TABLE II. Effect of Linoleic Acid on Growth of Fat-Deficient Guinea Pigs.

Supplement (mg daily for 1 wk)	Wt (g) 1 week before treatment	Wt (g) Time of starting treatment	Total gain (g) Wks after starting treatment 1	2	3	4
10	462	477	3	23	31	30
30	538	534	12	18	25	40
60	486	486	14	51	69	80
0	624	655	0	0	−12	4
0	576	587	8	12	15	6

acid in all 3 of the animals. Improvement in the animal which received the 10 mg dosage appeared to have subsided by the end of the third week. Eventually deficiency symptoms reappeared in all of the supplemented animals but latest in the one treated with the highest dosage of linoleic acid.

Summary. These experiments have shown that omission of fat from the diet of the guinea pig results in a syndrome characterized by: retardation of growth, dermatitis, marked drying of the inside of the ears and, in some animals, ulcers, loss of fur, and a tendency to a swollen, somewhat cyanotic condition of the feet. All of these symptoms were corrected by the oral administration of linoleic acid. These results afford the first demonstration of the necessity for the guinea pig of a dietary source of linoleic acid or, possibly, of some other fatty acid present in corn oil.

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Lipemia Clearing by Hyaluronidase, Hyaluronate, and Desoxycorticosterone, and its Inhibition by Cortisone, Stress, and Nephrosis. (21209)

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The lipemia clearing action of heparin(1) has been amply confirmed. A similar action has been reported for other compounds(2,3) but has been established with certainty only for sulfated polysaccharide anticoagulants (2,4,5). The apparent specificity of the type of compound that caused lipemia clearing and the anticlearing action of protamine and antiheparin dyes have suggested to some observers that heparin has a physiologic role in the transport and metabolism of lipids(5-9).

We have reported that hyaluronidase (H) has antihypercholesterolemic action(10). It was of interest therefore to determine whether it would also clear lipemia. Since in our studies of the ground substance(11-13) partially depolymerized hyaluronic acid (PDHA) and desoxycorticosterone (DCA) had effects resembling those of H while cortisone and stress were opposite in effect, it was of further interest to ascertain whether the same relationship prevailed with respect to lipemia clearing.

*Materials and methods.** Purified hyaluronic acid (HA) and PDHA were the same as that used in our previous studies(14). The HA had 3.4% N, less than 0.01% carbohydrate by the anthrone method(15,16) and no P, S, or halogen. The bovine testicular H (6000 TRU/mg N) used in all tests had 1.9 U proteolytic activity/mg by the method of Hadidian(17). Higher potency H (28000 TRU/mg N) was used to inactivate the lipemia clearing factor (CF) *in vitro* in order to eliminate significant proteolytic activity (less than 0.005 U/test). Sodium alginate was prepared by Dr. Harvey E. Alburn and was fractionated to yield a product with a molecular weight of 13,000-20,000 to approximate that of heparin. Plasma was decanted from citrated blood (0.1 ml M/10 Na citrate/ml) centrifuged at 2000 RPM until separation oc-

* All compounds were dissolved in 0.9% NaCl except DCA which was in oil (Cortate, Schering) and cortisone which was a 0.5% suspension of crystalline acetate in 0.5% acacia.

TABLE I.
Effect of Plasma from Treated Rats on Light Transmission of Lipemic Dog Plasma (LP).

Rat treatment	Route	mg/kg	Avg % light transmission					
			LP	LP + rat plasma min. after inj. 60	120	180	240	300
Heparin	IV	4	24	86	84	85	45	43
"	PO	50	26	32	34	25	30	28
H	SC	150 TRU	27	69[4]	85[4]	86	97[5]	86[4]
PDHA	IV	10	29	89	86	88	86	84
"	SC	50	29	77[5]	98	79	54	29
"	PO	50	18	78	93	70	54	25
HA	IV	25	16	72[5]	54	46	30	20
DCA	IM	5	30	41	28[5]	64	91	77
Cortisone	IM	5	10	9[5]	10[4]	10[5]	10[5]	10[5]

