TABLE I. Effect of Lysine on Rat Growth and Composition of Tibiae.

Diet	% L-lysine in diet	5-wk gain (g)	Pooled tibiae analysis (6 rats) Raw tibia wt (g)	% water	% fat extracted	% bone ash	% organic residue	% protein (N × 6.25)
Control bread	.29	84	1.7600	38.03	4.39	36.59	20.99	19.31
Bread + .2% L-lysine monohydrochloride	.45	143	2.1852	38.59	3.12	36.88	21.41	20.68
Bread + .5% L-lysine monohydrochloride	.69	186	2.5693	39.50	2.39	36.59	21.52	21.31
Bread + 1.0% L-lysine monohydrochloride	1.09	187	2.5566	39.11	2.70	36.20	21.99	20.12
Stock diet	1.10	204	2.7750	40.06	2.32	36.09	21.53	19.87

of the tibia. The per cent of bone ash and organic residue was constant with all 5 diets, but amount of moisture varied inversely with fat content. Thus lysine deficiency produced a fatty marrow with low moisture in the bones.

Protein content of the raw bones of each group was determined by Kjeldahl analysis of the tibiae from the left hind legs. It is evident that the organic residue is composed almost exclusively of protein.

These observations concerning changes in lipid content of tibiae of rats fed bread diets have been confirmed in experiments with other diets of low lysine content. Details of these experiments, as well as observations on the fat content of the tibiae of normal animals over their life-span, will be published separately.

Summary. Results with lysine-supplemented experimental diets and with stock diet show that the lipid content of the tibia is relatively low when sufficient lysine for optimum growth is available to young rats. With the lysine-deficient diet, growth is retarded and bone lipids are increased. Interference with production of normal bone marrow appears to be a definite indication of lysine deficiency.

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Received June 19, 1958. P.S.E.B.M., 1958, v98.

An *in vitro* Study of Fatty Acid Absorption.*† (24201)

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The form in which lipids are absorbed has been the subject of a number of investigations (1). Evidence has been accumulated in support of each of the 2 major theories of fat absorption, partition or lipolytic. The 2 theories have in common the necessity of free

* The author gratefully acknowledges the suggestions of Profs. H. C. Tidwell and T. Hastings Wilson during this investigation and technical assistance of Joanne Keeling and George Nunn.

† This work has been supported by the Williams-Waterman Fund and by U.S.P.H.S. Grant.

fatty acid production but to different degrees. However, the actual mechanism by which the fatty acids are absorbed is still not clear. One of the difficulties in interpretation of previous results is the absence of an *in vitro* procedure in which it is possible to measure precisely the material absorbed. A modification of an existing *in vitro* technic, employed in carbohydrate absorption(2), has been utilized in this study of absorption of C^{14}-labeled palmitic acid.

Methods and materials. Preparation of fatty acid-albumin complex. The complex was prepared by a modification of a method by Felts(3) in which 20 mg of palmitic acid and 0.7 μc of C^{14} palmitic acid were dissolved in ether and mixed with 4 ml of ethanol. The mixture was neutralized with 1.8 ml of 0.1 N NaOH and taken to dryness under nitrogen. Ten ml of a 10% albumin‡ solution in bicarbonate-saline, pH 7.0(4), was added and the mixture shaken in mechanical shaker for 1 hour. The solution was then filtered through a sintered glass filter, the pH adjusted to 7.4, glucose added to make a final concentration of 200 mg percent, and the volume made to 15 ml with albumin solution. *Preparation of tissue.* Segments of small intestine of male golden hamster were washed and everted according to procedure described by Wilson and Wiseman(2). In preparation of the sacs, a modification of the existing method was employed by introducing a small polyethylene tubing at one end during preparation of the everted sac, in order to fill the sac with the serosal solution. The tubing was stoppered with a small glass rod. By removing the stopper and introducing a small needle, the serosal solution could be removed without contamination. Sacs for aerobic and anaerobic experiments were prepared from the same animal. The sacs were filled with approximately 1 ml of bicarbonate buffer, (pH 7.4), containing 200 mg % glucose and were incubated 2 hr at 37°C in 3.5 ml of the C^{14} palmitic acid-albumin complex in an atmosphere of either 95% oxygen-5% CO_2 or 95% nitrogen-5% CO_2. At completion of incubation period, aliquots of serosal and mucosal solutions, and homogenized intestinal wall were plated for counting. The remainder of these samples were combined and fractionated into glycerides and fatty acids. *Fractionation methods.* Mucosal and serosal solutions and intestinal wall were extracted and fractionated into fatty acids and glycerides, with the aid of a carrier, according to the method of Borgström(5), at completion of incubation.

