1967 #19

EVIDENCE FOR GASTRIC INTRINSIC FACTOR IN THE EEL (Anguilla Rostrata)

Manuel E. Kaplan, Department of Medicine, Washington University School of Medicine and The Jewish Hospital of St. Louis, Mo.

In mammals vitamin B_{12} absorption occurs principally in the lower small intestine and is facilitated by intrinsic factor, a mucinous, non-dialyzable, heat-labile secretory product of the stomach. To date, there has been no report of a systematic investigation of lower vertebrates in order to define the phylogenetic level at which an intrinsic factor mechanism first appears. With this goal in mind the intestinal uptake of vitamin B_{12} was studied in the eel (Anguilla rostrata) utilizing the everted intestinal sac technique described by Wilson and Strauss (Amer. J. Physiol. 197:926, 1959). Eel intestines from upper duodenum to cloacal orifice were isolated, everted, and divided into four segments of equal length (1 = proximal segment, 2 and 3 = intermediate segments, 4 = distal segment). Sacs were prepared by filling the serosal compartments with gassed (87% O_2, 2% CO_2) fresh-water medium (Forster) containing 220 mg percent glucose and 20mM bicarbonate, pH 7.5. Individual sacs were incubated for one hour at 20°C in 7 ml of the same medium containing 11 millimicrograms Co^{57} cyanocobalamin. To some flasks 0.1 ml of a crude extract of eel stomach was added. This was prepared by homogenization and centrifugation of pooled eel gastric mucosa so that 0.1 ml of the clear homogenate represented approximately 6.5 mg (wet weight) of gastric mucosa. After incubation the sacs were washed repeatedly, opened, and carefully blotted before being counted. The results (Figure 1) indicate that eel stomach homogenate (ESH) (represented by shaded bars), significantly enhanced vitamin B_{12} up-

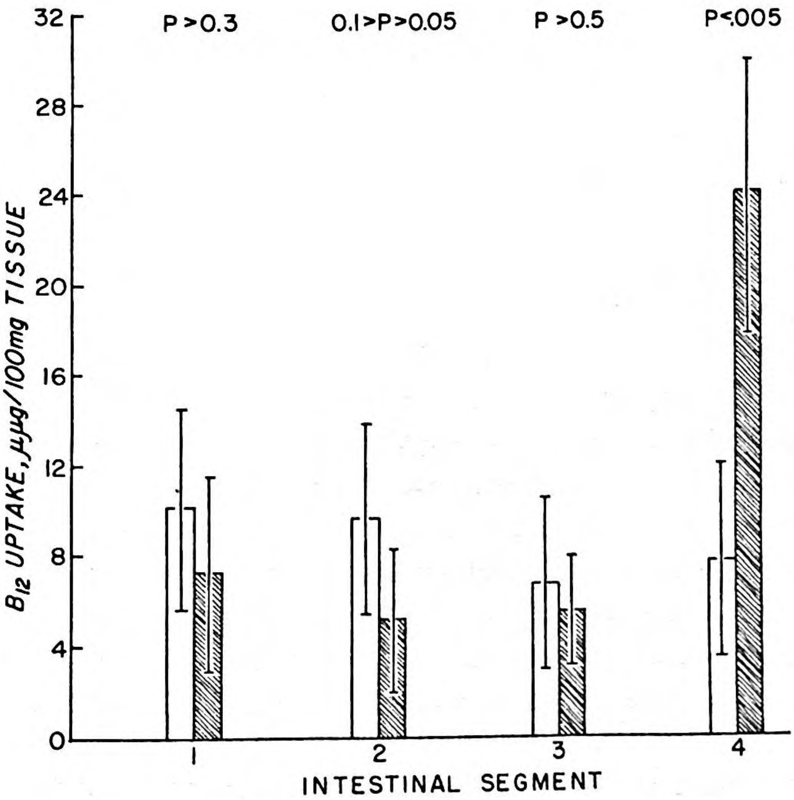

Figure 1

take over control values (open bars) only in the most distal portion of the intestine. The threefold 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.

Supported by U.S.P.H.S. General Research Support Grant #1SOLFR05491-03-5.

1967 #20

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

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

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.

*Advanced Research Fellow - American Heart Association.