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Received July 22, 1957. P.S.E.B.M., 1958, v97.

α and β Lipoproteins and Serum Cholesterol Levels Following Administration of Unsaturated Fatty Acids. (23709)

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Quantitative and qualitative alterations of ingested fat have been shown to regulate the composition of transported lipids in the human subject(1,2). Bronte-Stewart(3) postulated that atherosclerosis is possibly intensified by a dietary deficiency of essential fatty acids. The effect of lipotropic substances on dispersion of fat has been reported(3) and confirmed(4,5). The experimental data presented below indicate that serum alpha and beta lipoprotein and cholesterol levels are significantly modified by administration of essential fatty acids and lipotropic substances. The importance of these findings in the prevention of atherogenesis is discussed.

Material. Twenty-five patients, 16 males and 9 females, age 36 to 78, with clinical evidence of myocardial infarction, under our continuous observation for 4 to 5 years, were the subjects of this study. Controls were 20 subjects, ranging in age from 10 to 44 and one child of 3 (19 males and 2 females) with no demonstrable cardiovascular disease. The experimental as well as the control subjects were encouraged to remain on a normal diet with no restriction of fat, cholesterol-containing or any other food.

Methods. Determinations were done monthly on fasting serum of controls, experimental subjects and on pooled serum from healthy blood donors. Each blood sample was analyzed by a modified electrophoretic method for lipoprotein and protein fractions and by chemical analysis for cholesterol. The experimental subjects had been previously studied on an unrestricted diet while receiving a lipotropic mixture* and results reported(6). The same diet and lipotropic mixture were continued but to them was added 3 g of saf-

* Daily intake comprised 1 g of choline, 1 g of dl-methionine, 750 mg of inositol, 18 μg of vit. B_{12} and 750 mg of desiccated liver, supplied as Methischol Capsules, courtesy of U. S. Vitamin Corp., N. Y. City.

TABLE I. α/β Lipoprotein Ratio in 21 Presumably Healthy Subjects and in Pooled Serum from Apparently Healthy Donors (Multiple Runs).

Age	Sex	α/β lipoprotein ratio
22	♂	.58
29	♂	.72
19	♀	.67
?	♀	.47
10	♂	.89
21	♂	.69
22	♂	.82
27	♂	.52
44	♂	.41
11	♂	1.10
23	♂	.75
26	♂	.41
33	♂	.70
25	♂	.78
11	♂	.95
31	♂	.56
3	♂	.89
?	♂	.81
26	♂	.82
28	♂	.90
27	♂	.95
Avg		.734
Blood donors' pooled serum		.62-.67

flower seed oil and 4 mg pyridoxine per day.† Maximum effect on fat metabolism had been achieved by the earlier regimen, and significant changes were considered to be due to the added safflower seed oil.

Results. Table I lists the values for controls. The range of alpha to beta ratio is from 0.41 to 1.10; the mean is 0.734. This mean differs only insignificantly from the mean of pooled serum. The technic error for the alpha to beta ratio appears to be 0.05. This has been repeatedly confirmed in several thousand electrophoretic separations.

Table II lists the alpha to beta lipoprotein ratio and total serum cholesterol before medication and after 12, 18, 24 and 30 week intervals. The average alpha to beta lipoprotein ratio before administration of the safflower oil-pyridoxine-lipotropic product was 0.461. At the 18 week mark that ratio was 0.580, representing a difference of 0.120. The standard deviation of difference was 0.102; standard error of the mean difference was 0.022. Thus, the mean difference divided by the standard mean difference was 5.4, which is highly significant. The odds against occurrence of a deviation as great as, or greater than, 0.12 (increase in the alpha to beta lipoprotein ratio after 18 weeks) are approximately 2,000,000 to 1.

Shortly after the 18 week mark, patient F.E. (No. 22), 38-year-old white male, suddenly died of acute myocardial infarction while undergoing extreme physical and emotional stress. Autopsy revealed marked and widespread coronary atherosclerosis. It is of interest that this patient's initial alpha to beta ratio was 0.35 and 18 weeks after safflower oil administration it rose to 0.42. His total serum cholesterol had fallen from an initial 592 mg% to 525 mg% at 18 months.

