ectomized chicks indicate that the D-isomer has a similar action on cholesterol synthesis and turnover as does L-thyroxine.

Summary. The effect of L- and D-thyroxine on cholesterol synthesis and turnover in thyroidectomized and in normal untreated chicks has been compared using acetate-2-C^{14} and cholesterol-4-C^{14}. (a). Although liver weights and liver cholesterol levels were not significantly different between the normal (EU) and iodoamino acid treated thyroidectomized chicks and those thyroidectomized without treatment, serum cholesterol levels were elevated only in the last group. (b). Appearance of labeled serum cholesterol after acetate-2-C^{14} injection, cholesterol synthesis after incubation of liver slices with acetate-2-C^{14}, and cholesterol-4-C^{14} turnover in these various groups indicate that both L-thyroxine and D-thyroxine in the quantities used can stimulate cholesterol biosynthesis and turnover in a manner comparable to that found in the normal. (c). The data are discussed in the light of present concepts of the mode of action of thyroid substances on cholesterol metabolism, and the applicability of the findings to mammalian species.

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Variables Influencing Uptake of Radioactive Triiodothyronine from Plasma by Erythrocytes of Cattle.* (28957)

K. T. SZABO† AND J. P. MIXNER
Department of Animal Sciences, New Jersey Agricultural Experiment Station, Sussex

The *in vitro* uptake of tracer amounts of radioactive triiodothyronine (T_3-I^{131}) from plasma by red blood cells (RBC) has been suggested as a simple and rapid method for evaluating thyroid function in humans(1). This procedure has been shown to possess diagnostic accuracy in humans comparable to other standard tests such as: a) basal metabolic rate, b) chemical protein bound iodine, and c) thyroid I^{131} uptake(2-5). Many stud-

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† Present address: Smith Kline & French Laboratories, Research and Development, Philadelphia, Pa.

ies have indicated that RBC uptake values may be influenced by different factors and that even some relatively minor variations in the technique could produce widely divergent results(6-8).

A reliable *in vitro* method utilizing radioactive isotopes for estimation of thyroid function would be of great value in dairy cattle and veterinary research.

In this paper is presented the influence of certain variables on *in vitro* uptake of tracer doses of T_3-I^{131} by RBC in dairy cattle. A brief report has been made(9).

Materials and methods. The blood samples were obtained from Holstein-Friesian female cattle (from birth to 2 years of age) of the Rutgers University Dairy Research Center, Sussex, N. J. The experimental animals were maintained under normal farm conditions. Blood samples were drawn from the jugular vein, or in the case of cows, from the mammary vein directly into a Pyrex blood-collection tube containing an oxalate anticoagulant solution which had been previously evaporated to dryness.

The methods originally described and improved upon by Hamolsky *et al*(1,2) were followed. Briefly, 0.1 ml of tracer amounts of T_3-I^{131} solution (L-Triiodothyronine-I^{131}, list no. 0896, E. R. Squibb & Sons) was added to 3.0 ml of sample blood. The tubes were placed in a water bath (37°C) for 2 hours and were agitated by shaking at 15-minute intervals. After incubation the radioactivity was determined on a 1.0 ml aliquot using a well-type scintillation counter. The red blood cells (RBC) were then washed 5 times in physiological saline (0.9% NaCl) solution by centrifuging. The clear supernatant fluid was removed with care to avoid disturbing the erythrocytes. After the final washing, the volume of the original sample was restored by adding saline (approximately 1.0 ml) to the mark previously made on the tube. The radioactivity remaining in the cell mass was then determined and expressed as percentage uptake of the original radioactivity, using the following formula:

$$T_3\text{-}I^{131}\ (\text{RBC})\ \text{uptake}, \% = \frac{F \times 100}{H}$$

TABLE I. Effect of CO_2 Tension on Uptake of T_3-I^{131} by RBC.

