Triiodothyronine-I^{131} Uptake by Bull Spermatozoa.* (27395)

J. J. Sullivan and J. P. Mixner
Department of Dairy Science, New Jersey Agricultural Experiment Station, Sussex

Carter(1) reported that O_2 consumption of rabbit spermatozoa was not affected by thyroxine. Lardy and Phillips(2) reported that thyroxine stimulated glycolysis, but inhibited respiration of washed bull spermatozoa. Schultze and Davis(3) found that the respiration of bull spermatozoa was increased slightly by thyroxine. Gassner and Hopwood (4) reported that thyroxine or triiodothyronine stimulated respiration of spermatozoa in low concentrations and depressed both respiration and fructolysis in high concentrations. Freund and Mixner(5) found no apparent effects of either thyroxine or triiodothyronine on fructose utilization by bull spermatozoa.

In other studies labeled glucose(6,7), labeled fructose(8), labeled phospholipids(9), and labeled glycerol(10) have been used to study spermatozoa metabolism.

The present studies were to determine if there was active uptake of radioactive triiodothyronine (T_3-I^{131}) by bull spermatozoa and, if so, what were the effects of certain factors on this uptake.

Methods and materials. A preliminary experiment showed that the *in vitro* uptake of T_3-I^{131} was greater for freshly ejaculated bull spermatozoa than for spermatozoa which had been injured or killed by cold-shock. Accordingly, trials were undertaken further to characterize this phenomenon.

Semen from dairy bulls was used in 4 different trials, with each subsample being diluted to contain 250×10^6 spermatozoa per ml.

Stock solutions of commercial solutions of radioactive triiodothyronine (Squibb) were diluted to 50 ml with physiological saline solution and each shipment of isotope was used routinely for a period of 2 weeks. In each study 0.1 ml of the diluted T_3-I^{131} solution was added to each tube of diluted semen. This amount of isotope had a range of radioactivity from 0.02 to 1.32 μc and a range in T_3-I^{131} weight from 6.734×10^{-7} to 4.776×10^{-4} μg.

To determine the percent uptake of T_3-I^{131} by spermatozoa, stoppered test tubes containing diluted semen (2 ml in trial 1 and 3 ml in trials 2, 3, and 4) and tracer doses of T_3-I^{131} were incubated for 2 hours at 37°C. Duplicate tubes were run on each treatment and were gently agitated every 15 minutes during the incubation period. Radioactivity of a 1 ml portion of diluted semen from each tube was determined after incubation, using a well-type scintillation counter. The spermatozoa were then centrifuged down, the supernatant fluid removed, and the spermatozoa washed with 10 ml of physiological saline. Centrifugations and washings totaled 5 (trial 1) or 3 (trials 2, 3, and 4). Following the washings the radioactivity of the spermatozoa was then recounted.

$$\% \text{ uptake} = \frac{\text{Net counts/min./250} \times 10^6 \text{ spermatozoa after washing}}{\text{Net counts/min./diluted 1 ml semen sample before washing}} \times 100$$

Experimental procedures and results. Trial 1. *Effect of freezing and storage of spermatozoa at –79°C upon motility and uptake of T_3-I^{131}.* Six semen samples were diluted separately in an egg yolk-sodium citrate-glycerol diluent such that each ml contained 250×10^6 spermatozoa. Portions of the samples were sealed in 1-ml glass ampules, frozen to –79°C, and stored in dry ice according to procedures already described(11). The uptake of T_3-I^{131} of unfrozen portions of the semen samples was determined on the day of semen collection, at which time the percent of motile spermatozoa was also noted. Similarly the uptake of T_3-I^{131} and percent of motile spermatozoa of the frozen samples was determined after 1 week of storage at –79°C. The mean T_3-I^{131} uptake of the unfrozen samples

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TABLE I. Effect of Storing Spermatozoa at 5°C for 9 Days upon Motility and Uptake of T_3-I^{131}.

Day of storage	Mean motility	Mean uptake
	(%)	(%)
0	67.5	3.85
3	59.2	3.36
6	43.3	4.10
9	30.8	2.54

was 1.77% and of the frozen samples, 0.97%. Mean motility estimates were 71% and 38% for the unfrozen and frozen samples, respectively. In both instances the differences in mean values were statistically significant ($P<0.01$).

Trial 2. *Effect of storing spermatozoa at 5°C for 9 days upon motility and uptake of T_3-I^{131}.* Six semen samples were diluted in an egg yolk-sodium citrate diluent(11) such that each ml contained 250×10^6 spermatozoa. The uptake of T_3-I^{131} and mean percent of motile spermatozoa were determined on the day of semen collection (day 0) and on the 3rd, 6th, and 9th days of storage at 5°C (Table I). Motility of spermatozoa declined in a conventional manner upon storage. The uptake of T_3-I^{131}, however, seemed to be erratic in nature, particularly the determinations made on day 6, such that the correspondence between percent of motile sperm and the uptake of T_3-I^{131} by spermatozoa was not particularly good.

Trial 3. *Effect of weight of T_3 upon T_3-I^{131} uptake by spermatozoa.* Six semen samples were diluted separately in an egg yolk-sodium citrate diluent(11) such that each ml contained 250×10^6 spermatozoa. The amount of T_3 added per ml was varied. The mean percent uptakes of T_3-I^{131} were not significantly different for the 3 levels of T_3 added (Table II).

