seemed to show a slightly better correlation (20). To our knowledge systematic studies on this aspect have not been done with the rat.

Another controversy has arisen concerning use of turpentine to produce leucocytic exudates. Harris(19) reported it to be toxic to cells. Menkin(18) explains his different observations as due to use of different species. We can only say that in our experiments the leucocytes in the exudate which results from injection of turpentine in the granuloma pouch showed high myeloperoxidase activity after being washed with saline.

Summary. The rat granuloma pouch has been found to be a convenient source for collection of leucocytes rich in myeloperoxidase. Of a number of irritants tried, a suspension of turpentine was most satisfactory. Within a limited range, pH of turpentine suspension had no significant effect on cellular composition of the exudate.

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Received December 22, 1958. P.S.E.B.M., 1959, v100.

Effects of Dietary Fat upon Polyunsaturated Fatty Acids of Blood in Patients with Multiple Sclerosis.*† (24688)

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Among several hypotheses advanced concerning the etiology of multiple sclerosis (MS), 2 schools of thought have recently gained prominence; one(1) proposes that an allergic reaction is involved; the other(2,3) that an enzymatic or metabolic defect underlies recurrent attacks of demyelination. We reported previously that some patients with MS revealed higher plasma levels of dienoic acid than did controls(4). This observation, together with the dietary studies of Swank(5) and increased chylomicron index noted by Skillen, *et al.*(6), warrants further investigation of the lipid metabolism in this disease.

Methods. The plan of this study was similar to that of Holman, *et al.*(7). Diagnosis of MS was established as outlined previously (4). A baseline blood sample was obtained after patient had been on average hospital diet including approximately 20% protein, 28% fat, and 52% carbohydrate. Then, all individuals received a nearly eucaloric diet with soya bean oil as main source of polyunsaturated fatty acids (PUFA). Composition of diet, its lipids and vitamins added, is given in Tables I-A, B and C. Diagnoses of control patients are listed in Table II. Sequence of

* Supported by U. S. Veterans Admin., and by grants from Nat. Multiple Sclerosis Soc., N. Y., and U.S.P.H.S.

† Presented, in part, before Am. Inst. Biol. Sci., Stanford Univ., August 29, 1957.

TABLE I-A. PUFA-Enriched Diet Based on Approximately 2600 Calories/Day Intake.

	g
Non-fat milk solids	149.4
Soya bean oil	71.5
Lecithin (Granulestin)	52.7
2½ slices of bread	42.6
Gelatin	150.0
Glucose	31.2

Coffee, tea, green salad, raw carrots, radishes and celery *ad lib.*

TABLE I-B. Lipids of Diet in g (Avg of 3 Analyses).

	Soya bean oil	Granulestin
Total grams	71.5	52.7
Fatty acids:		
Unsaturated: mono-	20.2	10.8
di-	38.6	25.4
tri-	3.6	2.4
Saturated	6.0	6.7

TABLE I-C. Vitamins/Day.

Methionine	1 g
Pyridoxine	50 mg
Pentothenate	9 "
Nicotinamide	90 "
B_{12}	3 μg
Riboflavin	39 mg
Thiamine	36 "
Ascorbic acid	225 "
B-2	1,200 units
A	15,000 "

patients was kept alternating to exclude influence of seasonal factors. Blood was obtained after overnight fasting. Clotting of blood was prevented by addition of dry potassium oxalate. The cells were rapidly separated by centrifuging at 3,000 rpm. Plasma was extracted and total lipids and cholesterol determined as in previous investigation(4). The PUFA were measured by method of Herb and Riemenschneider(8). Precautions recommended by Holman(9) were followed. Measurement at 374 mμ was included, and the PUFA calculated according to Hammond and

TABLE II. Five Control Cases.

Race	Age	Diagnosis
White	21	Rheumatic fever
Negro	36	Epilepsy
"	47	Traumatic paraplegia T-4
White	31	
"	34	Epilepsy

TABLE III. Effects of Soya Bean Oil Diet upon Plasma Polyunsaturated Acids.

