Extent of Total Hydrolysis of Dietary Glycerides during Digestion and Absorption in the Human. (25192)

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The opportunity to obtain thoracic duct lymph from human beings(1,2) has prompted us to undertake a study of the extent of hydrolysis of dietary glycerides during digestion and absorption in man. From animal experiments it is known that glycerol once liberated from the triglycerides by hydrolysis in the intestinal lumen is not utilized for resynthesis of glycerides. The ratio of label in the acid to that in glycerol of the lymph glycerides after feeding double labeled glyceride, when compared with the same ratio in the fed glycerides, gives percentage of glyceride molecules which have been completely hydrolyzed during digestion and absorption process.

Methods. Subjects: Our subjects were 2 patients with diagnosed adenocarcinoma of the left lung. Patient A was a 60-year-old man, weight 56 kg. Patient B was a 56-year-old man, weight 72 kg. In both patients a supraclavicular biopsy of the lymph nodes was performed and a plastic cannula was inserted into the thoracic duct as described earlier(1). In Patient A the cannula was inserted into one of the 2 stems of equal diameter present on operation. In patient B the catheter was inserted into the thoracic duct in such a way that the major portion of lymph flow bypassed the catheter. Only a portion of total lymph flow was therefore obtained during collection periods from these patients. This fact does not, however, influence the results as these are based on the ratio of label in acid and glycerol of the glycerides. *Labelled material.* Linoleic acid-1-C^{14} and glycerol-1-C^{14} (obtained from Radiochemical Centre, Amersham, England) were synthesized into glyceride *via* the acid chlorides. Free fatty acids were removed by passing the reaction mixtures through Amberlite IRA-400 columns and the triglyceride isolated by chromatography on silicic acid(2). Two different batches of glycerides were synthesized. The test meal for patient A was made from 635 mg doubly labelled glyceride homogenized into 310 ml of test meal (Table I). The fatty acid mixture obtained on saponification had a specific activity of 750 cpm and mg. Glycerol had a specific activity of 493 cpm and mg. Three hundred ml of this test meal was fed this patient at 8 a.m. the day after operation. Patient B was fed 300 ml of a test meal made up from 1.520 mg doubly labelled glyceride in 310 ml formula. The fatty acid mixture had a specific activity of 680 cpm/mg and the glycerol part 880 cpm and mg. The test meal was fed at 8 a.m. the day after cannulation. Test meals furnished around 10 g of glycerides each. Lymph was collected in periods shown in the Tables. Food was given again at 11 a.m. and 2 p.m. *Extraction and fractionation of lymph lipides.* Total lipide of the lymph was extracted with 20 volumes of ethanol:ether 3:1. The solvent was evaporated in vacuo, and total lipides were taken up in petroleum ether (b.p. 60-70°) and made to volume. Aliquots were subjected to chromatography on silicic acid to obtain a neutral fat, non-esterified fatty acid fraction and a phospholipide fraction(2). Then, the non-esterified fatty acids were removed from the neutral fat by chromatography on Amberlite IRA-400(2). The neutral fat fraction (glycerides, cholesterol and cholesterol esters) was subjected to chromatography on silicic acid for separation of cholesterol esters and a triglyceride-cholesterol fraction(2). Lipides in aliquots of test meals were extracted in the same way as described for extraction of lymph lipides. Fatty acids from various glyceride fractions of lymph lipides were obtained after saponification at room temperature with 4% KOH in 95% ethanol and petroleum ether, followed by extraction with alkaline 50% ethanol and reextraction of the fatty acids with light petroleum after acidification. Then the glycerol in the saponification liquor was

TABLE I. Composition of Formula Mixture Fed Daily as Sole Source of Nutrients to Patient A. 1500 ml of this formula contains approximately 1900 calories. The patient was fed 300 ml 5 times a day.

Constituents	Wt, g
Triglycerides, sterol-free	210
Milk protein	470
Dextrose	670
Water	5000

oxidized to formaldehyde with periodate and the formaldehyde steam-distilled and precipitated with dimethyldehydroresorcinol(4). The resultant methylene bismethone was filtered, recrystallized and weighed. The different fractions were plated in infinitely thin layers on aluminum planchets for counting in a gas-flow counter. Determinations of specific activity were also performed by the gas-phase counting technic of Glascock* The results of these latter determinations coincided well with those obtained on plating.

Results summarized in Tables I and II, show that relative specific activities of the fatty acid part of the lymph triglycerides are greater than those of the glycerol part. As the fatty acids liberated by hydrolysis appear in lymph mainly as triglycerides, but liberated glycerol is not reutilized for glyceride synthesis, the lower relative specific activity in the glycerol part is a measure of amount of glyceride, which has been completely split during digestion and absorption. The figures obtained show that when dietary glycerides reach the thoracic duct lymph in man 1/5 to 1/2 have been completely hydrolyzed. Inside this range, extent of hydrolysis varies considerably with a tendency to lower hydrolysis during early period of digestion and absorption. The figures obtained include loss of glycerol taking place in lumen of intestine and in the mucosa cells. The correspondence of the figures obtained in our experiments and those obtained from studies of intestinal content(5) indicate that no appreciable loss of glyceride glycerol occurs in the cells. The absorbed glycerides must have had one or more fatty acids attached to the glycerol but the figures do not give any indication on the extent of hydrolysis of the glycerides absorbed as such(6). The results of this investigation on extent of total hydrolysis of glycerides during digestion and absorption are well in accord with those calculated for extent of hy-

TABLE II. Analysis of Thoracic Duct Lymph Lipides of Subject A after Ingestion of a Doubly Labeled Triglyceride. Form of fat administered: Linoleic acid-1-C^{14} as triglyceride. Spec. act. of TG-FA = 750 cpm/mg. Glycerol-1-C^{14} as triglyceride. Spec. act. of TG-glycerol = 493 cpm/mg. Glycerol counted as methylene bismethone. Lymph triglycerides were isolated after chromatography on silicic acid.