Trial 4. *Effect of seminal plasma removal and diluent composition upon uptake of T_3-I^{131} by spermatozoa.* Washed and unwashed spermatozoa of each of 3 semen samples were diluted with either an egg yolk-sodium citrate (11) or a 3% sodium citrate diluent, such that each ml contained 250×10^6 spermatozoa. The spermatozoa of the unwashed portions were centrifuged down and then resuspended repeatedly in the diluent to simulate the physical aspects of the centrifuging and washing of the other samples as closely as possible. Mean uptake of T_3-I^{131} for each treatment is presented in Table III. The difference in uptake of T_3-I^{131} by the washed and unwashed spermatozoa was highly significant with the washed spermatozoa having the greater uptake. Whether this effect is due to the washing manipulations or to removal of seminal plasma constituents is not known. Spermatozoa diluted in sodium citrate solution had a significantly higher uptake of T_3-I^{131} than those diluted in the egg yolk-sodium diluent. These results suggest that some constituents of both seminal plasma and the egg yolk may compete with spermatozoa for uptake or binding of available T_3-I^{131}.

TABLE II. Effect of Weight of T_3 Added Upon T_3-I^{131} Uptake by Spermatozoa.

Wt of T_3, μg.	Mean uptake, %
2.388×10^{-4}	3.65
4.776×10^{-4}	3.65
9.552×10^{-4}	3.80

TABLE III. Effect of Seminal Plasma and Diluent Upon Uptake of T_3-I^{131} by Spermatozoa.

Diluent	Seminal plasma	Mean uptake, %*
Egg yolk-citrate	not removed	6.40
" " "	removed	15.93
Sodium citrate	not removed	21.57
" "	removed	51.23

* Means are significantly different ($P < .01$).

Discussion. Our original interest was to determine whether the uptake of T_3-I^{131} by spermatozoa was related to viability of the spermatozoa. The preliminary experiment and trials 1 and 2 tended to show that there was such a relationship.

Within the limits of the experiment, the amount of T_3 added to diluted semen had no influence on amount of uptake of T_3-I^{131} by the spermatozoa. This would be a necessary prerequisite for the application to have general significance.

Crispell *et al.*(12) found that there was a greater uptake of T_3-I^{131} by erythrocytes suspended in saline than by those suspended in plasma. Other workers have found that washed spermatozoa have a greater uptake of labeled glucose(6) and labeled phospholipids

(9). Our results with spermatozoa in trial 4 showed a greater T_3-I^{131} uptake by washed spermatozoa and would indicate a possible competition between spermatozoa and seminal plasma constituents or complex diluent constituents for the T_3-I^{131}. This points out the need in semen evaluation work for washing the spermatozoa and suspending them in simple salt solutions prior to making determinations of T_3-I^{131} uptake.

These preliminary trials indicate that measurement of uptake by washed spermatozoa of T_3-I^{131} may be useful in semen physiology studies.

Summary. The uptake of triiodothyronine-I^{131} by bull spermatozoa was studied. Trials with frozen semen (−79°C) and stored semen (5°C) indicated that uptake of T_3-I^{131} by spermatozoa was related to viability of the spermatozoa. Uptake of T_3-I^{131} by spermatozoa was independent of the amount of T_3 added to the diluted semen within the range studied. The composition of the spermatozoa diluent greatly influenced uptake of T_3-I^{131} by spermatozoa. Washed spermatozoa suspended in a 3% sodium citrate diluent had a much greater uptake of T_3-I^{131} than washed spermatzoa suspended in an egg yolk-sodium citrate diluent, indicating that diluent constituents may compete with the spermatozoa for uptake or binding of T_3-I^{131}.

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Influence of Thyroid Function on Co^{60} Cyanocobalamin Metabolism in Various Animals.* (27396)

HAROLD L. ROSENTHAL AND GLORIA O'BRIEN
Dept. of Physiological Chemistry, Washington University School of Dentistry, St. Louis, Mo.

Previous studies(1-2) have shown that female rabbits undergoing starvation accumulate a greater amount of injected Co^{60} labeled cyanocobalamin in kidneys and excrete more of the dose in urine than *ad libitum* fed animals. Similar results were obtained with starved chickens, but not in rats or guinea pigs. Since total starvation tends to depress metabolic activity(3), it was conceivable that depressed thyroid function may be related to the mechanism of Co^{60} cyanocobalamin incorporation in kidney tissue. This hypothesis was supported by studies with starving rabbits which showed that large doses of thyroxin injected for 5 days tended to prevent the Co^{60} cyanocobalamin accumulation in the kidney(4). It was of interest therefore to study more completely the relationship between hypo- and hyperthyroidism and the metabolism of Co^{60} cyanocobalamin in liver and kidney tissue.

Materials and methods. Adult female white rabbits weighing 2 to 3 kg, female rats of the Wistar strain weighing 300 g and female guinea pigs weighing 400 g obtained from commercial sources were maintained in individual metabolism cages for measurement of food and water consumption and collection of urine. Water was available to all animals *ad libitum*. Food and water consumption,

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