	Diet	No. of individuals		Chol. Lipid	PUFA Lipid	Diene PUFA	Triene PUFA	Tetraene PUFA	Di/Tri* Ratio	Tri/Tetra* Ratio
				%						
MS patients	Free	6	Avg	26.2	15.8	62.3	13.1	16.0	5.16	.84
			Range	19.7–32.6	7.8–20.2	59.5–68.5	8.2–17.4	12.9–18.7	3.43– 8.38	.50–1.35
	PUFA (1 wk)	5	Avg	21.0	20.9	72.4	13.0	11.5	6.62	1.13
			Range	18.1–23.4	17.4–24.1	66.1–78.1	6.7–25.3	7.7–16.4	2.76–11.32	.55–1.54
	" (3 ")	4	Avg	21.9	20.7	70.7	13.9	9.6	5.49	1.49
			Range	10.8–27.7	18.3–23.9	66.7–79.7	8.8–17.9	7.7–11.7	3.82– 9.06	1.08–2.25
Controls	Free	5	Avg	25.3	16.0	61.1	13.9	16.5	5.11	.89
			Range	20.2–28.7	13.9–18.5	50.4–75.8	8.1–20.2	10.5–23.9	2.96– 9.40	.54–1.47
	PUFA (1 wk)	5	Avg	24.6	21.5	71.3	12.2	10.1	6.73	1.23
			Range	17.1–36.7	15.5–24.0	59.0–82.1	7.6–18.6	6.0–13.3	3.17–10.00	.69–1.71
	" (3 ")	4	Avg	22.4	21.8	74.8	10.2	9.8	8.79	1.06
			Range	18.8–24.7	17.5–25.5	69.8–83.9	5.2–14.6	6.5–13.0	4.79–16.25	.71–1.65

* Ratios calculated individually and then averaged.

TABLE IV-A. Average Difference from Baseline to 3rd Week of Diet.*†

Ratios	Control group No.¶	Control group Diff.	MS group No.¶	MS group Diff.
Chol. Lipid	4	-4.20‖	4	5.40
PUFA/Lipid	4	5.35	5	5.34§
Diene/PUFA	4	10.95	5	9.70§
Triene/PUFA	4	-3.95§	5	-.12
Tetraene/PUFA	4	4.77	5	6.32
Diene/Triene	4	3.39‡	5	.98
Triene/Tetraene	4	.09‡	5	.58§

* We are indebted to Mr. R. A. Schindler for assistance in statistical evaluation.

† Values with ref. marks (‡ § ‖) are significantly different from zero at levels indicated. (Two-tailed t test for correlated means.)

‡ $p < .10$ § $p < .05$ ‖ $p < .01$

¶ No. of individuals.

Lundberg(10). Separation and estimation of PUFA in red blood cells were carried out as described elsewhere.

Results. According to Holman, *et al.*(7), ratios of individual lipids to total lipids and those between the 2 groups of structurally related PUFA are more revealing than data on individual lipids. In agreement with this concept, our results are likewise reported as ratios.

Plasma lipids. Cholesterol/lipid ratio declined and PUFA/total lipid ratio increased in both MS and control groups during 3 weeks of PUFA-enriched diet (Table III). The diene/PUFA ratios showed a rise, and tetraene/PUFA ratios showed a fall in both groups during the study, but a statistically significant difference in degree of change was not observed in either case (Table IV). The triene/PUFA ratio declined in the control group while it remained practically unchanged in MS patients (Table III), resulting in a statistically significant difference (Table IV-B). Similarly significant was the difference in behavior of the diene/triene and of the triene/tetraene ratio (Table III). The former rose markedly in the control group and only slightly in MS patients; conversely, triene/tetraene ratio increased considerably in MS, but only to a minimal extent in control patients.

