(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)

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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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TABLE I. Effect of Thiouracil Feeding on Tissue Uptake and Urine Excretion of Co^{60} Cyanocobalamin Injected into Various Animals.

Diet	Days on Exp.	Food consumed (g/day)	Wt. change (%)	Kidney (% dose/g±S.E.)	Liver (% dose/g±S.E.)	Urine (% dose)
			Rabbit			
Control, Pair fed	26	44 (4)*	—13.3	.72 ± .36	.19 ± .01	5.30 ± 1.40
Thiouracil (.5%)	27	45 (4)	— 5.2	4.07 ± 1.06†	.19 ± .02	9.68 ± 2.01‡
			Rat			
Control, Pair fed	33	18.7(6)	— 1.8	18.16 ± .63	.44 ± .05	1.91 ± .44
Thiouracil (.5%)	34	23.8(6)	+ 3.1	14.58 ± .80	.44 ± .03	2.60 ± .19
			Guinea Pig			
Control, Pair fed	30	26.4(5)	+11.4	.37 ± .05	2.77 ± .39	1.99 ± .30
Thiouracil (.5%)	31	30.5(5)	+ 9.3	.29 ± .04	2.73 ± .36	2.05 ± .31

* No. of animals in parenthesis.
† Significantly different from control values at 1% level.
‡ " " " " " " 5% "

when necessary, was determined daily and the animals were weighed twice weekly. Rabbits and rats were fed ground commercial rabbit or rat checkers. Guinea pigs were fed ground commercial rabbit checkers containing 4 mg ascorbic acid/100 g diet to prevent scurvy. Thiouracil and desiccated thyroid powder were mixed into the chow in dry form while $KClO_4$ was given as a 1% solution in drinking water. Rabbits were totally thyroparathyroidectomized under nembutal anaesthesia and maintained on commercial rabbit checkers. The operated animals were given 1% calcium chloride to drink to prevent tetany due to removal of parathyroid glands. The calcium chloride was discontinued within one to 2 weeks after operation when the animals were eating adequately.

Four days before termination of the experiments, 24 hr control urine collections were obtained. The animals were then injected with 0.05 μC Co^{60} cyanocobalamin/kg body weight by intramuscular injection in rabbits and by intraperitoneal injection in rats and guinea pigs. The animals were sacrificed by cervical fracture 3 days after receiving the injection of radioactive cyanocobalamin. Livers, kidneys and daily urine collections were assayed for radioactivity as previously described(1).

Thiouracil and desiccated thyroid powder were purchased from Nutritional Biochemicals Co. Potassium perchlorate was reagent grade. Specific activity of the Co^{60} cyanocobalamin was approximately 1 μC/μg.

Results. The inclusion of 0.5% thiouracil in the diet depressed food consumption in rabbits to 45 g/day as compared with *ad libitum* control values of about 100 g/day thus necessitating paired feeding experiments (Table I). Thiouracil feeding resulted in a significantly greater accumulation of radioactivity ($P = < 0.01$) in the kidneys of rabbits as compared to control animals. Since partial food restriction alone may increase the accumulation of injected Co^{60} cyanocobalamin in kidney tissue(2), the value of 0.72% of the injected dose/gram kidney for the pair fed control rabbits was expected. This value is not significantly different from that obtained for *ad libitum* fed animals of 0.35% of the injected dose/gram kidney as shown in Table II. Thiouracil feeding also tended to increase the excretion of radioactivity in the urine. Concentration of radioactivity in rabbit liver tissue was not altered by feeding thiouracil.

In rats and guinea pigs, food consumption was somewhat greater for thiouracil fed animals, thus obviating the need for paired feeding. In these experiments, concentration of radioactivity in kidney, liver and urine of thiouracil fed animals was the same as the values obtained for the control animals. The very large accumulation of injected Co^{60} cyanocobalamin in the kidneys of rats and the livers of guinea pigs as compared with other animals(2,5) is interesting.

Thyroidectomy, $KClO_4$ administration or inclusion of desiccated thyroid powder in the diet did not alter the accumulation of radioactivity in liver and kidney or excretion in urine

TABLE II. Effect of Thyroid, Perchlorate and Thyroidectomy on Tissue Uptake and Urine Excretion of Co^{60} Cyanocobalamin in Female Rabbits.

Diet	Days on exp.	Food consumed (g/day)	Wt. change (%)	Kidney (% dose/g±S.E.)	Liver (% dose/g±S.E.)	Urine (% dose±S.E.)
Control, Fed	15	108(9)*	+25.0	.35 ± .09	.15 ± .03	4.41 ± .70
" Starved†	11	0(6)	−32.9	2.77 ± .45§	.25 ± .03	7.08 ± 1.17‖
Thyroidectomy	28	98(4)	+15.4	.30 ± .06	.11 ± .01	4.49 ± .62
$KClO_4$,‡ 1% H_2O	26	106(4)	+12.6	.40 ± .19	.17 ± .03	4.41 ± .37
Thyroid, 0.25%	28	157(6)	+ 6.8	.54 ± .25	.16 ± .01	5.18 ± 1.20
idem. + Thiouracil, .5%	28	128(6)	− 3.4	1.43 ± .33§	.20 ± .04	11.24 ± .53§

* No. of animals in parenthesis.
† From Rosenthal, 1960.
‡ Rabbits consumed 1.77 g $KClO_4$ per day per rabbit.
§ Significantly different from fed control animals at 1% level.
‖ " " " " " " " 5% "

(Table II). The concentration of thyroid powder in the diet (0.25%) increased food consumption and depressed growth of the animals. Inclusion of both thiouracil (0.5%) and desiccated thyroid powder (0.25%) in the diet resulted in a significant ($P = < 0.01$) increase in concentration of radioactivity in kidney tissue and urine, which is less than that found with the same concentration of thiouracil alone shown in Table I.

Discussion. Evidence that compounds which effect thyroid function may also have extrathyroidal effects have recently been found. Thus Duncan and Best(6) and Boyd (7) have shown that thiouracil exerts an effect on cholesterol metabolism in the athyreotic rat. A similar extrathyroidal effect of thiouracil on accumulation of Co^{60} cyanocobalamin in the kidney of rabbits is demonstrated in our experiments.

That hypofunction of the thyroid by thyroidectomy or $KClO_4$ administration(8) failed to increase the accumulation of radioactivity in the kidneys of rabbits while thiouracil is markedly effective, suggests that thiouracil is directly affecting the metabolism of cyanocobalamin in the kidney. That the altered kidney function due to thiouracil is not mediated *via* the thyroid is substantiated by the experiment in which inclusion of thyroid in the thiouracil containing diet did not eliminate the increased kidney accumulation or urine excretion of the injected Co^{60} cyanocobalamin.

The thiouracil effect appears to be specific for rabbits. Rats and guinea pigs fail to respond to thiouracil feeding. Rats and guinea pigs subjected to starvation also fail to accumulate increased amounts of injected Co^{60} cyanocobalamin while starving rabbits markedly accumulate the radioactivity in kidney tissue(2). The mode of action of thiouracil and its effect on vitamin B_{12} metabolism are not clear.

Summary. Rabbits, but not rats or guinea pigs, fed thiouracil, accumulate increased concentrations of injected Co^{60} cyanocobalamin in kidney tissue but not in liver tissue. Radioactivity excretion in urine also increases. The effect of thiouracil on cyanocobalamin metabolism by rabbit kidney appears to be extrathyroidal and not mediated through the thyroid gland.

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