lar origin. Thus, present experiments demonstrate that flounder tubular cells are able to transport accumulated protein to the incubation medium. Although partial modification of the protein molecule during its transcellular transport cannot be completely excluded, catabolism to polypeptides and amino-acids does not occur in significant amounts.

In addition, we have results of a preliminary nature, indicating that some catabolism of Ly takes place (arginine becomes detectable and Ly recoveries are less than 100%) when initial renal concentration of Ly is very high (above 30 μg/mg protein). This finding suggests an interesting speculation. The association of proteins with lysosome-like bodies in the renal cell, observed after the administration of massive doses of foreign proteins, has generally been interpreted as evidence that filtered proteins are catabolized within the kidney. In view of the present data, perhaps the catabolic pathway is utilized only in situations in which an overload of proteins occurs (e.g., nephrosis, increased serum levels of normal proteins and massive injections of foreign proteins) while transcellular transport of intact protein is the way the kidney handles a normal protein load. This hypothesis is currently under further investigation.

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1967 #22

GONADOTROPIC REGULATION OF DEHYDROGENASE ENZYMES IN THE OVARY OF *Squalus acanthias*

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Previous studies in mammalian systems had demonstrated the importance of activation of dehydrogenase enzymes of the ovary by gonadotropins for regulation of cellular replication and steroidogenesis (Biochim. Biophys. Acta 97:542-55, 1965 and 104:237-49, 1965). It was of interest to determine if various dehydrogenase enzymes of the dogfish ovary could be induced or activated by mammalian gonadotropins. First, it was necessary to determine if dehydrogenase enzymes similar to those found in mammalian ovarian tissues even existed in the dogfish ovary.

Ovarian tissue was removed from female dogfish at various stages of gestation. Homogenates were made from various tissue components of the ovary in dogfish intracellular Ringer solution. The homogenates were centrifuged for 45 min at 105,000 xg in a Beckman model L-2 ultracentrifuge. The supernatant was carefully removed and analyzed for enzyme activity and protein content. The various $NADP^+$ and NAD^+-linked dehydrogenases were measured by following the rate of reduction of added pyridine nucleotides at 340 mμ in a Beckman DU spectrophotometer.

Table 1 shows the cellular distribution of various dehydrogenases in the ovaries from a pregnant dogfish in late pregnancy. The most active dehydrogenase enzyme was glucose-6-phosphate dehydrogenase, showing the greatest activity in the interstitial tissue. Enzyme activity in ovarian tissue from dogfish at various stages of gestation found in June, July, and August were measured. Enzyme activity was high at all stages and no apparent variation in activities was detected.

The pituitaries were removed from a number of pregnant dogfish. This was accomplished by drilling through the roof of the mouth into the base of the brain with a cork borer and suck-

Table 1

DEHYDROGENASE ACTIVITIES IN OVARIAN COMPONENTS S. acanthias WITH PUPS

Substrates	Follicular membranes	Whole follicle	Corpora lutea	Interstitial tissue	Whole ovary
$NADP^+$ + Glc-6-P	2.73 ± .06	3.87 ± .09	5.80 ± .07	7.70 ± .18	6.85 ± .14
$NADP^+$ + 6-PG	1.30 ± .03	2.46 ± .07	2.51 ± .02	2.74 ± .06	1.91 ± .07
$NADP^+$ + isocitrate	1.05 ± .06	1.40 ± .11	5.50 ± .10	1.81 ± .05	1.55 ± .04
$NADP^+$ + malate	1.54 ± .08	2.73 ± .04	1.41 ± .04	2.90 ± .07	1.00 ± .03
NAD^+ + malate	1.01 ± .02	2.61 ± .08	1.40 ± .07	1.90 ± .02	1.24 ± .08
NAD^+ + lactate	1.85 ± .07	2.37 ± .07	3.00 ± .05	5.05 ± .03	4.80 ± .08

Activities are expressed as μ moles nucleotide reduced/ min/ mg protein and represent max. activity with substrates.