Six out of 25 patients showed decreased total serum cholesterol levels at the 18 or 30 week period.

Discussion. Major aberration in lipid metabolism as evidenced by greatly elevated total serum cholesterol, elevated fasting chylomicron index or even frank lipemia are predominant factors in premature and severe coronary atheromatosis(7,8,9). The difficulty lies in the borderline cases which comprise the majority of our clinical material, where one can apply only group correlation.

Paper electrophoresis is a reliable quantitative method for measurement of alpha and beta lipoprotein(10). Determination of the ratio of alpha to beta lipoprotein in fasting serum is in our experience the most sensitive index of the patient's individual ability to metabolize fat.

Since ingestion of large amounts of medication is disagreeable to many patients, we used *moderate* doses and the present report deals with the results *obtained with those doses.* Kinsell *et al.*(11) reported that the 24-hour intake of 2 g of linoleic acid resulted in prompt and profound decrease in plasma cholesterol and other lipid fractions.

Moderate doses of safflower seed oil resulted in reduction of serum cholesterol in 6 patients. A statistically significant shift from beta to alpha lipoprotein occurred in a large percentage of our patients after 18 weeks of

† The combination of safflower seed oil, pyridoxine and lipotropics were administered in capsule form, coded LUFA.

TABLE II. Effect of Safflower Seed Oil - Pyridoxine - Lipotropic Medication.

			Before medication		12 wk		18 wk		24 wk		30 wk	
Patient	Age	Sex	α/β ratio	Cholesterol, mg %	α/β ratio	Cholesterol, mg %	α/β ratio	Cholesterol, mg %	α/β ratio	Cholesterol, mg %	α/β ratio	Cholesterol, mg %
1	52	♂	.25	328	.51	348	.42	321	.30	296	.50	277
2	49	♂	.39	295	.46	340	.65	374	.52	343	.59	349
3	67	♂	.51	207	.72	220					.75	210
4	68	♂	.30	409	.43	396	.40	408	.44	395	.28	378
5	63	♂	.39	332	.35	319	.43	326	.39	313	.41	303
6	56	♂	.41	262	.45	272	.42	286	.62	287	.49	286
7	55	♂	.43	289	.58	303	.57	302	.47	305	.51	297
8	71	♀	.36	320			.58	316	.32	369	.39	306
9	61	♀	.37	429			.38	508	.47	517	.32	485
10	54	♂	.27	220	.44	264	.51	260	.40	241	.51	216
11	63	♂	.30	248	.55	264	.68	272	.49	241	.63	228
12	51	♀	.44	311	.42	325	.42	334	.44	257	.42	326
13	51	♂	.74	251			.93	248			.98	261
14	43	♀	.59	280	.62	260	.67	326			.75	257
15	78	♂	.55	198	.43	200	.71	178			.60	188
16	72	♀	.40	362							.61	326
17	59	♂	.71	284	.52	294	.72	290	.59	300	.69	248
18	62	♂	.43	330	.39	272			.56	290	.67	248
19	58	♀	.80	291	.60	295	.80	290	.63	284	.65	289
20	51	♀	.61	336	.64	216	.94	358	.64	314	.79	316
21	42	♂	.42	265	.52	272	.50	264	.46	282	.58	253
22	36	♂	.35	592	.33	524	.42	525				
23	51	♀	.61	250	.87	269	.67	272	1.04	296	.74	261
24	63	♂	.39	277	.39	248	.48	267	.58	260	.44	224
25	40	♀	.49	279			.85	238				

treatment. The alpha to beta ratio continued to rise at 24 or 30 weeks of medication in 18 cases out of 24. This consistent trend encourages us to proceed with the study over a longer period of time and with larger doses of safflower seed oil. Our studies indicate that essential fatty acids do exert an unequivocal effect on serum lipids; in larger quantities they apparently lower total serum cholesterol. In moderate doses, which we consider practical from the patient standpoint, there is a consistent and permanent shift from beta to alpha lipoprotein.

