exhibited an increased sensitivity to endogenous insulin during confinement at 5°C. Implications of an increased sensitivity to insulin during prolonged exposure to cold have similarly been inferred from the enhanced blood glucose lowering activity of exogenous insulin administered to normal cold-acclimated rats(9,10).

Summary. In the absence of administered insulin, severely alloxan diabetic rats are unable effectively to utilize a carbohydrate regimen at levels sufficient to sustain the elevated metabolic rate necessary for extended survival in cold. However, mild to moderately diabetic rats not only survive at 5°C for periods up to 3 months but show a substantial amelioration in the diabetic state in relation to control animals maintained at 26°C. This was attributed to the development of an increased sensitivity to endogenous insulin activity during confinement in cold.

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Proteins of Ovarian Cyst Fluid and Serum from Hypothyroid Gonadotrophin-Treated Rats.* (28497)

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Studies comparing follicular fluid, ovarian cyst fluid, and serum reveal similarities that suggest a common origin for their protein components. Human ovarian cyst fluid exhibits protein patterns of lower globulin content than serum(1), whereas no difference was noted in bovine follicular fluid and serum in protein patterns. Bovine follicular fluid, however, contains less total protein than serum and lacks beta lipoprotein(2). Changes in bovine serum electrophoretic patterns resulting from lung disease are similarly reflected in the follicular fluid(2).

Rat ovarian response to human chorionic gonadotrophin and pregnant mare's serum can be enhanced by feeding a diet containing 0.5% thiouracil. Chorionic gonadotrophin causes luteinization of the ovary in euthyroid rats, but in the hypothyroid rat response to this hormone is characterized by luteinization and formation of large follicular cysts(3).

Hypothyroidism increases total serum protein concentration, particularly the gamma globulin fraction of rat serum(4), which provides a basis for investigating the origin of cyst fluid protein. A preliminary study suggests that a major portion of cyst fluid protein may be of plasma origin(5). In an effort to establish the origin of cyst fluid, a comparison was made between this fluid and serum using protein and glycoprotein electrophoretic patterns, and incorporation of S^{35} from methionine as criteria.

Materials and methods. Long-Evans female rats (65-75 g) were fed a diet containing 0.5% thiouracil for 30 days. Ovarian cysts were induced by injecting 10 i.u. of human chorionic gonadotrophin (HCG) (Fol-

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lutein)† subcutaneously, daily, for 20 days starting on day 11. At the end of the experimental period the rats were injected intraperitoneally with 0.0045 μg of S^{35} DL-methionine (Volk Radiochemical Corp.) and autopsied at intervals of ½, 1, 2, 4, 12, 24, and 120 hours following injection. All doses were corrected to 100,000 cpm/μg of methionine/100 g body weight. Blood serum and cyst fluid proteins were separated electrophoretically by the hanging strip method of Block *et al.*(6). Paper strips were dyed for proteins with bromphenol blue 10 B dye, and for glycoproteins by the periodic acid Schiff technique(7). Concentration of protein and glycoprotein fractions was determined using a recording densitometer. Percentage of total radioactivity in each fraction was obtained by passing the paper strips through a Nuclear Chicago Model C-100 B Actigraph counter and measuring the fraction areas with a planimeter. The biuret method(8) was used to obtain total protein of serum and cyst fluid. Ovarian, cyst fluid, and serum proteins labeled with S^{35} from DL-methionine were extracted and purified(9).

Results. Thiouracil feeding for 30 days increased both total serum protein and gamma globulin fraction concentrations significantly, as compared with those of euthyroid rats of similar age. Total protein of cyst fluid, however, was significantly lower than serum from the same rats even though percentages of individual protein fractions were the same. In contrast, total protein concentration of euthyroid rat serum was the same as cyst fluid, but protein fractions differed significantly. A higher per cent of gamma globulin fraction of cyst fluid was the most striking difference. Total glycoprotein concentration of cyst fluid was significantly lower than the serum of the same animal but polysaccharide to protein ratios were unchanged (Table I).