Red blood cells. Because of scarcity of data available on lipids of red blood cells, these are reported in Table V. The diene/PUFA ratio remained nearly stable in MS patients and rose slightly in the controls. Triene/PUFA and triene/tetraene ratios increased in MS patients while declining in the controls; but none of the differences were statistically significant.

Discussion. This investigation of multiple blood lipid components was limited to a small number of patients, as in the study of Holman (7). Nevertheless, analysis of data obtained on our 2 groups allows certain tentative interpretations.

Cholesterol/lipid ratio decreased in both groups during the period of special diet. This corroborates the findings of Kinsell, *et al.* and of Ahrens, *et al.*(11,12). Increase of PUFA/lipid ratio was of similar extent in both groups, indicating that there was no defect in absorption of dietary fat in the MS group.

Dienoic/PUFA and trienoic/PUFA ratios in MS and control groups were almost identical at start of experiment. During the period of PUFA-enriched diet, the diene/PUFA ratio rose significantly in both groups (Table IV-A). The triene/PUFA ratio, however, declined significantly in the controls during this period, while remaining stable in the MS group, resulting in a significant dfference between the 2 groups (Tables IV-A and B). The tetraene/PUFA quotient also was alike at the start, and decreased to the same extent of p in both groups. The diene/triene as well as the triene/tetraene ratios were almost identical in the 2 groups before receiving the diet (Table III). The former increased significantly in the controls leading to a difference

TABLE IV-B. Significance of Difference between Means of MS and Control Groups.

Diet	Chol. Lipid	PUFA Lipid	Diene PUFA	Triene PUFA	Tetraene PUFA	Diene Triene	Triene Tetraene
Free	N.S.*	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
PUFA (3 wk)	"	"	"	.10	"	.10	.10

* N.S. = Not significant.

TABLE V. Effects of PUFA-Enriched Diet upon Red Blood Cell PUFA.

Diet			PUFA / Lipid	Diene / PUFA	Triene / PUFA	Triene / Tetraene
MS patients	Free	Avg	12.5	29.0	23.5	.77
		Range	10.3–14.5	25.5–35.4	15.7–31.2	.42–1.09
	PUFA (3 wk)	Avg	12.1	30.5	25.5	.87
		Range	10.4–15.7	29.0–33.0	19.1–30.4	.58–1.17
Controls	Free	Avg	13.5	29.5	21.9	.68
		Range	12.6–15.7	24.4–32.6	19.4–25.6	.62– .78
	PUFA (3 wk)	Avg	14.3	33.9	18.7	.63
		Range	12.5–16.6	29.3–38.4	10.5–26.8	.30– .92

with the MS group with the significance $p < 0.10$. Conversely, the triene/tetraene ratio rose in the MS group with the final difference being also $p < 0.10$.

Comparison of data presented here with those reported by Evans(13) and by Pikaar, *et al.*(14) is of but limited value since dietary intake of the latter 2 groups was not given. Diene/and tetraene/PUFA ratios in the "fasting normal" of Pikaar's group are close to ours, all other diene levels in these reports being slightly higher. A larger difference is noticeable in the triene/PUFA quotient, which is higher in our group than in the 10 fasting normals of Pikaar. Since the methods employed were identical(13) or were similar (14), a different food intake may well be responsible.

More illuminating is comparison with Holman, *et al.*(7) data. The triene/PUFA ratios in Holman's group were initially lower but, after feeding hydrogenated cocoanut oil (HCO) rose higher in 2 of their subjects than in any of ours. The tetraene/PUFA ratio, under the influence of diet, declined in all our patients, as it did in 3 out of 4 of Holman's subjects receiving corn oil. The triene/tetraene ratio at start of experiments, of same range in both Holman's and our groups, rose in the MS patients to the degree observed by Holman, *et al.*, after feeding HCO.

