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)

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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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TABLE I. $T_{1/2}$ of Labeled Thyroid Hormones in the Blood of Normal and Immunized Guinea Pigs and Serum Antibody Titer Response.

	No. of animals	$T_{1/2}$ of T_4^{131} (hr)	No. of animals	$T_{1/2}$ of T_3^{131} (hr)	Serum antibody titer
Untreated	7	30.4 (24- 37)*	6	33.0 (22-39)	—
Adjuvant treated	3	29.4 (25- 32)	2	27.3	—
Thyroglobulin and adjuvant treated 5 wk	6	37.3 (29- 38)	3	26.4 (23-28)	+ †
Thyroglobulin and adjuvant treated 12 wk	5	71.6 (69-125)	3	61.5 (49-72)	++

* Range. † Antibody titer expressed by Boyden's technic(1).

mones was carried out by injecting 10 μc of either radioactive l-thyroxine (T_4^{131})† or radioactive l-triiodothyronine (T_3^{131})† and blood radioactivity measured once daily for 4-5 days in a well-type scintillation counter (Picker X-Ray, Model 2804). To provide statistically valid counting, 0.1-0.5 ml of blood was obtained from either the marginal ear vein or from the heart for radioactivity measurements. The half-life ($t_{1/2}$), *i.e.*, the time required for half of the labelled hormone to disappear from the blood, was calculated from the slope of the regression line representing the I^{131} disappearance curve according to the formula $t_{1/2} = \frac{\log 2}{\text{slope}}$.

Results. The data obtained are shown in Table I. The mean $t_{1/2}$ of T_4^{131} and T_3^{131} was similar in untreated control guinea pigs (30.4 and 33.0 hours, respectively), a situation not usual in other mammals(6). Guinea pigs injected with adjuvant alone gave similar $t_{1/2}$ values for T_4^{131} and T_3^{131} (29.4 and 27.3 hours, respectively). Five weeks after completion of immunization, the $t_{1/2}$ for T_4^{131} was 37.3 hours, a slight increase over both groups of controls, but the $t_{1/2}$ for T_3^{131} at the same period was 26.4 hours, a slight decrease in comparison with untreated controls, and about the same as adjuvant treated controls. No particular significance is attached to these small variations, since the magnitude of deviation from controls is well within the range exhibited by individuals in any one group.

At 11-12 weeks after completion of immunization, there was a marked increase in the $t_{1/2}$ of T_4^{131} (201%) and T_3^{131} (86.4%) as compared with untreated controls and of 211% and 161% respectively in comparison with the $t_{1/2}$ values in adjuvant treated controls. The serum antibody titer estimation at 4-5 weeks after completion of immunization ranged between 1+ and 2+(1). At 8-9 weeks after immunization, most of the immunized animals exhibited an antibody titer estimate of 2+ and 3+, indicating a high antibody titer. Since at no time were significant differences in the $t_{1/2}$ values found between sexes, the data of both males and females have been combined in Table I.

Discussion. The myxedematous state associated with Hashimoto's disease is evidently attributed to antigen or thyroid exhaustion resulting from the replacement of functional parenchyma by reactive non-functional tissues(3). As far as we are aware, there have been no studies dealing with the influence of circulating thyroglobulin antibody on the peripheral disappearance of thyroid hormones. On the other hand, the present study indicates that a sufficiently elevated circulating thyroglobulin antibody level may have a considerable influence on the rate of disappearance of T_4 and T_3. Differences in the $t_{1/2}$ of T_4^{131} and T_3^{131} between controls and experimental animals at 11-12 weeks after immunization were highly significant (p less than .01); these guinea pigs were all within the same weight range (500-900 g).

The possibility that I^{131} might be metabolically separated from the thyroid hormones and recycled (thereby effecting $t_{1/2}$ values) is considered, although the magnitude of such recycling, if any, should be extremely small since normal I^{131} uptake in guinea pigs is notoriously low. Indeed, we have been unable to show by *in vivo* technics using I^{131},

† Purchased from the Abbott Laboratories, Oak Ridge, Tenn.

effect of recycling in guinea pigs regardless of the level of goitrogen (tapazole) employed to block thyroid uptake of recycled I^{131}. Also, similar $t_{1/2}$ values for T_4^{131} and T_3^{131} in the normal guinea pig is reminiscent of the situation in avians, where both the hormones are bound to the protein with equal intensity, and further, there is a lack of specific thyroxine binding protein (TBP). Hence, both T_4 and T_3 disappear from the blood at the same rate(5). However, it is difficult to give proper interpretation from the standpoint of normal thyroid physiology since T_3 has not been detected in guinea pig serum with certainty.

Unpublished electrophoretic studies have shown that both T_4^{131} and T_3^{131} are bound by thyroglobulin antibody. This finding, coupled with the present observations, of the progressive retardation of the rate of disappearance of labelled hormones from blood in immunized animals would suggest that the cells at the periphery are not able to utilize the hormone for their energy metabolism when it is bound to thyroglobulin antibody. If it is assumed that the pituitary is also unable to utilize hormone-antibody complex, such a situation would result in a transient hypersecretion of thyrotropic hormone (TSH) because of the lowered levels of circulating unbound hormone. This would be in accordance with the classically demonstrated homeostatic mechanism regulating the thyroid and hypophysis. While such a state might aggravate an existing pathological condition in the thyroid, it would not, however, provide a basis for explaining the initial leakage of thyroglobulin into the circulation which evidently causes antibody production.

Summary. The time required for the disappearance from the blood of one-half the radioactivity ($t_{1/2}$) of thyroxine (T_4^{131}) and triiodothyronine (T_3^{131}) has been studied in normal, adjuvant treated and thyroglobulin-adjuvant immunized guinea pigs. The antibody titer in immunized animals has been estimated by the tanned red cell hemagglutination technic. The titer at 8-9 weeks was greater than at 4-5 weeks after immunization. The $t_{1/2}$ of T_4^{131} and of T_3^{131} of experimental animals was not significantly different from that of untreated and adjuvant treated controls at 5 weeks after immunization. However, at 12 weeks after immunization the $t_{1/2}$ of both radioactive hormones was markedly increased; the increase was by 201% in the case of T_4^{131} and by 86.4% in the case of T_3^{131}. There was a gross inverse relationship between the $t_{1/2}$ of these radioactive hormones and the magnitude of the antibody titer. The implications of this inverse relationship have been briefly discussed.

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