take over control values (open bars) only in the most distal portion of the intestine. The three-fold enhancement found is somewhat less than that reported when similar studies were performed in higher vertebrates (Proc. Soc. Exptl. Biol. Med. 112:654, 1963). The active substance in ESH was found to be non-dialyzable and destroyed by heating (100° C for 10 minutes). The vitamin B_{12} binding capacity of ESH (as measured by equilibrium dialysis) was approximately 1.2 millimicrograms cyanocobalamin per mg (wet weight) which is about 1/100 of that reported in guinea pig, rat and man (Physiol. Reviews 43:529, 1963).

These studies strongly suggest that a rudimentary intrinsic factor mechanism for vitamin B_{12} absorption is present in the eel.

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1967 #20

LONG-TERM INCUBATION OF DISSECTED TUBULAR MASSES OF THE FLOUNDER KIDNEY

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It has been generally assumed that Forster's preparation of dissected tubular masses of flounder (Pseudopleuronectes americanus) kidney (Science 108:65, 1948) can survive only short periods of incubation. Recently, Trump and Bulger (Lab. Invest. 16:453, 1967) have used incubation times of the order of 12 hours with tubules from other species of flat fish. However, no systematic study of conditions for long-term incubation has been reported. Methods of in vitro incubation, temperature dependency, and effect of medium composition and pH were studied. Viability of the preparation was tested by its ability to form and maintain a concentration gradient of chlorophenol red between tubular lumens and medium. As a reference point, preparations were considered viable as long as they exhibited 50-100% dye-accumulating tubules. Under the microscope, non-viable tubules show extensive vesiculation of the cytoplasm and disappearance of tubular lumens.

Preparations were incubated in Forster's saline medium. Chlorophenol red was added at a concentration of 2.5×10^{-5} M. Three methods of incubation were tested: (a) bubbling air in an open system; (b) rocking in a closed system saturated with O_2; and (c) incubation in a stationary culture dish. The last method, which gave the best results, consisted of about 10 mg of dissected tubular masses on a 13 mm diameter Millipore filter (pore diameter = 0.8 μ) which was supported by a wire grid that fitted the central chamber of an organ culture dish (#3010 Falcon Plastics, Division of B-D, Los Angeles, California). A 1.0-1.3 ml volume of medium, just sufficient to cover the tubules with a thin liquid film, was added to the central chamber. Incubations were carried out at room temperature (19-22 C) at 14 C and at 4 C. At room temperature viability was retained for 6-12 hours. After that, autolysis of the tubular cells (vesiculation), reduction of lumen diameter and loss of dye from tubular lumen took place rapidly. At 14 C dye accumulation was similar and survival of the preparation extended to 24-36 hours. At 4 C, morphological integrity and limited dye concentration capacity were retained for as long as 4 days.

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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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1967 #21

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

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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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