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Effects of Unsaturated Fat on Serum Lipids in Idiopathic Hyperlipemia. (23266)

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It has been known for some time that dietary fat influences serum lipid levels. Beveridge *et al.*(1) found that diets high in animal fat produced elevations in serum lipid levels, while diets high in fats of vegetable origin did not. Ahrens and his associates(2) also produced lower serum lipid levels with vegetable oils, especially corn oil. The serum lipid lowering effect of vegetable fat has been attributed by Kinsell(3) and more recently by Bronte-Stewart(4) to its higher content of unsaturated fatty acids. Bronte-Stewart(4, 5), in particular, has shown that the lipid lowering effect of administered fats in normal individuals may be directly proportional to the unsaturated fatty acid content of the diet. It was reported by Lever(6), and confirmed in this laboratory(7), that the intravenous administration of a cottonseed oil preparation (Lipomul I-V)* on some occasions led to a drop in serum lipids in patients with idiopathic hyperlipemia. Administration of the oral product (Lipomul Oral)* failed to lower serum lipids. Since the oral preparation is primarily cocoanut oil, containing almost no unsaturated fatty acids, this may explain the lack of success with the oral product. According to Deuel(8), the iodine number of cocoanut oil is 8-40, while that of cottonseed oil is 105-115.

Methods. Two patients with idiopathic hyperlipemia were placed on diets carefully controlled with respect to fat content. One patient, E.B., was a male 29 years old, the other, D.L., a male 31 years old. Neither patient was hospitalized during the course of the experiments. The following diets were used in this study: Diet I, uncontrolled with respect to fat content; and Diet II, which contained 120 g fat consisting of 50 g of highly purified soybean oil,† 30 g of soy phospholipid concentrate,‡ and 40 g animal fat. Diet III, administered to only one patient (D.L.) had no added soy phospholipid concentrate. This diet, which was isocaloric with Diet II, contained 50 g of soybean oil,† 40 g of animal fat, and 10 g of vegetable fat. The caloric content of all diets was 2400-2600. Serum lipid partitions were done on these patients on fasting blood samples taken

* Supplied by the Upjohn Co., Kalamazoo, Mich.

† Supplied by the Procter and Gamble Co., Cincinnati, O. This soybean oil had the following composition:

Iodine No.	131.9
Total fatty acids	95.9 %
Free " "	.0
OH number	1.6
Unsaponifiable	.5
Tocopherol	.12
Trans fatty acids	.2

Spectrographic analysis yielded the following values:

Saturated fatty acids	16.6 %
Oleic acid	21.2
Linoleic acid	50.4
Linolenic acid	7.7

‡ Purchased from Associated Concentrates, Woodside, L. I., N. Y.

TABLE I. Effect of Unsaturated Fats on Serum Lipids. Patient E. B.

	Diet I*	Diet II* (Fed 10/16-12/22)			Diet I* (Fed 12/22-3/1)	
	10/15	11/3	12/3	12/21	1/31	3/21
Total lipid†	2137	1314	1219	1012	1454	1705
Total cholesterol†	560	471	302	274	448	478
% cholesterol esters	77	81	76	80	77	80
Phospholipids†	413	418	366	263	369	463
Triglycerides†	888	184	395	325	403	505

* See text for description of diets. † Values expressed as mg %.

at intervals before, during, and after the administration of the special diets. Total lipids were determined by the method of Bragdon (9), total cholesterol and cholesterol esters by the Schoenheimer-Sperry method(10), and phospholipids by the Fiske-Subbarrow method (11).

Results. The serum lipid values resulting from the present study are listed in Tables I and II. It can be seen from Table I that the first patient, E.B., showed a marked drop in serum values for total lipid, total cholesterol, phospholipid, and triglycerides during the period of administration of Diet II. When the patient resumed eating Diet I, these values all showed marked increases. Although the absolute values did change, there was no significant change in percentage of cholesterol present in esterified forms.

Table II shows the results obtained on the second patient, D.L. The same qualitative picture is repeated. The extraordinarily high total lipid values obtained on this patient under Diet I showed marked decreases during the administration of Diet II. Resumption of Diet I produced very great elevations in all serum lipid values studied. This patient was then placed on Diet III, which contained high levels of unsaturated fatty acids, but without added phospholipid concentrate. During the period of Diet III, the patient again demonstrated marked decreases in all the serum lipid fractions studied. It should be noted that this patient also demonstrated no significant change in the percentage of cholesterol present in esterified forms. Both patients had opaque, milky sera, which cleared during the periods on Diets II and III. Both patients had tuberous xanthomata of the extensor joint surfaces. One patient, D. L., had generalized papular or eruptive xanthomas and lipemia retinalis. The papular xanthoma and lipemia retinalis disappeared, and the tuberous xanthoma diminished in this patient during the administration of Diet II. Patient E.B. showed some disappearance of the tuberous xanthomata.