Transmission values are averages of 6 rats except where superscripts indicate smaller groups.

curred, immediately stored at 5°C, and used only on the day of collection. Hemolyzed plasma was discarded. Rat blood was collected from the heart exposed under light ether anesthesia. Lipemic plasma (LP) was from dogs receiving 20-25 ml cottonseed oil (CSO)/kg intragastrically and was considered satisfactory if the light transmission did not exceed 30%. Light transmission was read at 650 μ in a Beckman quartz spectrophotometer using silica microcuvettes. Standardization was against distilled water. All readings were made at 22°C. In most instances the effect of a compound on lipemia was determined in a 2-stage test(18,19) by incubating 1.0 ml LP with 0.1 ml rat plasma for 5 minutes at 22°C and recording the change in light transmission. Each of the following compounds was administered to 15 male and 15 female albino rats weighing approximately 130 g: heparin intravenously and orally, 4 and 50 mg/kg , respectively; H subcutaneously, 150 TRU; HA IV, 25 mg/kg; PDHA IV, 1, 5, 10, 25, and 50 mg/kg; PDHA SC and PO, 25 and 50 mg/kg, respectively; DCA intramuscularly, 5 mg/kg; and cortisone acetate IM, 5 mg/kg. All oral administrations were in fasted rats. Groups of 3 male and 3 female rats were killed at hourly intervals thereafter for determination of plasma clearing activity. The effect of PDHA (10 and 20 mg/kg IV, and 50 mg/kg PO), HA (25 mg/kg IV) and alginate were also tested in dogs. The course of lipemia induced in fasted dogs was established for 6 hours in 3 males and 3 females. Lipemia was repeatedly induced at 3-4 day intervals for the purpose of testing the effect of a compound or for restandardization. When PDHA was tested for efficacy by the oral route the dogs did not receive CSO; instead the plasma taken at hourly intervals was added to lipemic plasma *in vitro* in the ratio of 0.1 ml:0.5 ml. Plasma from the following groups of rats was tested for ability to inhibit (CFI) the clearing action of plasma (CF) from rats receiving 4 mg heparin/kg IV: one hour after cortisone (5 mg/kg IM); 24 hours after exposure to 2°C; 14 days after nephrotic syndrome was induced by administration of anti-kidney serum (AKS). CFI from cortisone was also tested against CF 3 hours after DCA (5 mg/kg IM) and one hour after PDHA (10 mg/kg IV). LP (2.0 ml) was incubated 5 minutes at 22°C with 0.1 ml CF + 0.1 ml CFI. Heat stability of CFI was determined by heating on a water bath for one hour at 60°C and adding to LP + CF. Susceptibility of CF to inactivation by H was determined by incubating plasma obtained one hour after 4 mg heparin/kg IV with 10 TRU H/ml at 36°C for one hour. Aliquots were withdrawn every 5 minutes and tested against LP. Average potency H was used in one test and high potency H in another.

Results. Table I lists the various compounds that were administered to rats for determining the effect on LP. The degree of lipemia of the control plasma used for testing the particular compound is also listed. The CF activity of plasma samples taken from rats sacrificed at the times indicated after injection of the compound is indicated as per cent transmission. Since normal plasma gave transmission readings of 85% or better, LP (30% or

TABLE II. Effect of Hyaluronate and Alginate on Lipemia in Dogs.

