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Effect of Experimental Allergic Encephalomyelitis on Guinea Pig Plasma Lipid Fractions.* (29125)

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Kies *et al*(1) noted the occurrence of visible lipemia in sera of severely paralyzed guinea pigs after the induction of experimental allergic encephalomyelitis (EAE). This lipemia included markedly elevated triglyceride and less marked increases of cholesterol and phospholipid. There was also a significant increase in the lipid-staining material which migrated electrophoretically with albumin.

Dole(2) and Gordon and Cherkes(3) described the small but metabolically active free fatty acid (FFA) fraction of plasma which has subsequently been recognized to have a major role in fat metabolism(4). Goodman(5) noted that free fatty acids in the plasma are bound to albumin, and described the albumin binding sites.

On the hypothesis that the lipemia of EAE might be brought about by increased mobilization of FFA from fat depots with its subsequent reappearance as triglyceride, phospholipid, and cholesterol esters, plasma FFA concentrations were measured during early and late stages of EAE. Just as had been observed with total plasma lipids, plasma FFA values were highest in severely affected animals, but even at 15 or 16 days after injection, when many of the animals had not yet shown definite neurological signs, the FFA values were significantly higher than normal.

Since paralyzed animals do not eat and since fasting can increase FFA(2,3,6), the effect of fasting was compared to the effect of EAE on blood lipid fractions. Although fasting may increase all lipid fractions, certain quantitative differences were noted between fasting and EAE.

Experimental. Animals: Disease-free mixed color guinea pigs bred in a closed colony in the Animal Production Section at the National Institutes of Health were used for all of the experiments described. The animals were young males varying in weight from 250-500 g depending upon the individual experiment. All animals were housed in large general purpose cages, 6 to a cage, on a bed of cedar shavings. They were obtained from the stock colony one week prior to injection and given a regular stock diet for guinea pigs (NIH formula A) plus a daily supplement of greens (lettuce and kale).

Induction of experimental allergic enceph-

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TABLE I. Comparison of Plasma FFA and Visible Lipemia in Guinea Pigs with Paralytic EAE.

FFA	Visible Lipemia	Total* Lipid	FFA	Visible Lipemia	Lipid
1.85	+++	—	.38	0	—
1.27	+	—	.78	0	—
1.13	+	—	.71	0	—
1.00	+	—	.70	0	—
.93	+++	1550	.98	0	—
.88	+	590	.98	0	—
.77	+	890	.59	0	422
1.09	+	1150	.63	0	390
1.21	+++	1620	.79	0	386
.80	+	870			
1.60	+	750			
1.17	++	800			
$\bar{x} \pm$ S.D. 1.14 ± .32		1028 ± 379	.73 ± .19		399 ± 20

* Total lipid concentrations available are included to illustrate relationship of visible lipemia to total lipid.

alomyelitis (EAE) and experimental allergic aspermatogenesis: EAE was induced in the guinea pigs by one intracutaneous injection of 0.1 ml of the following emulsion: 4 mg lyophilized bovine cord, or cord protein, 1 ml phenol-saline (0.5% phenol-0.9% NaCl), 1 ml melted Aquaphor (Duke Laboratories) and 2 ml mineral oil (Fisher Scientific Co.) containing 4 mg ground heat-killed *Mycobacterium butyricum* (Difco). The mixture was homogenized in a glass homogenizer, heated to 60°C and rehomogenized immediately before injection. One-tenth ml of the warm emulsion was injected into a shaved area over the sternum.

Animals were weighed daily and examined for signs of neurological damage, such as hind leg weakness or paralysis, inability to right themselves after being placed on their backs, fecal impaction, tremors, etc. The disease index used for quantitative measure of disease severity was the same as that described by Alvord and Kies(7). The scale of 0 (no disease) to 10 (maximum response) is based on independently evaluated clinical and histological evidence of disease.

Allergic aspermatogenesis was induced in the guinea pigs by 7 intracutaneous injections over the back and neck of 0.1 ml of the following emulsion: 40 mg *Mycobacterium butyricum* (Difco), 8.5 ml mineral oil (Fisher Scientific Co.), 1.5 ml melted Aquaphor (Duke Laboratories), 10 ml phenol-saline (0.5% phenol-0.9% NaCl), and 30 mg of a testicular protein fraction designated as ASPM(8). The animals were sacrificed 21 days after inoculation.

Determination of FFA and blood lipids: All blood was collected for 30 seconds from the severed vessels of the neck in silicone-coated test tubes, each containing 3 drops of heparin (100 mg/ml). Blood samples were immediately placed in ice. Duplicate plasma samples were extracted with a mixture of 2,2,4-trimethylpentane (iso-octane), glacial acetic acid, and acetic anhydride. After 2 washings with 0.18 N H_2SO_4, the extracted FFA were determined by titration with 0.02 N NaOH(9). The remaining plasma was then frozen at −21°C and stored for determination of other blood lipids later.

