TABLE II. Absorption of Sr^{85} and Ca^{45} from Perfused Small Intestine as Affected by pH.

pH of perfusate	% of total dose*		Ratio
	Sr^{85}	Ca^{45}	Sr^{85}/Ca^{45}
1	4.4 ± .7	4.4 ± .7	1.01 ± .09
2	13.4 ± 5.0	13.9 ± 4.6	.97 ± .14
3	10.3 ± 8.1	13.8 ± 4.7	.75 ± .34
4	6.3 ± 2.4	15.3 ± 4.6	.41 ± .04
5	3.7 ± .4	14.0 ± 1.1	.27 ± .03
6	4.5 ± .8	13.9 ± 1.7	.32 ± .05
7	3.4 ± .6	12.3 ± 2.1	.27 ± .01
8	3.8 ± .9	14.5 ± 2.6	.26 ± .10
9	3.2 ± .6	15.0 ± 3.5	.21 ± .04
10	4.6 ± .9	16.3 ± 2.8	.28 ± .02
11	4.7 ± .3	15.0 ± 3.0	.31 ± .05
12	6.0 ± 1.0	8.7 ± 1.2	.68 ± .13

* Mean values from 6 rats ± 95% confidence interval.

city of beryllium as compared to the other alkaline earths.

The results of additional studies to determine the effect of pH are shown in Table II. There was remarkably little effect of pH in the range from pH 5 to pH 11. In the range from pH 4 to pH 2 there was a marked increase in absorption of Sr-85, but not in Ca-45, suggesting that absorption of the two isotopes occurs by different mechanisms. The greater variability of the results in this acid range may be due to animal variation in reserve buffering capacity. The sharp drop in Ca-45 absorption at the extremes of pH 1 and pH 12, and the similarity of the results obtained with Ca-45 and Sr-85 at these extremes, suggests that the mechanisms responsible for the preferential absorption of calcium over strontium are inactivated under these conditions.

The inconstancy in the ability of the small intestine to discriminate between strontium and calcium at varying levels of the alkaline earths and pH of the perfusion fluids, emphasizes the need for caution, expressed by Kornberg(10), in using the ratio concept in hazard assessment.

Summary. Using an *in vivo* perfusion technic the absorption of Sr-85 and Ca-45 from the small intestine of the rat was observed to be influenced by the alkaline earths and pH. Addition of calcium or beryllium to the perfusate reduced Ca-45 absorption more than Sr-85 absorption. Magnesium and barium had a greater effect on Sr-85 than on Ca-45, while strontium reduced absorption of Ca-45 and Sr-85 to the same extent. Extremes in pH tended to increase the ratio of Sr-85 to Ca-45 absorption.

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Further Studies on Milk Fat Synthesis.* (27490)

J. R. LUICK AND J. M. LUCAS
Department of Animal Husbandry, University of California, Davis

We have reported evidence(1) for the *de novo* synthesis of milk fat by mammary tissue based on a study of the radioactivity found in milk fat glycerol following intramammary infusion of various C^{14} labeled metabolites. The synthesis of glycerol from glucose(2) and subsequent incorporation of the newly synthesized glycerol into milk fat suggested that

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milk fat was synthesized within the udder from "pools" of fatty acids and glycerol. An alternate pathway, equally possible but lacking experimental proof, envisions milk fat synthesis as a process of fatty acid transacylation onto preformed triglyceride molecules which were previously absorbed from the blood(3). The present study was undertaken to extend our earlier work and to test our hypothesis of *de novo* fat synthesis by mammary tissue.

Experimental. Details pertaining to the method and site of infusion, the isotope infused, and the milk yield of the various cows used in these studies were reported earlier for all trials except stearate C^{12}(1). The cows were milked at 3 and 8-10 hours following intramammary infusion of the isotope, and the milk was stored at 4°C until the cream separated from the skim milk. Milk fat was extracted from the cream fraction and purified by the Roese-Gottlieb technic. Ten grams of each milk fat sample were then saponified with alcoholic KOH and the constituent fatty acids were isolated from the reaction mixture by ether extraction. The fatty acids were dried, combusted in a microfurnace, and the resulting CO_2 precipitated as $BaCO_3$. Planchets of "infinite thickness" were prepared for radiochemical assay in a standard G.M. counter.

In the stearate 1-C^{14} trials, 1.6 mc sodium stearate were blended with 100 ml of "HP" vehicle† prior to infusion through the teat canal into the right half of the udder. Milk fats obtained after infusion of stearate C^{14} were subjected to silicic acid chromatography prior to hydrolysis. This technic removes free fatty acids and was employed to prevent inadvertent contamination of milk fatty acids with unabsorbed stearic acid.

Results and discussion. The criterion by which we decide whether these C^{14} labeled fatty acids are used by mammary tissue for synthesis of milk fat is based on a comparison of the relative counting rates of milk fatty acids isolated from the infused and uninfused quarters. If the fatty acid is not incorporated directly into milk fat (or synthesized into a higher homolog that is) it would have to be taken into the general circulation and returned to the udder in a "usable" form before it could appear in milk fat. In this case, the C^{14} specific activity of precursor C would be the same for all 4 quarters and no difference in specific activity would be noted in the milk fatty acids isolated from them.

