The basal diet used in this study was deficient in one or more factors as indicated by the low growth response as well as the occurrence of the bone deformity described. Some of the beneficial effect of the added fish meal and gelatin may have been due to the glycine and/or arginine content, but not all, since the addition of 7.5% gelatin in the previous study did not completely prevent the occurrence of this bone deformity, nor did it allow maximum growth response. This diet contained adequate amounts of the required minerals and known vitamins. The diet also contained 100,000 units of strepogenin per 100 g, which is in excess of that found necessary for growth by Scott, Norris, and Heuser.[2]

Summary. A new bone deformity which is characterized by a twisting of the tibia at the proximal end has been observed in chicks fed purified diets. Under the conditions of this experiment gelatin and fish meal decreased the incidence and severity of the malformation. The diet used appears to be deficient in an unknown factor or factors necessary for maximum growth and the prevention of the bone deformity.

[2] Scott, M. L., Norris, L. C., and Heuser, G. F., *J. Biol. Chem.*, 1947, **167**, 261.

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The Effect of Ingested Mineral Oil on Plasma Carotene and Vitamin A.

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Due to the shortage of biological fats and oils during the war, the use of mineral oil in foods, in the form of imitation mayonnaise or salad dressing replacing the usual food oils, increased considerably. Such food substitutes are also used extensively in weight-reduction diets.

There are numerous reports concerning the potentially harmful effects of mineral oil based upon its interference with the absorption of fat-soluble vitamins,[1-8] particularly vitamin A and its precursor, carotene.[1-6] Most of the work is based on animal experiments in which huge amounts of mineral oil were used compared with what would ordinarily be taken by man. Nevertheless, the Council on Foods and Nutrition of the American Medical Association concluded[9] that "the indiscriminate use of mineral oil in foods and cooking is not in the interest of good nutrition, and any such use should be under careful supervision of a physician."

Curtis and his collaborators[10,11] showed in man that the ingestion of mineral oil will prevent blood carotene rises consequent to simultaneous intake of carotene-rich foods. They[11] also reported a decrease in blood carotene in subjects on a normal diet who took 20 cc of mineral oil immediately before

[1] Burrows, M. T., and Farr, W. K., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 719.

[2] Dutcher, R. A., Ely, J. O., and Honeywell, H. E., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 953.

[3] Dutcher, R. A., Harris, P. L., Hartzler, E. R., and Guerrant, N. B., *J. Nutr.*, 1934, **8**, 269.

[4] Mitchell, H. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 231.

[5] Jackson, R. W., *J. Nutr.*, 1934, **7**, 607.

[6] Curtis, A. C., and Horton, P. B., *Am. J. Med. Sc.*, 1940, **200**, 102.

[7] Smith, M. C., and Spector, H., *J. Nutr.*, 1940, **20**, 19.

[8] Elliott, M. C., Isaacs, B., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 240.

[9] Council on Foods and Nutrition, *J. A. M. A.*, 1943, **123**, 967.

[10] Curtis, A. C., and Kline, E. M., *Arch. Int. Med.*, 1939, **63**, 54.

[11] Curtis, A. C., and Ballmer, R. S., *J. A. M. A.*, 1939, **113**, 1785.

2 or 3 meals daily; if the mineral oil was saturated with carotene at body temperature, it did not produce any change.

Since these authors observed the effects only over a period of 15 days and since spontaneous daily fluctuations in blood carotene content are frequently observed, it was deemed desirable to make observations over a longer interval. Blood vitamin A levels also were determined simultaneously.

Twenty-five subjects, comprising 5 groups, were studied.

Group I—3 normals taking no form of mineral oil.

Group II—6 normals taking 1 tablespoonful of Leanermaise* with noon and evening meals daily.

Group III—4 normals taking 1½ teaspoonfuls of Leanermaise* with noon and evening meal daily.

Group IV—3 normals taking 1 tablespoonful of mineral oil with noon and evening meal daily.

Group V—9 patients with psoriasis vulgaris† of whom 6 ingested 1 tablespoonful of mineral oil with noon and evening meals and 3, 1 tablespoonful with each of 3 meals.