Plasma samples were thawed and then extracted with a 1:1 mixture of absolute alcohol and acetone. Aliquots of this mixture were then measured for total lipids, phospholipid, and free and total cholesterol. Triglycerides were calculated by difference as suggested by Bragdon(10). Total lipids were determined by the method of Bragdon(10), phospholipids by the method of Fiske-SubbaRow after digestion(11), free and total cholesterol by the $FeCl_3$ method(12).

The two-tail Student's *t* test and the standard correlation coefficient were used for statistical evaluation. Values are expressed as means ± standard deviations.

Results. Table I shows the relationship of FFA to visible lipemia in 21 paralyzed

TABLE II. Effect of Early EAE on Plasma FFA.

Time after disease induction	N	FFA ($\bar{x} \pm$ S.D.)	D.I.
No induction	11	.41 ± .12	0
15 days	8	.62 ± .26*	1.6
16 "	7	.75 ± .20†	3.1

* $P < .05$ } EAE vs control.
† $P < .001$ }

guinea pigs chosen at random with an average disease index of 9.3. Those with visible lipemia are on the left; those without it are on the right. The animals were sacrificed from 13 to 21 days after inoculation with antigen Freund's adjuvant. The normal guinea pig has an FFA concentration of 0.41 ± 0.12 meq/l with a range of 0.23 to 0.55 meq/l(6). All but one of the 21 animals had FFA values out of the normal range (above 0.55 meq/l), the mean FFA of the paralyzed animals being 0.96 ± 0.34 meq/l, a value which is significantly higher than the normal mean ($p < 0.001$). The 12 animals with visible lipemia also had higher FFA concentrations ($p < 0.01$) and higher total lipid concentrations ($p < 0.001$) than those animals without visible lipemia.

The effects of early EAE on FFA are shown in Table II. The animals sacrificed 15 and 16 days after EAE inoculation showed minimal signs of EAE involvement but nevertheless had mean FFA values of 0.62 ± 0.26 and 0.75 ± 0.20 meq/l, respectively. One of these animals had visible lipemia, and this one had the highest FFA of the group (1.08 meq/l). The disease indices of these two groups were 1.6 and 3.1. Histologic evidence of EAE was found in 3 of 8 animals killed on the 15th day and in 5 of 8 animals killed a day later.

From the experiments presented, one can recognize that the plasma free fatty acid (FFA) concentration is an excellent index of the severity of EAE. Of 49 animals with EAE there is highly significant correlation between the FFA and disease index ($r = +0.50$ and $p < 0.001$). Increased FFA consistently appears earlier in the development of EAE than visible lipemia, although animals with visible lipemia had the highest FFA.

In Fig. 1 the effect of fasting is compared to that of paralytic EAE on plasma total lipid, triglyceride, cholesterol and phospholipid concentrations. EAE produced proportionately larger increases in total lipid, triglyceride, and phospholipid ($p < 0.001$) than fasting. Plasma cholesterol was not significantly different from fasting levels noted.

Twelve guinea pigs with severe allergic aspermatogenesis on a normal diet had a mean FFA concentration of 0.33 ± 0.06, which is within the normal range. Visible lipemia was not observed in any of these plasmas.

Discussion. The previous study from this laboratory(1) reported a lipemia in guinea pigs with EAE in which the amount of plasma lipid was positively correlated with the severity of this disease. Electrophoretic studies revealed 2 areas of plasma protein which were associated with increased lipid, the albumin and β globulin. The animals in the previous and in the present study were on the usual low fat diet. The increases in plasma FFA reported in this study would most likely coincide with the lipid peak in the albumin area. The other elevated lipids would appear to be a β-lipoprotein constituent.

As reviewed by Olson and Vester(13), the β-lipoproteins are synthesized by the liver largely from that portion of FFA not utilized by the animal for other purposes. It seems plausible, therefore, that the increased FFA

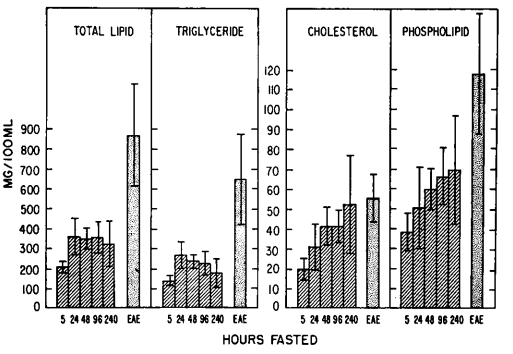

FIG. 1. Effect of fasting and paralytic EAE on plasma lipids. Lipid concentrations expressed as mean ± 2 S.E.M. Data were obtained from 11 guinea pigs with paralytic EAE and 6 guinea pigs in each fasted group.

of these animals with EAE results in increased synthesis of triglyceride, phospholipid, and cholesterol. If so, the lipemia associated with EAE resembles other states of lipemia with increased FFA mobilization, as in diabetes(14), injections of long-acting epinephrine compounds(15), and injections of anterior pituitary gland extracts(16-19).