Protein synthesis, measured by the relative amount of incorporation of S^{35} from DL-methionine, was comparable in serum and cyst fluid (Table I). Four hours after injection of S^{35} DL-methionine whole serum

† Follutein was generouly supplied by E. R. Squibb & Sons, New Brunswick, N. J.

TABLE I. Protein, Polysaccharide, and S^{35} Concentration of Electrophoretic Fractions of Serum and Ovarian Cyst Fluid of Control and Hypothyroid Rats.

	Protein (g/100 ml)			Glycoprotein g polysaccharide/100 g protein		Incorporation of S^{35} from methionine % of total activity		
		Hypothyroid		Hypothyroid			Hypothyroid	
	Control serum	Serum	Ovarian cyst fluid	Serum	Ovarian cyst fluid	Control serum	Serum	Ovarian cyst fluid
No. rats	9	18	18	18	18	9	18	18
Total	6.09 ± .22*	7.34 ± .12	6.03 ± .16	1.22 ± .04	1.15 ± .04			
Albumin	3.33 ± .29	3.56 ± .45	3.12 ± .16	.49 ± .03	.44 ± .04	36.2 ± 3.9	36.2 ± 1.1	39.7 ± 1.2
$Alpha_1$	.57 ± .03	.86 ± .03	.62 ± .03	4.23 ± .21	4.55 ± .29	14.1 ± 2.0	16.5 ± 1.0	12.6 ± 1.2
$Alpha_2$	.58 ± .06	.57 ± .02	.47 ± .02	1.93 ± .10	2.26 ± .19	14.9 ± 2.3	10.9 ± .6	12.0 ± 1.1
Beta	.88 ± .07	1.03 ± .03	.83 ± .04	1.36 ± .06	1.12 ± .06	17.2 ± 1.4	16.2 ± .6	14.7 ± .9
Gamma	.72 ± .06	1.26 ± .04	1.01 ± .09	.93 ± .04	.98 ± .08	18.8 ± 1.9	20.1 ± 1.0	20.8 ± 1.4
A/G	1.25 ± .12	.99 ± .03	1.07 ± .07					
Total mg%				91.10 ± 4.09	69.33 ± 3.17			

* Standard error.

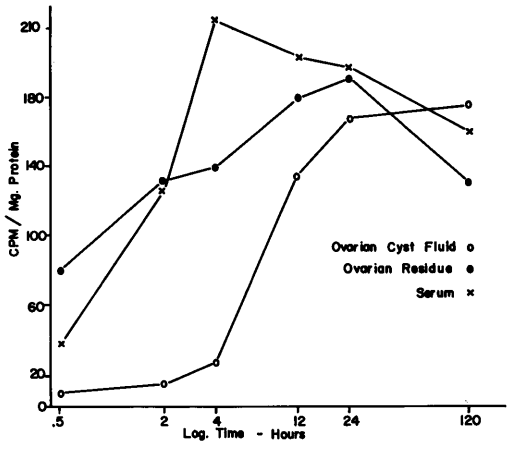

FIG. 1. Distribution in cpm/mg of purified tissue proteins from animals fed thiouracil and injected with human chorionic gonadotrophin for 20 days.

reached its peak specific activity which was maintained approximately at this level up to 120 hours, but cyst fluid had a negligible uptake at 4 hours. After 24 hours the difference in specific activity between serum and cyst fluid was no longer significant. Ovarian residue (ovarian tissue minus cyst fluid) had a higher specific activity at ½ hour than either serum or cyst fluid. After 120 hours, residue activity was the same as serum and cyst fluid (Fig. 1).