Collection periods after admin., hr	Lymph vol, ml	Total fat, mg	Lymph glycerides: Fatty acid (Relative spec. act.)	Lymph glycerides: Glycerol (Relative spec. act.)	% total hydrolysis
1–2	21	577	44.5	30.5	32
3–4	12	218	20.5	16.2	22
4–5	22	772	38.0	31.0	19
5–6	35	2228	55.5	32.2	42
7–8	12	551	16.4	12.4	25

TABLE III. Analysis of Thoracic Duct Lymph Lipides of Subject B after Feeding a Doubly Labeled Triglyceride. Form of fat administered: Linoleic acid-1-C^{14} as triglyceride. Spec. act. of TG-FA = 680 cpm/mg. Glycerol-1-C^{14} as triglyceride. Spec. act. of TG-glycerol = 800 cpm/mg. Glycerol counted as methylene bismethone.

Collection periods after admin., hr	Lymph vol, ml	Total fat, mg	Lymph glycerides: Fatty acid (Relative spec. act.)	Lymph glycerides: Glycerol (Relative spec. act.)	% total hydrolysis
0–2	39	959	13.2	10.4	21.5
2–3	7.5	473	12.9	6.7	48
3–4	21	381	5.9	3.7	37.5

* These determinations were performed by Dr. S. Lindstedt.

drolysis from thoracic duct studies in the rat (7,8).

The technical assistance by J. Gürtler is greatly acknowledged.

This work is part of investigations supported by Swedish Medical Research Council.

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Received July 16, 1959. P.S.E.B.M., 1959, v102.

Comparative Toxicity of 3-Nitro 4 Hydroxyphenylarsonic Acid and Its Formaldehyde Dimer to Chicks. (25193)

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The arsenical, 3-nitro 4 hydroxyphenylarsonic acid (3-nitro), is widely used in poultry and swine rations. In the feed or drinking water of poultry, 3-nitro has a growth stimulating and coccidiostatic effect(1). In swine feeds, 3-nitro is used for growth promotion and as an aid in controlling infectious enteritis(2). Toxicosis has been reported in both species when 3-nitro was fed at recommended levels for growth stimulation or disease control(3,4). Polybenzarsol, a mixture of polymers formed from the reaction of formaldehyde and 4-hydroxy benzenearsonic acid, has been reported to have a low order of toxicity in rats, mice, dogs and man, following oral administration(5). This report suggests the possibility that the reaction product of formaldehyde and 3-nitro 4 hydroxyphenylarsonic acid (hereafter called the dimer) might have reduced toxicity to poultry.

Methods. The dimer of formaldehyde and 3-nitro was synthesized using the method of Faith(6). This was fed to chicks in 2 experiments. The basal diet was a practical broiler feed deemed adequate in all known nutrients. The birds were housed in electrically heated batteries equipped with raised wire floors and allowed access to feed and water *ad lib.* In the first experiment, duplicate lots of 10 male broiler strain chicks were given the following treatments: control; 3-nitro at levels of 49.5 and 198.0 mg/kg of feed; the dimer of 3-nitro at levels of 51.15 and 204.6 mg/kg; and procaine penicillin at a level of 4.4 mg/kg. All treatments in the second experiment were triplicated. Each lot contained 5 male and 5 female chicks. Treatments were as follows: control; 3-nitro at levels of 49.5, 74.25, and 198.0 mg/kg of diet and the dimer of 3-nitro at levels of 51.15, 76.72, and 204.6 mg/kg. At end of the 4-week test, triplicate liver arsenic determinations were made for each level of arsonic fed.* In both experiments all chicks were wing banded and individual weights taken at initiation and at termination of test. Feed consumption was determined by group at weekly intervals. Acute toxicity was studied using 4-week-old male chicks given a single oral dose of 3-nitro at levels of 100 and 200 mg/kg body weight and the dimer at levels of 103.2 and 206.4 mg/kg. The technic has been described(7).

Results. Data obtained in Exp. 1 and 2 are shown in Table I. In the first experiment, 4.4 mg penicillin, 49.50 mg 3-nitro and 51.15 mg of the dimer/kg diet respectively significantly improved growth over that obtained on the basal diet ($P = 5\%$ or less). 3-nitro appeared to be more effective in stimulating growth than either penicillin or the dimer, the latter 2 being essentially identical in their

* Arsenic determinations were made by J. B. Thompson of Trace Metal Research Labs., Chicago Heights, Ill.