An increase of triene/tetraene quotient has been discussed by Holman(7) as possibly signifying a PUFA deficiency. In view of abundant supply of PUFA and of similar tendency of diene/PUFA ratio in both groups, a deficiency seems unlikely. The decline of both triene/ and tetraene/PUFA ratios in the controls suggests a withdrawal of the higher PUFA from plasma when there is a plentiful exogenous supply of dienoic acid. In MS patients, however, this mechanism seems to be effective only for tetraenoic acid. The results, further, emphasize Holman's conclusion that single samples of plasma do not allow evaluation of the nutritive status of an individual as pertaining to PUFA.

Findings of red blood cell lipids did not reveal any significant changes in the ratios, though reflecting to a slight degree the changes in plasma lipids.

Summary. 1. Effects of diet providing adequate protein and vitamin intake and enriched with PUFA were studied in a group of patients with multiple sclerosis and compared with those in control patients. 2. A decline of cholesterol/lipid and of tetraene/PUFA, and an increase of PUFA/lipid as well as diene/PUFA ratios were observed in both groups. 3. Differences between the 2 groups are represented by significant decline of triene/PUFA ratio in controls and, conversely, by a rise of triene/tetraene ratio in the MS group.

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Received December 23, 1958. P.S.E.B.M., 1959, v100.

Effect of Digitalis on Potassium Toxicity in Isolated Turtle Heart. (24689)

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It is well accepted that potassium administration can antagonize toxic effects of digitalis(1). Patients receiving digitalis may exhibit digitalis toxicity when their potassium intake is restricted, and patients who are overdigitalized frequently respond to administration of potassium. The converse of these situations has not been satisfactorily investigated. Does digitalis antagonize the effects of potassium toxicity on the myocardium? A perfusion experiment in the isolated turtle heart was devised to study the effects of rapidly acting digitalis preparations on induced potassium intoxication. According to Szent-Gyorgi, the mineraloid hormone of adrenals abolishes "Treppe" in the isolated perfused turtle heart as does digitalis(2,3). The protective action of water soluble desoxycorticosterone glucoside against potassium toxicity was also investigated.

Methods. Hearts were removed from 30 unanesthetized turtles to include 1 cm of the truncus. The dorsal aorta was cannulated and the myocardium perfused through the coronaries, as shown in Fig. 1, with various test solutions(4). The phrenum was attached by suture to metal bar which acted as an isometric lever. Tension developed in the bar was transformed into electrical energy through suitable transducer and recorded on oscillograph. The basic solution (Howell's solution) for perfusion contained the following constituents: NaCl 0.7%, KCl 0.3%, $CaCl_2$ 0.026%, $NaHCO_3$ 0.003%, MgCl 0.01%, glucose 0.1%. Potassium was added to basic solution to augment the final concentrations of potassium by 4, 6, 8, and 12 meq/liter of potassium chloride. The substances to be tested, lanatoside C, acetyl strophanthidin and desoxycorticosterone glucoside were added in varying concentrations to the potassium enriched perfusion medium. Lanatoside C and acetyl strophanthidin were added to the perfusion media to give following concentrations: .4, .8, 1.2, and 1.6 mg/liter. Between each experiment with potassium enriched solution the heart was permitted to return to normal beat by thoroughly perfusing with plain Howell's solution.

Results. A slow infusion of KCl in Howell's solution was capable of producing cardiac arrest in most experiments when the concentration of KCl was augmented by 8 meq/l. There was individual variation, and occasionally arrest was produced with additional 6 meq of potassium/liter. Similarly, some hearts did not arrest even with an additional 10 meq/l. In lower concentrations, KCl impaired development of tension by the myocardium but did not cause arrest. Both amplitude of isometric contraction and rhythmicity were recorded. Potassium solution caused linear decrease in development of tension until asystole occurred (Fig. 2a). Cardiac rate remained constant during perfusion with potassium. Changes in rhythmicity were not progressive as were changes in amplitude of contraction. Rhythmicity was sud-