	% transmission (avg 6 dogs) min. after CSO orally									
	Pretreatment	60	90	120	150	180	210	240	300	360
Lipemia control (standardization)	93	36		21		29		35	25	46
PDHA IV (20)‡	70	55*	77	85		88		59	46	37
PDHA IV (10)	94	55*	92	92	84	64		52	41	8
Restandardization	89	38		32		28		40	44	48
Alginate IV (50)	92	48*	16	17		18†	42	56	61	
Restandardization	80	33		28		18		30	32	36
PDHA PO (50)	86	66		87		95		64	46	
LP	87	43		14		17		36	37	

* Compound administered. † HA administered. ‡ ( ) mg/kg.

less) was considered to be cleared when light transmission approximated these. Each clearing value listed in Table I is the average of 6 rats except where smaller groups are indicated by superscripts. When pronounced lipemia clearing was present, transmission above 60% had a S.D. of ±4-6% and below 40% the S.D. was ±10-20%; the increase in the S.D. can be attributed, in part, to the shorter sojourn of the CF in some animals than in others. When no lipemia clearing occurred the S.D. was ±4-6%. In addition to confirming the effectiveness of intravenous heparin the data show that H, PDHA, and DCA released CF into the plasma. HA appeared also to have this property but to a much lesser degree. Cortisone did not release CF. Release of CF following oral administration of PDHA is in contrast to the ineffectiveness of orally administered heparin.

The values in Table II are averages obtained on 6 dogs and show 1) the lipemia clearing action of parenteral PDHA and HA in dogs receiving large doses of CSO by stomach tube, 2) the failure of alginic acid, a plant polyuronic acid, to exert such action, and 3) the effectiveness of PDHA when administered orally.

Incubation of CF with medium and high potency H preparations (10 TRU/ml) at 36°C resulted in progressive loss of clearing activity. With both preparations the decrease was 20% in 15 minutes of incubation, 50% in 25 to 30, and 100% in 45 minutes. CF maintained at this temperature for 60 minutes had no loss of activity. Table III lists data showing that cortisone treatment, stress, and the nephrotic syndrome inhibited the lipemia clearing action of CF plasma. Dilution of CFI with physiological salt solution decreased the inhibitory action. The inhibitor was stable when heated at 60°C for one hour.

Discussion. Our chief interest was in establishing the effect of H, HA, PDHA, DCA, cortisone, stress, and nephrosis on lipemia clearing. The method employed is quantitative for establishing the potency of a compound with respect to total clearing and for duration of action. These studies reinforce decisively the current concept that lipemia clearing is unrelated to anticoagulant action because of all the compounds used only heparin prevents clotting. The data establish that CF activity may be completely abolished by CFI. They are not consistent with the growing concept that heparin is the physiologic agent responsible for lipemia clearing. It appears that mucopolysaccharides of the ground substance or agents that activate them may release CF; conversely, those agents that depress their activity may release CFI. Thus H and DCA which increase permeability of the ground substance by depolymerizing HA also released CF; cortisone and stress, which have opposite effects on the ground substance released CFI. Alginate of the same molecular weight as heparin did not release CF, although in some respects it resembles mucopolysaccharides, the chief difference being that it does not contain amino sugar residues.

The fact that subcutaneous injection of H released CF *in vivo* is not inconsistent with the finding that H inactivated CF *in vitro*. The amount of H injected subcutaneously would not achieve an adequate concentration in the blood to duplicate the *in vitro* results. It is more likely that injection resulted in the formation of PDHA which released CF. It

TABLE III. Effect of CF and CF + CFI Rat Plasma on Lipemic Dog Plasma (LP).

No. rats CF	Source of CF	No. rats CFI	Source of CFI	Avg % light transmission LP	LP + CF	LP + CF + CFI
6	Heparin	15	Cortisone	9	96	10
6	PDHA	6	"	22	86	22
6	DCA	6	"	20	97	21
6	Heparin	6	Stress	20	96	21
6	"	4	.1 AKS*	18	86	41
6	"	3	.2 "	23	86	27
6	"	5	.4-.8 "	21	86	23

* ml anti-kidney serum/100 g of rat.
CF = Lipemia clearing factor.
CFI = " " " inhibitor.

has been established that CF is readily destroyed by heat, high concentrations of salt, and chymotrypsin digestion. It has also been established that CF contains a lipoprotein which requires a non-lipoprotein co-protein in order to produce clearing(8). H is not known to act on lipoproteins and it may be assumed that it may have hydrolyzed a mucopolysaccharide moiety of the co-protein. It is thus indicated that it is not likely that heparin is the mucopolysaccharide attached to CF. It has already been pointed out that the proteolytic activity of the H used can be considered as negligible.

