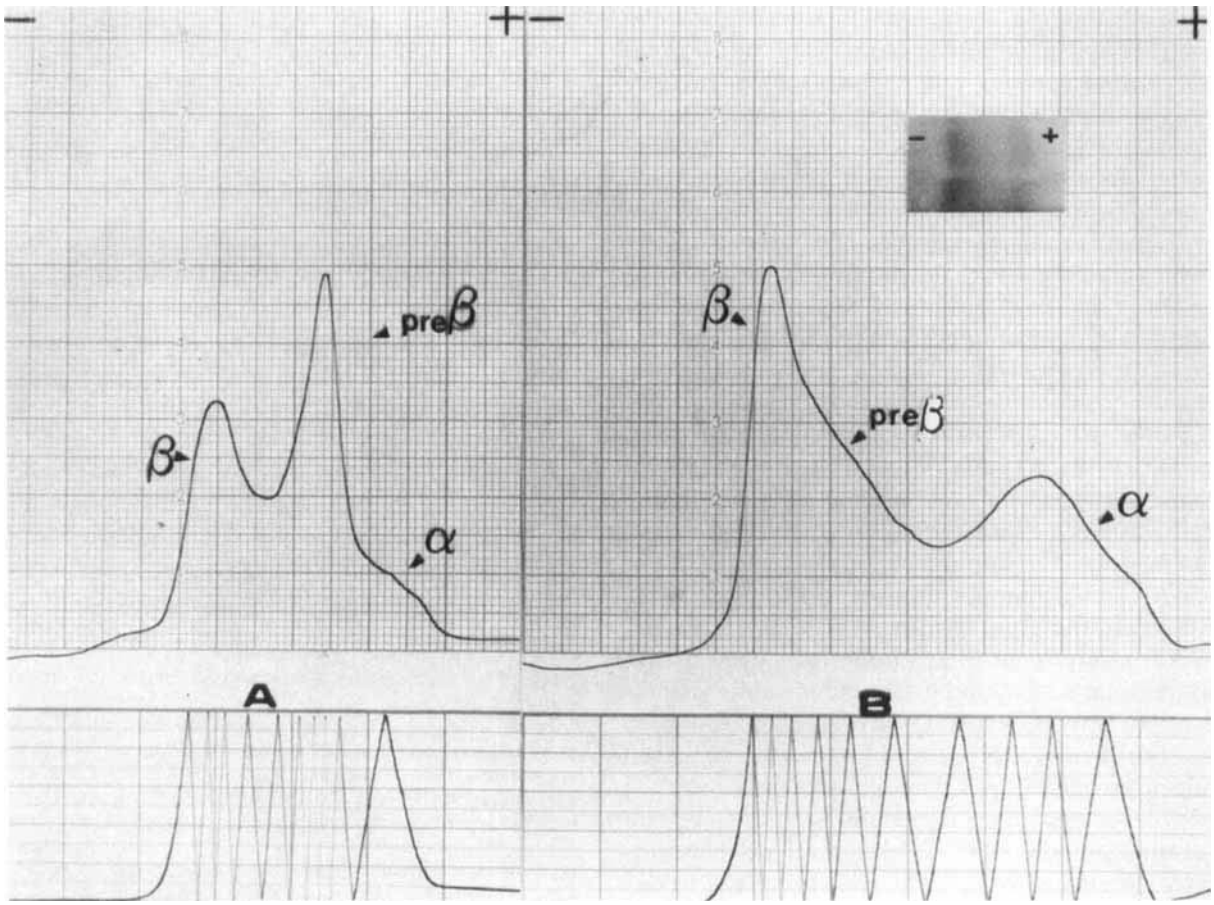

FIG. 7. Tracings from densitometric scanning of lipoprotein electrophoregrams on postadmission hospital days 2 and 47, A and B, respectively, reveal a significant decrease in the vertical peak amplitude of the pre-β fraction which accords with a corresponding decrease in the serum triglyceride from the hypertriglyceridemic into the eutriglyceridemic range (*i.e.*, 314 → 134 mg/dl). *Inset:* Photograph depicts relatively close alignment of the patient's bands in the upper strip vis-a-vis the control, lipotrol, in the lower strip. A return to normal electrophoretic mobility (compare and contrast with findings in Figure 6 coincides with a decrease in the patient's free fatty acid concentration from the hyperfatty acid into the eufattyacidemic range (*i.e.*, 3100 → 650 μEq/l).

by malondialdehyde and peroxides and reduced deformability imposed by PG E_2 should promote erythrophagocytosis by Kupffer cells, splenocytes, and proliferating histiocytes including those in the bone marrow.[11,26,27]

The hyperfattyacidemia in FEL can be explained on the basis of an interplay of the known effects of PG E_2 and PG $F_2\alpha$ on factors that influence the adipokinetic/antiadipokinetic balance. That is to say, because PG $F_2\alpha$ (and to some extent PG E_2) can effect a release of several adipokinetic factors in man to include growth hormone, adrenocorticotropin, and (subsequently) glucocorticoids and because PG E_2 can suppress the release of the principal antiadipokinetic agent, insulin,[11,18] we believe that elevated levels of these prostaglandins in FEL could result in excessive lipolysis with mobilization of free fatty acids and hyperfattyacidemia. Reesterification of this increased fatty acid load in hepatocytes would result in panlobular steatosis[28] and following complexing of the triglycerides with apolipoproteins, in a type IV hyperlipidemia.

Conversely, increased levels of certain of the mobilized fatty acids such as linoleic, by serving as precursor substrate in endoperoxidation,[25] could contribute to the state of hyperprostaglandinemia; and thereby, a propagation of the disease process. The decline in our patient's free fatty acid level after the institution of hypertonic glucose therapy alone and the associated amelioration of his disease process support this contention.

In short, we propose that the pathogenesis of FEL may involve an inherent hyper-responsiveness on the part of the patient's lymphohistiocytic cells to any of a number of the expected postnatal antigenic challenges resulting in exaggeration of their usual state of endoperoxidation.[20,29] Furthermore, many of the clinical manifestations and biochemical and hematological aberrations that characterize FEL could then be ascribed to known effects of PG E_2 and PG $F_2\alpha$. Finally, the efficacy

of histiocytolytic (VP-16) and cyclo-oxygenase inhibitor (indomethacin) therapies in returning the prostaglandin levels and lipid profile to a nearly normal state coincidental with clinical recovery supports our thesis and provides hope for controlling an otherwise fatal disease utilizing relatively nontoxic therapies.

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