Animal No.	Treatment during incubation: Open tubes	Stoppered tubes	Tubes flushed with CO_2, then stoppered
	(Uptake, %)		
H-303	10.12*	9.93*	13.07*
H-305	8.86	8.76	11.76
H-327	8.98	9.13	12.72
H-329	10.47	11.37	15.90
H-341	8.65	9.15	12.18
H-342	7.98	8.06	11.31
Mean	9.18	9.40	12.82

* Each value represents mean of duplicate determination.

where F = measured fraction of T_3-I^{131} taken up by RBC, and H = hematocrit of the blood sample expressed as a decimal fraction. All determinations were made in duplicate.

Results and discussion. Effect of CO_2 tension on uptake of T_3-I^{131} by RBC. Earlier investigators(1,2,10) have reported a marked decrease in uptake of T_3-I^{131} by RBC using non-stoppered tubes during incubation. Additional studies(11,12), however, have shown different results on the role of CO_2 in the incorporation of labeled thyroid hormones into RBC. To investigate the effect of CO_2 tension on uptake of T_3-I^{131} by RBC, 6 blood samples were incubated with T_3-I^{131} in: (a) open tubes, b) stoppered tubes, and c) tubes flushed with CO_2 and then stoppered. Otherwise, the general procedure previously outlined was carried through.

Stoppering the tubes during incubation resulted in a negligible increase in T_3-I^{131} uptake as compared to control unstoppered tubes. However, the uptake of T_3-I^{131} by RBC was strikingly increased when the incubation was carried out in tubes flushed with CO_2 and then stoppered (Table I).

The increases obtained were: 39.65 and 36.38% in comparison with unstoppered tubes and stoppered tubes, respectively. Since it was evident that elevated CO_2 tension had a definite positive effect on T_3-I^{131} uptake, this method was adopted as standard procedure for subsequent experiments(13).

Effect of antibiotics and storage time of blood on uptake of T_3-I^{131} by RBC. Ham-

TABLE II. Effect of Storage of Blood at 5°C and Antibiotic Treatment on Uptake of T_3-I^{131} by RBC.

	Storage period (days)	No. of blood samples	No antibiotics	Antibiotics*
			(Uptake, %)	
Trial 1	0	3	13.3	16.6
	1	3	12.0	15.6
	4	3	11.8	15.3
	8	3	9.6	12.6
	15	3	9.5	10.6
Trial 2	0	12	13.6	—
	1	12	13.2	—

* 1000 units of penicillin and 1.0 mg of streptomycin per ml of blood.

olsky *et al*(2) have reported that reproducible results were obtained with non-sterile human blood stored in a refrigerator for 7 days and with sterile blood kept under refrigeration for as long as 32 days. If storage of blood were possible, blood samples could be mailed from distant areas to diagnostic laboratories without undue distortion of uptake values. It was hoped that such data would provide basic information for preservation or mailing blood samples.

Three samples of blood were stored at 5°C for 0, 1, 4, 8, and 15 days. Subsamples of blood in addition were treated with 1000 units of penicillin and 1.0 mg of streptomycin per ml of blood (Table II, trial 1).

In non-treated blood samples stored at 5°C for 0, 1, 4, 8, and 15 days, a gradual decrease in uptake of T_3-I^{131} by RBC with storage time was observed. In the treated blood, addition of penicillin and streptomycin increased uptake of T_3-I^{131} by RBC in comparison to the values obtained in control tubes, but did not prevent the decline in uptake upon storage.

No significant difference was found in comparing uptake of 12 blood samples stored for zero and one day without antibiotics (Table II, trial 2).

These results indicate the importance of running the assays on fresh blood.

