Evidence for Gastric Intrinsic Factor in the Eel (*Anguilla rostrata*)* (33978)

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In mammals, vitamin B_{12} absorption occurs principally in the distal small intestine (ileum) and is facilitated by intinsic factor (IF), a secretory product of the stomach (1). The IF is a nondialyzable, heat-labile glycoprotein that binds vitamin B_{12} (2). To date, there have been few reports of studies designed to investigate the presence of IF in lower vertebrates (3, 4). Consequently, the phylogenetic level at which an intrinsic factor mechanism first appears is unknown. With this goal in mind, the intestinal uptake of B67 was studied in the freshwater eel (*Anguilla rostrata*) utilizing the everted intestinal sac technique described by Strauss and Wilson (5).

Methods. Freshwater eels were trapped without trauma and kept fasting for 1–5 days in 25-gallon aquaria with continuously running fresh water. Eighteen animals were studied, nine experimental and nine control. The eels were anesthetized by immersion in ice water. Intestines, from first portion of the duodenum to cloacal orifice, were stripped from the mesentary, washed by perfusion with tap water, 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 modified "freshwater medium" (F-medium) described by Forster (6) containing the following concentrations of salts (mmoles/liter: NaCl, 100; KCl, 2.5; $CaCl_2$, 1.5; $MgCl_2$, 1.0; NaH_2PO_4, 0.5; $NaHCO_3$, 20. Just before use, glucose was added (220 mg/100 ml) and the medium was gassed with 98% O_2–2% CO_2 (pH 7.4). Individual sacs were placed in 25-ml Erlenmeyer flasks that contained 7 ml of F-medium and 11 mμ of ^{57}Co cyanocobalamin (Abbott Laboratories; sp act 13.1 μC/μg). To alternate flasks 0.1 ml of a crude supernatant extract of eel stomach was added; control flasks received 0.1 ml of buffer. Incubation was performed in a Dubnoff shaking incubator under 98% O_2–2% CO_2 for 1 hr at 20°. After the sacs were washed by repeated immersion in tap water (3 changes) and the serosal fluid was aspirated, they were opened, blotted gently on paper towels, weighed, and transferred to disposable counting tubes. Radioactivity measurements were performed with a Nuclear-Chicago automatic gamma spectrometer. The counting period was either 10 min or until 10,000 counts were obtained. Data were expressed as B_{12} uptake in picograms/100 mg of intestine.

Eel stomach homogenate (ESH) was prepared by scraping with a glass slide the washed gastric mucosa from four freshly killed eels, suspending and homogenizing the pooled mucosa in 10 ml of freshwater medium, and centrifuging at 800*g*, 4° until a clear supernatant was achieved (approx 2 hr). Aliquots were frozen at —20° and thawed once when needed. 0.1 ml of the clear supernatant represented 6.5 mg (wet wt) of gastric mucosa.

Results and Discussion. Eel stomach homogenate enhanced vitamin B_{12} uptake only in the most distal quarter (segment 4) of the intestine (Fig. 1). In all instances, serosal fluids were devoid of radioactivity. Although statistically significant ($p<.005$), the threefold enhancement of B_{12} uptake is considerably less than that observed in analogous studies utilizing intestinal sacs and isologous IF from higher vertebrates. In the more proximal portions of the eel intestine (segments 1–3), ESH caused a modest, statistically in-

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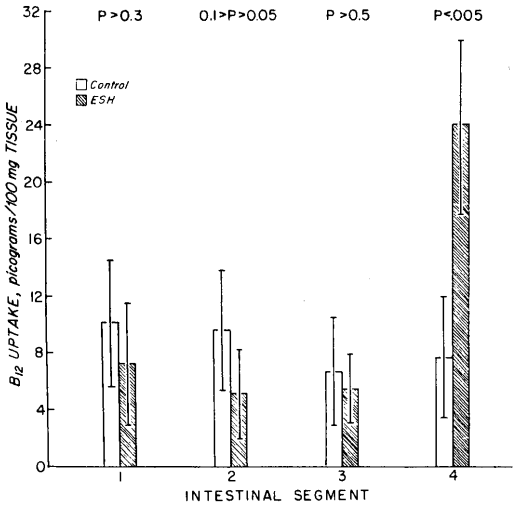

FIG. 1. Effects of eel stomach homogenate on the uptake of ^{57}Co cyanocobalamin by various segments of eel intestine. One standard deviation of the mean and significance of mean differences are shown.

significant fall in B_{12} accumulation. The failure to achieve statistical validity (particularly for segment 2) may be due to the limited number of intestines studied. A similar observation has been made by Donaldson and coworkers (8) who reported that IF reduced the uptake of vitamin B_{12} by brush border from the proximal small intestine of the hamster and stimulated B_{12} uptake by distal intestinal brush border.