Discussion. Degeneration of the follicular epithelium increased colloid osmotic pressure, allowing serum to diffuse into the cystic cavity(10). Other research has demonstrated an inter-follicular enzyme capable of increasing osmotic pressure(11). Using a fluorescent technique, both fluorescent and non-fluorescent granules were observed within mouse follicular antra, thus suggesting that selective transmission of serum macromolecules probably occurs normally during growth of the mouse oöcyte(12). Direct histological evidence supports the view that circulating serum protein may be transported *via* capillary endothelium, theca and granulosa layers into the egg cytoplasm of maturing follicles(13). Paper electrophoresis, indicated that the protein of bovine follicular fluid is of serum origin, filtered through the blood vessels of the theca interna(2). Further evidence derived from the similarity of their respective polysaccharides to protein ratios for total and component protein fractions indicate that the protein of both serum and cyst fluid may have a common origin. Cyst fluid protein and glycoprotein concentrations were reduced below serum levels, suggesting that the entrance of serum protein into the follicle is regulated to some degree by the size of the molecule.

Radioactivity in the serum, indicative of serum protein formation, reaches a peak in 4 hours, and not until 8 hours does an appreciable amount of activity appear in cyst fluid protein. However, *in situ* synthesis is unlikely since ovarian residue retains its peak activity at a time when cyst fluid is rapidly increasing to maximum. If cyst fluid proteins were derived mainly from *in situ* synthesis, a supply of cells capable of accomplishing this task would be required. The most obvious cellular source of follicular fluid appears to be the granulosa cells(14); however, it has also been shown(3) that the cystic follicle is lined with a simple cuboidal, simple squamous epithelium, or in some cases, by only the theca interna. Absence of granulosa cells, in experimentally induced follicular cysts, is additional evidence against *in situ* synthesis of the major portion of labeled cyst fluid protein.

Summary. Thiouracil feeding increased total protein and gamma globulin in the serum of female rats above that of their euthyroid controls. Ovarian cyst fluid in these gonadotrophin treated hypothyroid rats had an electrophoretic protein pattern which simulated the serum and was characterized by an increase in gamma globulin. Total glycoprotein of serum was greater than cyst fluid but total protein to polysaccharide ratio and ratios of respective fractions did not differ. Following injection of S^{35} DL-methionine, serum reached peak specific activity in 4 hours; however, cyst fluid activity, negligible for 12 hours, did not reach that of serum until 24 hours. Increasing specific activity of cyst fluid was not coincident with a drop in activity of ovarian residue. Thus these data suggest that protein portion of cyst fluid may be of plasma origin.

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Comparison of UDPG-Glycogen Synthetase Activity of the Tongue and Uterus.* (28498)

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The two enzymes, phosphorylase and uridinediphosphoglucose (UDPG)-glycogen synthetase, synthesize 1,4 linkages of glycogen with glucose-1-phosphate (G-1-P) and UDPG as their respective substrates. The action of phosphorylase is readily reversible so that this enzyme may be involved in both synthesis and breakdown of glycogen while UDPG-glycogen synthetase is concerned only with synthesis of glycogen. According to Stetten and Stetten(1) there seems to be conclusive evidence that in most tissues the synthesis of glycogen proceeds largely through the action of UDPG-glycogen synthetase and the branching enzyme, while the function of phosphorylase in conjunction with that of a debranching enzyme (amylo-1,6-glucosidase) is involved in the breakdown of glycogen to G-1-P.

Glycogen synthesized from UDPG cannot be demonstrated histochemically in the uterus of the rat and rabbit while glycogen synthesized from G-1-P can be demonstrated in the myometrium of these two species(2). However, glycogen synthesized from UDPG and G-1-P can be observed histochemically in the tongue(2).

The present investigation was designed to determine whether UDPG-glycogen synthetase is present in the uterus of the rat and, if present, how its activity is altered by the ovarian hormones. The investigation was also designed to compare UDPG-glycogen synthetase activity of skeletal muscle of the tongue and smooth muscle of the uterus.

Materials and methods. Thirty-nine young adult Wistar rats (Charles River) were maintained on Purina Chow and water *ad libitum* throughout the experiment. All rats were bilaterally ovariectomized and 10 days following ovariectomy 9 animals were sacrificed without further treatment. The remaining rats were divided into groups receiving the following daily hormonal† treatment: I. 1 μg of estrogen (10 animals); II. 1 mg of progesterone (10 animals); III. 1 μg of estrogen plus 1 mg of progesterone (10 animals). The hormones were injected sub-

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