Results. After incubation under aerobic conditions, approximately 5% of the original activity, initially placed on the mucosal side, was found in the serosal compartment. When the pooled serosal solutions were fractionated into fatty acids and glycerides, approximately 90% of the serosal activity was found in glyceride form and 10% as free fatty acid. The intestinal wall contained approximately 20% of the original activity distributed as 60% glyceride and 40% free fatty acid. The mucosal solution at completion of experiment, contained 65% of original activity. Fractionation of the mucosal solution into glyceride and fatty acid gave values of 10% and 90% respectively. When a sample of the original palmitic acid-albumin complex, which had not been exposed to the intestine, was subjected to an identical fractionation, average values of 99% fatty acid and 1% glycerides were obtained.

In contrast to above results, total activity on the serosal side, after incubation under

TABLE I. Total Activity Recovered from Mucosal and Serosal Solutions and Intestinal Wall after Placing C^{14}-Palmitic Acid-Albumin Complex on Mucosal Side of the Isolated Intestine.

Incubation atmosphere*	Total activity after incubation based on initial activity		
	Mucosal side	Intestinal wall	Serosal side
	(%)		
Aerobic	63.3	19.4	7.2
"	62.9	22.6	4.8
"	67.2	21.4	4.6
Avg	64.5	21.1	5.5
Anaerobic	90.3	1.0	.4
"	90.6	1.4	.2
"	89.7	1.4	.15
Avg	90.2	1.3	.25

‡ Bovine albumin powder (Fraction V), Armour Labs., Kankakee, Ill.

* Aerobic and anaerobic atmosphere 95% O_2–5% CO_2 and 95% N_2–5% CO_2, respectively.

anaerobic conditions was on the order of 0.2%. Activity of this fraction was too low for fractionation. The intestinal wall and mucosal solutions gave average values of 1.3% and 90.2% of the total activity, respectively. The resulting mucosal solution after fractionation gave a distribution of activity between fatty acid and glyceride similar to that of the original solution. The intestinal wall under these conditions contained only a small fraction of activity as compared to that found under aerobic conditions. This trace of activity was distributed as 90% glyceride and 10% fatty acid.

Discussion. With this procedure a fatty acid, a product produced during digestion of fats, has been shown to penetrate the intestinal wall, be converted into a glyceride, and be transported into the serosal solution. The resulting adsorbed material found on the serosal side was in the form of a glyceride, which is the major product appearing in lymph, when either fatty acids or the esterified fatty acids are fed to the intact animal. An investigation to determine the nature of this glyceride is presently underway.

Evidence has been presented to indicate that the transport from mucosal to serosal side is an energy requiring process, since this transport is decreased approximately 20 fold under anaerobic conditions. It has been possible during the process of absorption and transport to demonstrate the incorporation, which in itself is an energy requiring reaction, of fatty acid into a glyceride. This evidence is substantiated by analysis of the intestinal wall at completion of the experiment. Actual uptake of activity by the intestine, under anaerobic conditions, is decreased 16-fold as compared to aerobic conditions. In addition to decreased energy supply under anaerobic conditions, a factor which may contribute to the lower levels of transformation of the fatty acid into glyceride is a stripping of intestinal mucosa under anaerobic conditions. However, this loss of some of the mucosa does not account for the decrease in activity of the intestinal wall glycerides, since the glyceride in the mucosal solution is not increased over control value.

The increasing percentage of glyceride in the direction, mucosal to intestinal wall to serosal compartment, is paralleled by a decreasing percentage of fatty acid. This finding is consistent with the postulate of entrance of fatty acid into the mucosal cell, transformation into glyceride form, and restricted passage of the glyceride into the serosal solution. The 10% glyceride found on the mucosal side is presently under investigation. This phenomenon may be involved in (a) secretion of glycerides into the intestine(6, 7), (b) reported mechanism of transesterification(8), and (c) controlling factor for rate of fat absorption.

Summary. An *in vitro* procedure has been adapted for study of fatty acid absorption. Free palmitic acid enters the intestinal mucosa and is transformed into a glyceride, which is subsequently transferred to the serosal side. The resulting activity on the serosal side is present as 90% or more in the form of glycerides. Transfer of activity to the serosal side and incorporation of activity in the intestine, is inhibited 20- and 16-fold, respectively, under anaerobic conditions. The relationships of the reported findings to fatty acid absorption are discussed.

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Received June 19, 1958. P.S.E.B.M., 1958, v98.