Flounder plasma, glucose and antibiotics (penicillin and streptomycin), added to the incubation medium, had no influence on either function or survival time of the tubules. Substitution of the bicarbonate buffer system of Forster's medium by a 1.0 mM phosphate buffer system has no effect on the preparation when the pH was kept high (above 8.0). However, tissue incubated in phosphate substitute medium at a pH of 7.2 exhibited a lower dye accumulation capacity and survival time.

The results show that dissected tubular masses of the flounder kidney can be incubated for long periods of time. The single most important factor in the survival time is the temperature. When incubation is carried out without mechanical agitation, at 14 C and at an alkaline pH the preparation retains its morphological integrity and ability to actively transport organic acids for at least 24 hours.

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TRANSPORT OF A SMALL MOLECULAR WEIGHT PROTEIN, LYSOZYME, IN DISSECTED AND INCUBATED TUBULAR MASSES OF THE FLOUNDER KIDNEY

Thomas Maack[*] and William B. Kinter, State University of New York, Upstate Medical Center, Syracuse, N. Y.

It is now well established that filtered endogenous and exogenous proteins are taken up by proximal tubular cells. Unknown is the fate of the reabsorbed protein inside the renal cell. The present study was designed to test if flounder (Pseudopleuronectes americanus) kidney cells catabolize reabsorbed protein or if transcellular transport of the intact protein occurs. Lysozyme (Ly), a 14,000 molecular weight protein that can be measured with accuracy and reproducibility, was used as the tracer protein.

Intact flounders, 50-150 g body weight, were injected i.p. with 2x crystallized egg-white Ly. As expected, the protein accumulates in the kidney (tissue/plasma ratios, T_{Ly}/P_{Ly}, ranged from 1.2 to 3.7. Clearance, C, experiments indicated that the accumulation was due to renal uptake of filtered proteins, i.e., C_{Ly}/C_{inulin} = 0.3 to 0.8 when P_{Ly} varies from 0.03 to 0.2 µg Ly/µl serum.) At several time intervals after Ly injection, fish were sacrificed and the kidney removed and transferred to cold Forster's saline medium. The cephalic portion was discarded and the caudal kidney teased grossly and transferred to fresh cold medium where dissection was completed. 5-10 mg of dissected tubular masses were placed on Millipore filters and incubated from 1-24 hours at 14 C as described in the preceding paper. Non-incubated tubular masses served as controls for calculating Ly recoveries. Tubular masses were homogenized in 0.5 ml distilled water with a tightly fitting teflon over glass homogenizer. Non-incubated and incubated tubule homogenates and medium were analyzed for Ly, total protein and arginine.

The main features of the results, presented in Tables 1 and 2 are: (1) Ly specific activities are always higher in medium than in the tubular masses, showing that the protein is released to the incubation medium. (2) Within the error of the method, Ly recoveries are complete, showing that during incubation the protein is not activated, inactivated or broken down to smaller active

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[*]Advanced Research Fellow, American Heart Association.

Table 1

RATIOS OF LYSOZYME SPECIFIC ACTIVITIES IN INCUBATION
MEDIUM AND TUBULAR MASSES OF 7 FLOUNDER KIDNEYS

Initial concentration of lysozyme in kidney (μg/mg protein)	Average: 15.3 Range: 5.7 - 26.4
Lysozyme in medium/lysozyme in tubules (μg/mg protein)　　　(μg/mgprotein)	11.2 ± 2.9
Lysozyme recovery (%) Non-incubated tubules = 100%	102.8 ± 3.3

Table 2

TIME SEQUENCE OF LYSOZYME RELEASE AFTER INCUBATION OF FLOUNDER
TUBULAR MASSES IN FORSTER'S SALINE MEDIUM

Time of incubation (hours)		Total lysozyme (μg)	Total protein (μg)	Lysozyme specific activity (μg/mg protein)	Recovery %
0		-	-	26.4	-
1	Tubules	7.2	449.5	16.1	98.0
	Medium	5.8	56.2	103.2	
6	Tubules	3.8	322.6	11.9	97.7
	Medium	6.4	75.9	84.3	
12	Tubules	3.0	339.9	9.0	108.7
	Medium	10.8	144.1	74.9	

units. (3) Release of Ly from tissue to medium increases with the time of incubation. (4) Arginine (the main amino-acid of Ly) was undetectable in the incubation medium (lower limit of the method, 0.3 μg/0.1 ml medium). Together with the complete recovery of Ly, this indicates that the protein is not being catabolized in significant amounts during incubation.

The following experiments show that the results presented effectively represent transport of Ly from cells to medium: (1) Integrity of each preparation was tested by the morphological aspect of the tubules under the microscope and ability to concentrate chlorphenol red in the lumen. Both, morphological integrity and dye concentration capacity, were retained for at least 24 hours. This observation together with the fact that Ly concentrations are expressed in specific rather than in total activities demonstrates that the release of Ly to the medium is not due to cell breakdown or nonspecific increase in the permeability of the basement membrane to proteins. (2) Ly space in the incubated tissue was 19.3 to 62.2 times greater than the measured inulin-C^{14} space, demonstrating that Ly is not confined to the extracellular compartment, including tubular fluid. Since inulin-C^{14} space did not change during 24 hours of incubation, it can be assumed that very little or none of the tubular fluid is transferred to the medium. Contamination with extracellular fluid and trapped plasma can account for no more than 0.1% of the Ly content in medium. Since no Ly binding to control tubular masses occurred when Ly was added to the incubation medium, it can be concluded that almost all of the Ly found in the medium is of cellu-

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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GONADOTROPIC REGULATION OF DEHYDROGENASE ENZYMES IN THE OVARY OF Squalus acanthias

Kenneth W. McKerns, Marty Criss, and Wayne Criss, College of Medicine, University of Florida, Gainesville, Fla.

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-