The diet of all subjects was unrestricted. The mineral oil or salad dressing was taken immediately before or during the meal. Plasma carotene and vitamin A concentrations were determined by the method of Kimble[12] before, and at frequent intervals during, the observation periods of from 3 to 8 weeks.

Results and Comment. The results (Table I) indicate that, concomitant with the prolonged daily ingestion of mineral oil either as such or in the form of mayonnaise dressing at meal time, the plasma carotene concentration decreased about 50%. The decline was about the same for those using 2 tablespoonfuls as for those using 3 teaspoonfuls; no difference was evident between those subjects on Leanermaise and those on regular mineral oil. Some decrease was noted within 1 to 2 weeks, and it progressed further in many cases as intake of the oil continued. No significant changes in plasma vitamin A content occurred, and no untoward clinical manifestations appeared.

These alterations are probably due to extraction by mineral oil of food carotene which is thus prevented from entering the body. That extraction was not complete, even in those cases who received 1 tablespoonful of mineral oil with each meal, is indicated by the considerable carotene concentration even after many weeks on this regime.

Since in normal subjects blood carotene parallels the amount of this substance which enters the body[9,13,14] it may be concluded that under the conditions described above mineral oil interferes with the absorption of the provitamin from the gastro-intestinal tract. It is noteworthy, however, that even after prolonged use plasma carotene levels remained substantial, indicating that considerable amounts of the material were still entering the body.

One tablespoonful of mineral oil can theoretically dissolve 70,000 I.U. of carotene (in terms of vitamin A) at body temperature,[11] an amount of the provitamin far greater than that usually provided in one meal (1100-2200 I.U.[9]), or even 3 meals. That the plasma carotene did not fall precipitously to negligible levels in those subjects taking 1 tablespoonful of mineral oil with each meal for 6 weeks indicates either that the extraction of food carotene by mineral oil in the gastro-intestinal tract is far from complete, or that sizable amounts of carotene were ingested between meals, an unlikely possibility which, however, cannot be excluded.

Nevertheless, since from ½ to ⅓ of the vitamin A supply is derived from carotene

* Generously supplied by Leanermaise Company, Boston, Mass. Composition: 83% mineral oil, 2% fat. According to our analyses, the material used during the first 7 weeks of the study (Lot 1) contained 27 μg vitamin A per g; during the 8th week (Lot 2) it contained 43 μg per g. Both lots were carotene free.

† Subjects under study of vitamin A and carotene metabolism in *Psoriasis vulgaris.*

12 Kimble, M. S., *J. Lab. Clin. Med.*, 1939, **24**, 1055.

13 Wald, G., *Am. J. Physiol.*, 1942, **137**, 551.

14 Brenner, S., and Roberts, L. J., *Arch. Int. Med.*, 1943, **71**, 474.

TABLE I.
Effect of Mineral Oil Ingestion on Plasma Carotene and Vitamin A.

		Plasma carotene—μg per 100 cc									Plasma vitamin A—μg per 100 cc	
		Control* (Before	Weeks								Control* (Before	† (After
	Subject	min. oil)	1	2	3	4	5	6	7	8	min. oil)	min. oil)
Group I—No mineral oil	1	134	130	153	155	114	118				46	44
	2	132	121	169	150	134	134				53	55
	3	137	125	93	120						48	56
Group II—	4	124	113	94	88	79	106	83	78	58	36	39
Leanermaise—2 tbs.		143									42	
		149									38	
	5	245	204	168	131	144	125	128	125	104	38	38
		215									40	
		225									38	
	6	173	101	96	98	85	87	65			53	47
		162									56	
	7	86	61	46	57	71	65	37			53	46
		115									57	
	8	151	100	112	96	79	72				53	54
		200									63	
	9	153	119	119	104	92	95				38	46
		174									39	
Group III—	10	131	121	101	84	62					41	39
Leanermaise—3 teaspoons		136									43	
	11	97	106	88	95	83	80				35	44
		146									45	
	12	207	136	140	128	104					50	47
		145									45	
	13	164	129	102	114	114	102	113	94		34	33
		179									38	
Group IV—	14	168	139	145	139	116					33	36
Mineral oil—2 tbs.		181									39	
	15	156	118	107	101	83					26	31
		144									34	
	16	159	137								56	59
		161									32	
Group V—	17	232			91			129			64	60
Mineral oil—3 tbs.	18	166			91			77			52	48
	19	72				26					33	30
Mineral oil—2 tbs.	20	111		76			71				66	35
	21	109			106				77		60	46
	22	187	143		108	62					47	36
	23	85		76			99			65	37	72
	24	95				50					65	63
	25	134		102		125		108			63	61

* Multiple values given in this column represent determinations spaced one week apart.