The finding that an inhibitor of lipemia clearing was released by cortisone, stress, and renal disease is of considerable importance. The effect of cortisone and stress indicates that release of CFI is another manifestation of the general adaptation syndrome. CFI was present in all rats manifesting the nephrotic syndrome but only those in the more severe phases had gross lipemia in addition. The question is raised whether development of pathological lipemia is preceded by increase of inhibitor to a critical level necessary to completely suppress clearing. CFI effectively neutralized considerable amounts of CF and it therefore remains to be established whether administration of heparin in non-anticoagulant doses and of other CF releasing agents would be of benefit in the treatment of pathological lipemia.

The mechanism by which CFI suppresses CF is not known. The heat stability suggests that CFI is not a complex protein.

Summary. 1. Partially depolymerized hyaluronic acid released a lipemia clearing factor following intravenous, subcutaneous and oral administration to rats. Hyaluronidase and DCA which were administered only to rats hypodermically were also effective. 2. Plasma from rats treated with cortisone prevented the lipemia clearing by heparin, DCA and partially depolymerized hyaluronic acid. The effect of exposure to cold was tested only against heparin lipemia clearing and was also found to inhibit it. 3. Release of a lipemia clearing inhibitor appears to be another manifestation of the general adaptation syndrome. 4. Plasma from rats made nephrotic by administration of low doses of anti-kidney serum also had an inhibitor of the clearing factor, and those given larger doses of anti-kidney serum had in addition gross lipemia suggesting that pathological lipemia may be preceded by the appearance of the inhibitor. 5. The lipemia clearing factor was inactivated by incubation with hyaluronidase *in vitro* suggesting that this factor contains a mucopolysaccharide moiety which is not heparin. 6. The activity of the inhibitor of the lipemia clearing factor was not affected by heating at 60°C for one hour.

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Failure of Newborn Rat to Respond to Hypoxia with Increased Erythropoiesis.* (21210)

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It is known that the newborn young of many mammals have a normal or higher than normal erythrocyte count and hemoglobin(1) and that these values decrease immediately after birth, reaching minimal and subnormal levels within a matter of days or weeks depending on the species. One of the proposed explanations of this neonatal anemia is that in intra-uterine life the fetus exists under reduced oxygen tension, to which it responds by active erythropoiesis(2). After birth the newborn, having adequate oxygen, no longer requires the excess of red cells formed during intra-uterine life. Red cell production decreases somewhat, the physiological anemia of the newborn supervenes, and the count only gradually returns to a stable, normal level.

If the hypothesis were sound that hypoxia is the direct determining factor for the high count at birth and, therefore, indirectly responsible for the ensuing anemia, then continued hypoxia after birth should prevent the neonatal anemia or perhaps even result in polycythemia. It is the object of this paper to report the hematological changes found in the newborn rat when exposed to low oxygen tension, as compared with the well-known erythropoietic response of older rats to hypoxia.

Materials and methods. Rats of the Long-Evans strain were used throughout these experiments. Two litters of 6 male rats each were divided into 2 groups; 3 were chosen from each litter. One group, 6 rats, was exposed in a large decompression chamber to a simulated altitude of 15,000 feet for 6 hours a day from the 4th to the 18th days of age. At the end of each exposure, the young were returned to their own mothers. The remaining 6 rats, which served as controls, were also removed from their mothers over the same period daily for 6 hours. The temperature of the chamber was maintained at 25° ± 2°C. The relative humidity was in excess of 95% throughout the experiment. To further protect the young from chill and desiccation, they were placed in a small feeding can in a deep well of shavings, which simulated a nest and prevented their scattering. The controls, during the period when they were separated from their mothers, were similarly protected. The nursing mothers were maintained on a

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