Table 2

DEHYDROGENASE ENZYME CHANGES IN WHOLE, HYPOPHYSECTOMIZED, AND GONADOTROPIN-TREATED DOGFISH

	$NADP^+$ + Glc-6-P	$NADP^+$ + 6-PG	$NADP^+$ + Isocitrate	$NADP^+$ + Malate	NAD^+ + Malate	NAD^+ + Lactate
			Experiment 1			
Whole	7.21 ± .13	2.07 ± .07	1.63 ± .06	1.21 ± .05	1.21 ± .05	5.61 ± .14
Hypophysec. - killed 4 hrs later	3.20 ± .10	0.50 ± .05	0.62 ± .09	0.40 ± .04	0.47 ± .07	2.59 ± .07
*Hypophysec. - killed at 4 hrs	7.31 ± .17	1.81 ± .09	1.60 ± .16	1.25 ± .07	1.23 ± .08	5.20 ± .09
			Experiment 2			
Whole	7.01 ± .13	1.93 ± .06	1.56 ± .09	1.07 ± .07	1.20 ± .04	5.74 ± .10
Hypophysec. - killed 16 hrs later	3.54 ± .08	0.90 ± .08	0.82 ± .06	0.27 ± .05	0.52 ± .07	1.25 ± .10
†Hypophysec. - killed at 17 hrs	7.30 ± .10	1.75 ± .11	2.22 ± .14	1.24 ± .09	1.23 ± .11	4.11 ± .16

Activities expressed as μ moles nucleotide reduced/ min/ mg/ protein.

*Gonadotropin infused at hour 2 - 4.

†Gonadotropin infused at hour 15 - 17.

ing out the pituitary lobes with a mouth syringe. Hypophysectomy was confirmed by visual observation of removed lobes and by decrease in pigmentation of the skin within two hours. A number of hypophysectomized dogfish were also perfused with 100 IU of human chorionic gonatropin (Ayerst) and 500 mg of bovine follicle-stimulating hormone (NIH) dissolved in 10 ml of dogfish intracellular Ringer solution. The total amount of gonadotropins was injected slowly at 20 min intervals into the dorsal aorta over a two hour period. Table 2 shows the results of studies with the hypophysectomized and gonadotropin-treated dogfish. There was a marked drop in activities of all dehydrogenase enzymes by four hours. Infusion of gonadotropins over a two hour period restored enzyme activities. It is not known whether this response to gonadotropins represents enzyme induction or activation of existing enzymes. It is interesting, however, that the enzymes of the dogfish ovary responded to gonadotropins from a mammalian source. Skin pigmentation was also largely restored by the infusion of the gonadotropin preparation which, no doubt, represents contamination of the preparations with melanophore hormone (MSH).

Ovarian tissue was also extracted before and after incubation with various substrates such as glucose-6-phosphate and isocitrate and analyzed by thin-layer chromatography by methods described by K. W. McKerns (Chapter 12 in Functions of the Adrenal Cortex, K. W. McKerns, editor, Appleton Century Crofts). Large amounts of cholesterol were found, in addition to four unknown steroids which have not, as yet, been characterized. The unknown steroids did not correspond in thin-layer chromatography systems to any of the known androgenic, progestational, or estrogenic steroids of mammalian endocrine tissues.

In summary, it was found that dehydrogenase enzymes corresponding to those found in mammalian systems exist in the ovary of the dogfish. These enzymes regress rapidly on removal of the pituitary and are synthesized or activated rapidly by the administration of mammalian gonadotropins.

1967 #23

THE ACTION OF EPINEPHRINE ON GASTRO-INTESTINAL MOTILITY IN THE SPINY DOGFISH

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It is known that epinephrine has an inhibitory or relaxant effect on the gastrointestinal motility of mammals. In elasmobranchs, epinephrine is stimulatory to the smooth muscle of the GI tract. This effect has been demonstrated both in isolated strips of gastrointestinal smooth muscle and in the living animal (Nicholls, J.V.V., Proc. Soc. Exptl. Biol. and Med. 30:54-56, 1932; Hiatt et al., Bull. M.D.I.B.L. 6:22-23, 1966).

Experiments in the living dog have shown that epinephrine exerts its inhibitory effect in mammals through alpha and beta receptors apparently located in the smooth muscle of the gut (Levy and Ahlquist, Ann. N. Y. Acad. Sci. 139, 781-87, 1967). The possibility that similar receptors mediate the stimulatory response of epinephrine in Squalus acanthias was explored. Eighteen female dogfish, average weight 3.5 kg, were used. A balloon was inserted in the lumen of the distal stomach of the dogfish and the pressure measured by a saline column. The average increase in pressure in response to epinephrine, IV, 10-100 μg/kg, was 25 cm water. The alpha