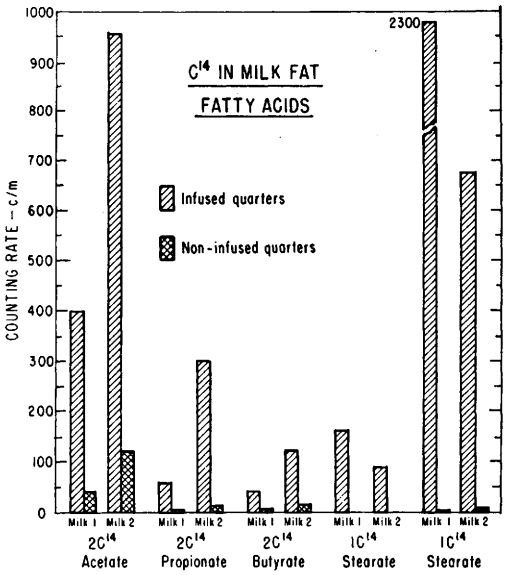

FIG. 1. C^{14} in milk fat fatty acids following intramammary infusion of C^{14} labeled acetate, propionate, butyrate, and stearate into one quarter of the gland. The height of the columns does not necessarily reflect the importance of the metabolite as a precursor of fatty acids. Column height also depends upon amount of isotope infused, extent to which it is absorbed, amount of milk synthesized during collection period, and dilution with preformed milk.

The results of these trials are shown in Fig. 1. The high radioactivity found in the fatty acids of the infused quarter following infusion of acetate, propionate and butyrate might have been anticipated since these acids (especially acetate) are generally acknowledged to be prime precursors of the lower fatty acids in milk fat. Acetate contributes 2 C units for synthesis of the characteristic short chain fatty acids of milk fat(4) whereas propionate is thought to be the 3 C unit to which 2 C units are added for synthesis of the odd chain fatty acids(5). The relatively high label in milk fatty acids following butyrate C^{14} infusion suggests that this compound entered di-

† An emulsion of neutral pH used for intramammary infusion of fat soluble substances. Hamilton Pharmacal Co., Inc.

rectly into the C 4 pool from which the short chain fatty acids, especially butyrate, originate.

There is little evidence to suggest that stearic acid is synthesized in the udder. On the contrary, this acid is thought to be transported to the mammary gland as a part of the absorbed plasma lipids. Lauryssens *et al.* (6) reported that 1-C^{14} stearate was incorporated directly into the glycerides of perfused mammary tissue. In this study we have shown that infused stearic acid also becomes a part of the newly formed milk fat molecule. The incorporation of C^{14} stearate into milk fat following its infusion into mammary tissue suggests that the plasma lipids are hydrolyzed in the udder and thereby provide a "pool" of fatty acids for milk fat synthesis. These results, therefore, supplement our earlier work and support the hypothesis that milk fat is synthesized in mammary tissue from the free fatty acids and glycerol. Additional support for this hypothesis has recently been published by Patton *et al.*(7).

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Peripheral Metabolism of Thyroid Hormones in Guinea Pigs Immunized Against Bovine Thyroglobulin.* (27491)

B. N. PREMACHANDRA, A. K. RAY AND H. T. BLUMENTHAL
Institute of Experimental Pathology, The Jewish Hospital, St. Louis, Mo.

Circulating antibodies against thyroglobulin have been demonstrated in some normal humans without thyroid disease and in cases with various forms of thyroiditis(2,4) as well as in experimental animals immunized with thyroglobulin(7). The precise mechanism which accomplishes leakage of thyroglobulin from the follicles and leads to struma lymphomatosum (Hashimoto's disease) is not clear. Once such leakage has occurred, subsequent pathologic and serologic findings indicate a continuing antigen-antibody reaction leading to extensive inflammatory changes in the thyroid culminating in a myxedematous state. In those individuals in whom antithyroglobulins can be demonstrated, and in whom there is no evidence of any perceptible aberration in thyroid function, the effect of such antibodies, if any, on peripheral metabolism of thyroid hormones has not been sufficiently investigated. The present report deals largely with this problem.

Experimental. A total of 35 guinea pigs of both sexes and ranging in weight between 500 and 900 g were used in this study; they were housed in cages and maintained at uniform room temperature (78 ± 2°C). One group of 17 animals was injected with electrophoretically pure bovine thyroglobulin mixed with Freund's complete adjuvant once weekly for 3 weeks. The antibody response was then estimated utilizing their serum and the Boyden(1) tanned red cell (TRC) hemagglutination technic. A control group of 5 guinea pigs was injected with adjuvant alone (treated controls) and the remaining 13 served as untreated controls. In the experimental group, antibody titers were estimated at 4-5 weeks and at 8-9 weeks. Determination of the rate of disappearance of I^{131}-labelled thyroid hor-

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