Discussion. This dietary study would seem to support the concept that the lipid lowering effect of administered fats may be dependent on the unsaturated fatty acid content of these fats. The recent findings of Bronte-Stewart(4,5) suggest that unsaturated dietary fat may prevent elevations of serum lipid in the presence of moderately large amounts of dietary animal fat. We believe that the study of hyperlipemic patients provides a very convenient means of evaluating this hypothesis, because of the very high initial serum lipid values found in idiopathic

TABLE II. Effect of Unsaturated Fats on Serum Lipids. Patient D. L.

	Diet I*	Diet II* (Fed 10/16-12/21)		Diet I* (Fed 12/21-2/1)	Diet III* (Fed 2/1-3/1)
	10/15	11/21	12/20	1/31	3/1
Total lipid†	20316	1985	3627	23327	3724
Total cholesterol†		454	489	2344	582
% cholesterol esters		75	72	71	71
Phospholipids†		429	728	2104	877
Triglycerides†		871	2171	17741	1984

* See text for description of diets. † Values expressed as mg %.

hyperlipemia.

Summary. Two patients with idiopathic hyperlipemia were placed on 2400-2600 calorie diets containing 50 g of highly purified soybean oil, with or without the addition of a phospholipid concentrate. Marked decreases in serum lipids occurred during the period of administration of both diets. The serum lipid decreases were independent of phospholipid concentration in the diet, and believed attributable to the presence of unsaturated fatty acids in the dietary fat. Clinical signs of the disease regressed or disappeared coincident with the decrease in serum lipids.

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Effect of 5-Hydroxytryptophane Upon Electroencephalogram in Hepatic Coma.* (23267)

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Liver failure is usually associated with serious disturbances of consciousness. These episodes are accompanied by electroencephalographic changes of a diffuse sort with an increase in amount and amplitude of slower frequencies and a diminution of faster frequencies. Some investigators have reported what they considered to be characteristic neurologic signs(1) and electroencephalographic patterns(2-3) in liver disease, but these have not been observed uniformly(4). Observations of increased ammonia content of blood in liver failure(5-6), caused by inability of the liver to remove or "detoxify" ammonia from the portal blood because of shunt or deficiency of the urea synthesizing system have led to an hypothesis for the mechanism of ammoniagenic coma(7). The liver, however, does far more than detoxify substances. It has been pointed out recently that some metabolites essential to brain are probably synthesized in the liver and that liver failure may lead to serious deficiency of these substances(8). Among these may be the precursor material for serotonin, which appears to play a significant role in brain metabolism(9-11). Parenteral injection of moderate amounts of serotonin causes no change in the brain content of this intermediate, but administration of small amounts of a precursor substance, 5 hydroxytryptophane, (5HTP), does cause a large increase of serotonin in brain(12-13) together with symptoms similar to those seen after a block in serotonin metabolism in brain (14) caused by lysergic acid diethylamide. Although the synthesis of 5 HTP in liver preparations has not been demonstrated, other hydroxylations of a similar nature have occurred in that organ. Indirect evidence for failure of supply of the serotonin precursor comes from observations that urinary excretion of 5 hydroxyindole acetic acid, (5HIAA),

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a major terminal metabolite of serotonin, is diminished in liver disease(15).

Methods. In view of these observations 10 mg of dl 5-hydroxytryptophane were given intravenously to 3 patients with liver failure and to 3 individuals who were not in liver failure. Electroencephalograms were made before, during, and after infusion. Measurements were made of the excretion of 5 HIAA, and of blood levels of serotonin during the experiment. These will be reported elsewhere.

Results. The electroencephalograms of all patients with hepatic failure exhibited the usual diffuse slow activity. During infusion of the 5 HTP all 3 showed a diminution of the slow activity and an increase of fast activity, representing a change in the record toward a more normal pattern. None of the patients developed a completely normal pattern, however. Fig. 1 is an example of the tracing from one patient in liver failure who had a very slow record before administration of the 5 HTP. The lower half presents the recording after infusion of 5 HTP (which took 20 minutes) was completed. With due allowance for the EKG artefact, it is obvious that there is an increase in faster frequencies and a decrease in the slow activity. These changes lasted for about an hour and the record gradually reverted to its previous form. The patient expired about 8 hours later.