Gross and microscopic examinations of the eel intestine revealed no clear-cut demarcation between "small intestine" and "colon." Gradual simplification of the mucosal structure was noted progressing from duodenum to cloaca. The mucosal villi of the distal intestine were much more clubbed and discrete than their filamentous, anastomotic duodenal counterparts.[1] Consequently, although maximal vitamin B_{12} uptake occurred at a position in the eel intestine equivalent to the lower ileum of higher vertebrates, there are no morphologic criteria to support this analogy.

Previous investigations utilizing the everted intestinal sac technique have convincingly demonstrated gastric IF in man, monkey, hog, rat, guinea pig, hamster, beaver, and rabbit (9). In these studies, a significant degree of species specificity was found. Thus, only rat IF will enhance B_{12} uptake by rat intestine, whereas guinea pig intestine will respond to intrinsic factors from many species (9). Previous efforts to demonstrate IF in nonmammalian vertebrates were limited mainly to clinical studies wherein desiccated gastric mucosa from a variety of animals was fed to individuals with pernicious anemia in relapse. Almost 20 years ago, Wilkinson reported that gastric extracts from a number of reptiles and amphibians were incapable of eliciting hematologic responses in such patients. In view of the species specificity of IF and the relative insensitivity of the test system employed, this information does not provide convincing evidence for the absence of IF in these lower vertebrates. More recent *in vitro* studies demonstrated that frog stomach homogenate failed to enhance the B_{12} uptake of monkey intestine *in vitro* (10). This again suggested the absence of IF in amphibia. However, in a relatively limited number of studies performed in this laboratory, frog gastric homogenate clearly facilitated B_{12} uptake by frog intestine.[2] These findings emphasize that test systems involving enormous phylogenetic disparity between the source of IF and the small intestine are difficult, if not impossible, to interpret.

In the present study, the active material in ESH which enhanced B_{12} uptake in the distal eel intestine was nondialyzable and was destroyed by heating at 100° for 10 min. Dialysis studies indicate that ESH bound vitamin B_{12} rather poorly, 1.2 mμ of cyanocobalamin/mg gastric mucosa (wet wt). This is approximately 1% of that reported in guinea pig, rat, and man (1). The observed characteristics of ESH, *viz.*, (a) the ability to influence selectively B_{12} uptake by various portions of the eel intestine, (b) the capacity to bind vitamin B_{12}, and (c) the nondialyzable character and heat lability of the active material strongly suggest that a substance closely related to IF is present in the

[1] I am indebted to Dr. Robert Ahlvin of the Dept. of Pathology of Jewish Hospital for these studies.

[2] Kaplan, M. E., unpublished observations.

eel gastric mucosa.

Summary. By the everted intestinal sac technique, a crude homogenate of eel gastric mucosa was shown to enhance selectively the vitamin B_{12} uptake of the distal portion of the eel intestine. The active principal in ESH was nondialyzable and destroyed by boiling for 10 min. Weak binding of vitamin B_{12} to ESH was demonstrated. These findings suggest that a rudimentary intrinsic factor mechanism for vitamin B_{12} asborption is present in the eel.

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The Salt Appetite of Adrenalectomized Rats as Influenced by Deoxycorticosterone and Hydrochlorothiazide* (33979)

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Administration of the diuretic agent, hydrochlorothiazide (HCZ), to rats was accompanied both by a spontaneous NaCl appetite and a reduction in the preference (taste) threshold for NaCl solution (1). These changes have also been reported to accompany bilateral adrenalectomy in rats (2, 3). The similarities of the responses to these two diverse experimental procedures are striking and suggest that a common mechanism may exist for both. Since administration of the proper dose of deoxycorticosterone acetate (DOCA) to adrenalectomized rats reduced their exaggerated NaCl intake (4, 5), it seemed worthwhile to determine whether a similar response occurred when DOCA and HCZ were administered simultaneously. The present experiments were designed to study the interrelationship between these two drugs as well as to test the effect of HCZ on the spontaneous NaCl intake of adrenalectomized rats.

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Methods. Two separate experiments were performed. Both used male rats of the Blue Spruce Farms Strain which were bilaterally adrenalectomized 5–7 days prior to the experiment. During this period and throughout the experiment the rats were maintained on finely powdered Purina laboratory chow and given choice between distilled water and 0.15 *M* NaCl solution to drink. Food containers were spill-proof and have been described in detail (6). Fluid containers consisted of infant nursing bottles with cast aluminum fountains (7). The NaCl intake of each rat was measured during 1 week prior to beginning the experiment to assure that all rats were completely adrenalectomized. Only those rats ingesting 65% or more of their total fluid intake as NaCl solution were used.

Expt. 1. The purpose of this experiment was to study the interrelationship between simultaneous administration of DOCA and HCZ with respect to the spontaneous NaCl intake of adrenalectomized rats. Thirty rats were placed into three equal groups. Group 1