Summary. 1. The present study confirmed the group correlation between clinically demonstrable coronary atherosclerosis and abnormally low alpha to beta lipoprotein ratio. 2. Daily administration of 3 g of safflower seed oil and 4 mg of pyridoxine for a period of up to 30 weeks, added to the previously administered lipotropes, resulted in a statistically significant increase of alpha to beta lipoprotein ratio starting at the 18 week period. The trend continued for the 24 and 30 week periods in over 70% of cases. 3. Twenty per cent of cases showed lowered total serum cholesterol levels. 4. In view of the potential significance of the observed results, further study is merited.

Acknowledgement is hereby expressed to Mr. Albert Breazeale and Mr. Marvin Dyess for valuable technical assistance; to Miss Margaret Rice and Miss Catherine Powell for help in statistical analysis; to Miss Eva Perry and Miss Shirley Jones for assistance in preparation of the manuscript.

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Received July 24, 1957 P.S.E.B.M., 1958, v97.

Tentative Pattern for Renewal of Lymphocytes in Cortex of the Rat Thymus.* (23710)

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It is not known how the 4 cell types present in the thymus (reticular cells, large, medium and small lymphocytes) are involved in lymphocyte production(1-3). In the hope of clarifying the problem, the numbers of these cells and their mitoses were counted in different regions of the organ, and an attempt made to elaborate a pattern of lymphocyte renewal consistent with the experimental data. The present article is a preliminary report on the results and conclusions reached so far.

Methods. Twenty-eight 10-week-old male albino rats were divided into 4 groups of 7 animals each, sacrificed at 10 a.m., 4 p.m. and 10 p.m. during same day, and at 4 a.m. the next morning respectively. After fixation for 2 days in modified Bouin-Hollande (4 g picric acid; 2.5 g copper sulfate; 20 ml neutral formalin; 0.75 ml acetic acid; 0.75 g trichloracetic acid and 100 ml water), the tissues were sectioned at 5 μ and stained by the Dominici technic.

Results. Cytological criteria (Fig. 1). The *reticular cell* has a pale cytoplasm which is little or not visible and a large nucleus (to 11 μ diameter), oval or diamond-shaped, which appears very light. Fine chromatin dots are associated with the nuclear membrane. There is usually one, and sometimes 2-4 large oval nucleoli, the center staining orange-red, the surface light blue. A loose network of fine chromatin threads connects the nucleolus to nuclear membrane.

The *large lymphocyte* has a basophilic, well-limited cytoplasm, arranged into a more or less regular ring around the nucleus. The nucleus is usually round, diameter equal to or greater than 5.9 μ and stains slightly darker than in the reticular cell. Larger chromatin dots than in the reticular cell are associated with the nuclear membrane. There are 1-4 nucleoli of variable shape (oval, stellate, elongated) with an acidophilic core and a more bluish chromatin coat than in the reticular cell. The thread network within the nucleus is slightly denser than in the reticular cell.

The *medium lymphocyte* usually has a small ridge of basophilic cytoplasm, less in amount than in the large lymphocyte. The nucleus which measures 4.6-5.9 μ is often round and the nuclear sap appears blue with a purplish hue. Coarser chromatin masses than in the large lymphocyte are associated

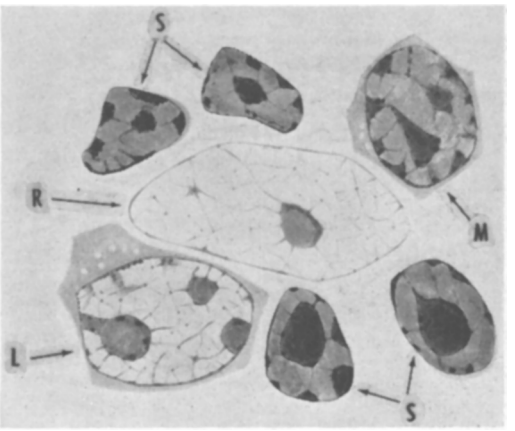

FIG. 1. Drawing of cells of thymus: R, reticular cell; L, large lymphocyte; M, medium lymphocyte; S, small lymphocyte.

* This work was supported by a grant of the Nat. Cancer Inst. of Canada. Suggestions made by Dr. Maria Stux and L. Genender are acknowledged.

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