The mechanism of the elevated FFA concentrations during EAE is unknown. Although paralyzed animals eat less than normal, it seems unlikely that fasting can explain all the FFA changes noted in EAE (Fig. 1). Furthermore, of the guinea pigs killed 15 and 16 days after injection of the encephalitogenic vaccine (Table II), 4 animals were gaining weight and eating well without outward manifestation of encephalitis but nevertheless had elevations of FFA.

The belief that lipemia associated with EAE results from the general inanition and fasting of paralyzed animals has been stated by Wender(20). In the present experiments plasma lipid levels even after as much as 10 days of fasting did not compare with those observed in animals whose clinical signs had been apparent no more than 24-28 hours.

The high concentrations of FFA and visible lipemia noted in EAE do not appear to be related to delayed hypersensitivity *per se*. Guinea pigs with severe allergic aspermatogenesis induced by large amounts of testis extract in Freund's adjuvant had normal FFA concentrations and no visible lipemia. The lipid changes would therefore be more readily attributed to encephalitis rather than delayed hypersensitivity.

There are, among others, two hypotheses which might relate the increased FFA and hyperlipemia to the neurological damage noted in EAE. The first is increased sympathetic discharge due either to severe stress to the animal or to the irritation of neural tissue itself. Epinephrine(2,3), norepinephrine(21), and even psychic stress(22-24) have been shown to increase FFA. Recently Correll has shown that neural stimulation itself will increase FFA(25).

The second possibility is that during the inflammatory reaction occurring in the CNS there is elaboration of the fat-mobilizing substance in the pituitary which has been studied by Rudman and associates(16) and others(17-19). The composition of the lipemia noted by Rudman *et al* in rabbits injected with such fat mobilizing extracts is similar to that of the guinea pigs with severe EAE in that the plasma triglyceride and phospholipid concentrations are increased proportionately more than total plasma cholesterol.

Summary. Significant elevations of plasma free fatty acid (FFA) concentrations have been observed in guinea pigs with experimental allergic encephalomyelitis (EAE). FFA values correlated well with disease index. Increased values were observed prior to marked clinical signs of disease and preceded development of visible lipemia. Those animals with lipemia had the highest FFA concentrations observed. Significant increases in other plasma lipid fractions were noted in guinea pigs fasted from one to ten days but these were smaller in all fractions than increases noted in encephalomyelitic guinea pigs, except for plasma cholesterol. Normal FFA concentration and no visible lipemia were noted in guinea pigs with severe aspermatogenesis. Two possible hypotheses are proposed which might relate these increased lipid fractions to the neurological damage noted in this encephalitis rather than to delayed hypersensitivity.

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Erythrocyte Fatty Acid Composition and Apparent Permeability to Non-Electrolytes.* (29126)

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The presence of lipids in the cell membrane was inferred from permeability studies(1), because the ability of non-electrolytes to penetrate the cell increased as the oil-water partition coefficients increased. Thus the composition of the membrane lipids might be expected to exert some control over the rate of penetration of solutes into the cell. DeGier and his co-workers(2) subjected the lipids extracted from the erythrocytes of various animal species to analysis and attempted to correlate the lipid composition with permeability towards certain non-electrolytes. The results indicate a possible correlation between the mixed fatty acid composition of the erythrocyte membrane and the permeability of the cell to non-electrolytes(3). The response of the cellular fatty acids to dietary fat(4,5) provides a means of testing this hypothesis in a single species.

Materials and methods. Male weanling albino rats were fed a diet containing 68% casein, 10% dextrose, salts, vitamins(6) and 10% corn oil, castor oil, butterfat, or hydrogenated coconut oil. The fatty acid compositions of the erythrocytes were determined by gas liquid chromatography of the methyl esters after the animals had received the diets for a period of 22 weeks(5).

Permeability to 3 non-electrolytes, glycerol, thiourea, or diethylene glycol, was determined by following the rate of hemolysis of the cells in isotonic solutions of the non-electrolytes. The procedure was based on that employed by Jacobs *et al*(7). Isotonic solutions of the non-electrolytes (0.3 M) were prepared using water distilled in an all glass apparatus. The temperature of the solution was adjusted to 25°C and 3.0 ml were pipetted into a 1 cm glass cuvette. The tails of representative rats were washed with absolute ethanol, 20 mm^3 of blood was drawn from a fresh cut in the tail, added to the solution of the non-electrolyte and mixing accomplished by gently blowing through the pipette employed for making the addition. The cuvette was immediately placed in the well of a Cary recording spectrophotometer, the chart set in motion, and the change in optical density of the cell suspension at 600 $m\mu$ recorded against time. This graph represented the clearing of the cell suspension

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