† The value given in this column is the last one obtained during the observation period which is the same number of weeks as that indicated for carotene. Intervening values are omitted for the sake of brevity.

in the usual American diet it must be presumed that the use of mineral oil under the conditions described above precludes entrance to the body of sizable amounts of vitamin A in the form of its precursor. In subjects with already restricted diets, such a loss may become clinically significant. If, however, the diet provides sufficient excess of carotene or vitamin A to meet this loss no derangement might occur.

The unchanged vitamin A levels are not surprising since it is well known[13,14] that the concentration of this vitamin in the blood remains relatively constant for a very long time

(6 months) in normal individuals who are subjected to restricted vitamin A and carotene intake. Therefore, no conclusion can be drawn on our subjects with regard to the effect of mineral oil on the supply of vitamin A as such to the body. Experiments on animals indicate that mineral oil interferes far less with absorption of vitamin A than carotene.[3,5,9,15] Furthermore, mineral oil containing vitamin A provides a ready source of this vitamin to vitamin A-deficient animals.[6]

[15] Rowntree, J. I., *J. Nutr.*, 1931, **3**, 345.

Similar experiments on man are contemplated.

Summary. Continued ingestion of mineral oil as such or in the form of mayonnaise dressing by 20 subjects on a normal unrestricted diet resulted in a moderate decrease in plasma carotene concentration. Vitamin A levels remained unchanged.

It is concluded that simultaneous ingestion of mineral oil with food prevents substantial amounts of food carotene from entering the body.

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Nutritional Requirements of *Tritrichomonas foetus* with Special Reference to Partially Digested Proteins.

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The purpose of this group of experiments is to review the metabolism of the endozoic parasite, *Tritrichomonas foetus,* in order to determine if this organism requires the same general type of nitrogen as that required by other animals, and also to determine the effects of partially-digested proteins on the growth rate of this organism. It is believed that this is the first more or less detailed study of protein nutrition, using synthetic media, in a bacteria-free endozoic protozoan which does not inhabit the blood.

Several methods have been proposed for the cultivation of *Tritrichomonas foetus.* A medium commonly used is egg-serum-Locke's medium (ESL). Collier and Boeck's modification[1] was used in this experiment for the maintenance of the culture. The test tubes used were 4″ x ⅜″ and were filled with 2 cc of liquid medium. After the media tubes had been prepared, they were each inoculated with approximately 380 organisms, this number being obtained from 2 bacteriological loopfuls of a well-mixed, 5- to 7-day-old, ESL culture. All culture tubes were incubated at 25°C in an oven with a saturated atmosphere to prevent evaporation as far as possible. Maximum growth in an ESL culture was obtained in 5 to 7 days.

The method of counting used throughout this experiment is a very simple one. The test tube is shaken thoroughly. Then a bacteriological loopful of liquid is removed aseptically to a clean slide. The number of organisms present in the center of the drop for one ocular field is counted. This procedure is followed 3 consecutive times to insure reasonable accuracy in a minimum amount of time. This method has been calculated statistically to be accurate with a maximum error of ±5%. Two cc of liquid were maintained as the standard volume in all media so that the figures obtained by this counting method in different media would be comparable. Bacteria-free cultures were maintained throughout the experiment.

Three standard media were compared to determine the differences in growth response in dissimilar types of media. Broth-serum-glucose (BSG) described by Andrews and

[1] Collier, J., and Boeck, W. C., *J. Parasit.*, 1926, **12**, 131.

[2] Andrews, J., and von Brand, T., *Am. J. Hyg.*, 1938, **28**, 138.