Effect of length of storage of T_3-I^{131} at 5°C prior to use on uptake of T_3-I^{131} by RBC. Due to the cost of the radioactive triiodothyronine, it would obviously be advantageous to be able to use, with comparatively accurate results, the radioactive material by extending storage time of the T_3-I^{131} prior to use. Different lots of diluted T_3-I^{131} were stored at 5°C for 1, 3, 5½ and 7½ weeks prior to its use in incubating with 3 blood samples. The mean per cent uptakes were, respectively, 14.5, 14.4, 14.4 and 12.5, for the 1, 3, 5½ and 7½ weeks storage period. Since the mean uptake values were very uniform up to 5½ weeks, storage of the diluted isotope (T_3-I^{131}) for this period would be satisfactory. The drop in uptake for T_3-I^{131} stored for 7½ weeks would indicate that a chemical breakdown of the triiodothyronine had occurred.

Effect of exogenous tracer levels of T_3 upon uptake of T_3-I^{131} by RBC. It has been reported that RBC uptake of T_3-I^{131} depends upon thyroid hormone–plasma protein interactions and indirectly measures the ability of the plasma to bind T_3-I^{131}(2). Other investigators(14,15) have noted that when increasing amounts of stable L-thyroxine were added to the erythrocyte uptake test system, T_3-I^{131} was displaced from the plasma to the erythrocytes.

In our study no conclusive indication was found that the changed concentration of exogenous tracer levels of T_3 had any effect upon uptake of T_3-I^{131}, within the levels studied (Table III).

Summary. Some of the possible variables influencing the uptake of radioactive triiodothyronine from plasma by red blood cells (RBC) of dairy cattle were investigated. The *in vitro* uptake of T_3-I^{131} by RBC was strikingly increased when incubation was carried out in tubes flushed with CO_2. The antibiotic treatment and storage of blood at 5°C prior to incubation delayed but did not prevent the decline in uptake of T_3-I^{131} by RBC upon storage. No significant difference was found in the uptake of blood

TABLE III. Effect of Exogenous Tracer Levels of T_3 upon Uptake of T_3-I^{131} by RBC (4 Blood Samples).

Exogenous T_3, μg/ml of blood	Uptake, %
79.6×10^{-6}	14.1
159.2×10^{-6}	14.3
318.4×10^{-6}	14.8

samples stored for zero and one day. Uniform uptake values were found by extending the storage time of T_3-I^{131} up to 5½ weeks prior to use on uptake of T_3-I^{131} by RBC. The lot of T_3-I^{131} stored for 7½ weeks indicated a chemical breakdown of the triiodothyronine since a significant drop in its uptake was found. Within the levels studied, the changed concentration of exogenous tracer levels of T_3 was found to have no significant effect upon uptake of T_3-I^{131}.

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Metabolism and Excretion as Factors Influencing Serum Cholesterol Level in Two Lines of Chickens.* (28958)

C. E. CLARK,† F. H. WILCOX AND C. S. SHAFFNER
Dept. of Poultry Science, University of Maryland, College Park

It has been demonstrated in various vertebrates including rats(1), humans(2), and chickens(3,4) that genotype influences blood level of cholesterol. The evidence is insufficient, however, to establish the precise mode of genetic transmission or to explain the physiological mechanisms involved. Hardy, Auger, and Wilcox(5) attempted to determine physiological contributors to blood serum cholesterol levels in 2 lines of White Leghorn chickens that differed in both combined and free cholesterol. Birds of both lines reacted similarly to fasting, injection of various hormones, and temperature stress. No line differences were observed with respect to: serum levels of fatty acids and phospholipids; cholesterol levels in testis, brain, adrenal, or liver; weights of various organs; or hematocrit values.

This study was undertaken further to investigate the physiological mechanisms that may be responsible for the different serum cholesterol levels in these 2 lines(4) of chickens. The immediate influence of the liver was checked by analyzing serum of post- and pre-hepatic blood of both lines. Comparative rates of sterol synthesis were studied *in vitro* by using acetate-1-C^{14} in whole blood and liver slices. The comparative catabolism, distribution, and excretion of injected cholesterol-4-C^{14} were determined in each line.

Materials and methods. Immediate liver effect. The chickens were progeny of high

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† Present address: Dept. of Poultry Husbandry, Utah State Univ., Logan.