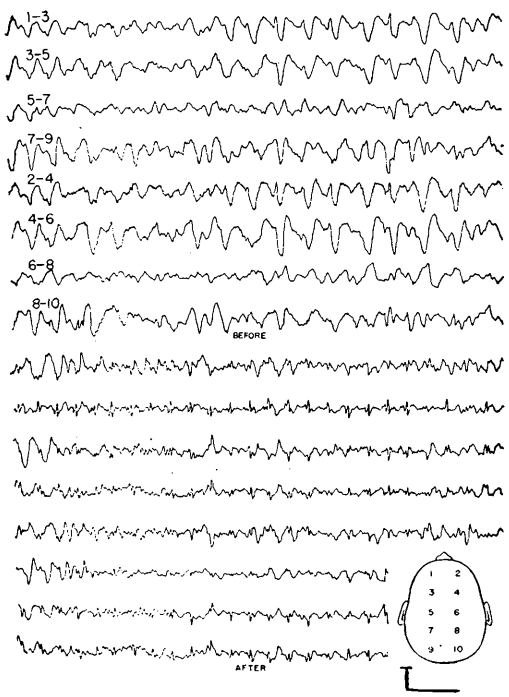

FIG. 1. Calibration 100 microvolts and 1 sec.

In the controls there was no change in the electroencephalogram of a patient with subdural hematoma on administration of 5 HTP, even though this record showed markedly slow activity bilaterally. In one patient with peptic ulcer and normal EEG, and in another patient with uremia and a mild diffusely slow EEG, the administration of 5 HTP produced no gross EEG changes.

All patients showed an increased excretion of 5 HIAA, amounting to about 80% of the expected amount of 5 HTP administered (calculated as the 1 form) within 6 hours. This is somewhat more rapid than the excretion noted on larger dosage(13). The fact that all patients did convert the 5 HTP to 5 HIAA equally well shows that the liver disease had no effect on conversion of 5 HTP. This furnishes further indirect evidence that the liver is concerned only with the hydroxylation of tryptophane to 5 HTP, since the 3 patients with liver failure also had low initial urinary excretion of 5 HIAA.

This evidence of a deficiency of an essential metabolite for brain caused by failure of the liver adds to the increasing understanding of the dependence of cerebral upon hepatic function. It also suggests that there might well be other essential precursors required by the brain, many of which might be supplied only by the liver. This would broaden the concept of the mechanism of hepatic coma to include substances which fail to appear in normal amounts in the circulation as well as those which accumulate excessively.

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Ovarian Weight and Responsiveness to Gonadotrophin Throughout the Estrous Cycle of Mice. (23268)

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Information about ovarian weight and capacity to respond to gonadotrophin at each stage of the estrous cycle is necessary for investigation of the effects of gonadotrophin on the ovary of the intact adult female. Astwood(1) reported the average uterine weight during estrous cycle of the rat, but data on weight of ovary during the cycle are not available for either rat or mouse. Folley and Malpress(2) stated that the stage of the estrous cycle had no effect on response of the cow ovary to exogenous gonadotrophin. Rowson (3) and Willett *et al.*(4) reported that cows gave a greater ovulatory response when gonadotrophin was administered during the follicular phase of the cycle. The purpose of this investigation was to determine if the weight of the mouse ovary and its capacity to respond to a constant level of equine serum gonadotrophin fluctuated during the estrous cycle.

Methods. Virgin female mice of the C57BL/6JAX strain, 90 to 120 days of age were used. To determine the stage of the estrous cycle, a vaginal smear was taken the day the mouse was put on experiment. The smears were air-dried and stained with 0.5% methylene blue in saline solution; the stage of the cycle was designated according to the scheme of Allen(5). Routine daily vaginal smearing prior to use of animals was not done because this tended to upset the cycle in a high percentage of the mice. *Average ovarian and uterine weights during the estrous cycle.* For this phase of the investigation, 74 mice were chosen at random from the colony and vaginal smears were made. They were sacrificed and the right ovary and uterine horn excised, dissected free of fat, and weighed on a torsion balance. The average weight data obtained are summarized in Table I. With the exception of late metestrus (M_2) and proestrus (P), the average and median ovarian weights were similar throughout the cycle. The values for those 2 stages were not reliable because the number of mice was not sufficient. Uterine weights shown here are at variance with similar data that Astwood compiled for the rat(1). The rat data showed a peak uterine weight at proestrus, and a decline that began at estrus and continued until it reached its lowest ebb on the first day of diestrus. The failure to decline rapidly at estrus (O) and early metestrus (M_1) reported here may represent a fundamental difference in the ovarian hormone secretion pattern of rat *vs.* mouse. In a group of 60 mice from 5 to 11 months of age, the average uterine weights at

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