Our data confirm the evidence of the presence of a potent diuretic and natriuretic factor in rat atria. Moreover, they strongly suggest that the specific granules may be the place of storage for this factor. The fact that its activity is destroyed by trypsin but not by boiling suggests that it is a small peptide. It is also possible that the cardiac tissue contains proteases, which may break down this peptide and which are inhibited by EDTA and PMSF.

The absence of inactivation of the atrial factor by prolidase, a protease known to destroy the activity of the natriuretic factor described by de Wardener[5], suggests that this natriuretic factor and the 'peptide' we have observed are not related.

It is tempting to speculate that mammalian atria, through the release of a polypeptide material produced in the specific granules, may play a role in the regulation of salt and water balance.

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Differential effects of cadmium and mercury on amino acid and sugar transport in the bullfrog small intestine[1]

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Summary. Amino acid transport in the bullfrog small intestine was specifically inhibited by application of Cd^{2+} to the mucosal fluid. This inhibition was dose-dependent, reversible and competitive in nature. In contrast, Hg^{2+} inhibited both amino acid transport and sugar transport: the inhibition by Hg^{2+} was non-graded, irreversible and non-competitive in nature.

Heavy metals can serve as tools for exploring the chemical nature of sites of membrane function[3,4], because these agents interact with several ligands (particularly sulfhydryl, carboxyl and imidazole groups) of biologically important molecules. The effects of several heavy metals on the transport of amino acids and sugars were examined in the bullfrog small intestine, in order to obtain structural information about these transporting sites in the intestinal brush border membranes.

Bullfrogs of either sex were used. The experimental procedures were similar to those described by Hoshi and Komatsu[5]. After decapitation, the initial short (about 4 cm) segment of the upper intestine below the opening of the common bile duct was isolated. The transepithelial potential difference was measured in the everted sac of the intestine incubated in an aerated saline solution via calomel cells and salt bridges with respect to the reference electrode in the mucosal fluid. The basic saline solution employed in the present study had the following composition (in mM): Na_2SO_4 11.5, $KHCO_3$ 2.5, $CaSO_4$ 1.0, mannitol 187.7, and Tris-H_2SO_4 5.0 (pH 7.4±0.1). To change the Na^+ concentration, mannitol was replaced with equiosmolar amounts of Na_2SO_4, or vice versa. All experiments were done at room temperature (24–29 °C), unless otherwise noted.

The magnitude of the transepithelial potential difference (PD) was dependent on the Na^+ concentration in the bathing solution ($[Na^+]_o$), and the serosal positivity decreased (or reversed) as the $[Na^+]_o$ was decreased. The mean PD value measured in 26 preparations was -6.2 ± 0.6 (SE) mV in the basic solution ($[Na^+]_o = 23$ mM). The addition of sugars or amino acids to the mucosal fluid resulted in an immediate decrease in the serosal negativity or in reversed polarity. It has been well established[6–9] that such evoked potentials correspond quantitatively with the Na^+-dependent, 'secondary' active transport of these organic solutes, although there is still room for debate about the origin of the evoked potentials[10]. The PD changes evoked by 5 mM D-glucose and 10 mM D-galactose were 6.5 ± 1.0 mV (n = 11) and 6.2 ± 1.0 mV (n = 8), respectively. Amino acid-evoked potentials were 13.8 ± 1.6 mV (n = 8) for 10 mM L-α-alanine and 9.2 ± 1.0 mV (n = 10) for 20 mM glycine. These sugar- or amino acid-evoked potentials exhibited the Michaelis-Menten-type kinetics versus the $[Na^+]_o$ values as well as versus the concentrations of those organic solutes, as observed in rat[11] and toad[5] small intestine. These evoked potentials were markedly suppressed by cooling the tissue to 2 °C, or by adding 0.3 mM ouabain to the serosal solution.

The addition of $CdCl_2$ (up to 3 mM) to the mucosal fluid neither affected the transepithelial potential difference in the absence of sugar and amino acid, nor the sugar-evoked potential (fig. 1, b). However, the alanine- or glycine-evoked potential was rapidly suppressed by the mucosal application of Cd^{2+}. This inhibition was dose-dependent (fig. 1, a) and reversible. A similar rapid, graded and reversible inhibition of amino acid- (but not of sugar-) evoked

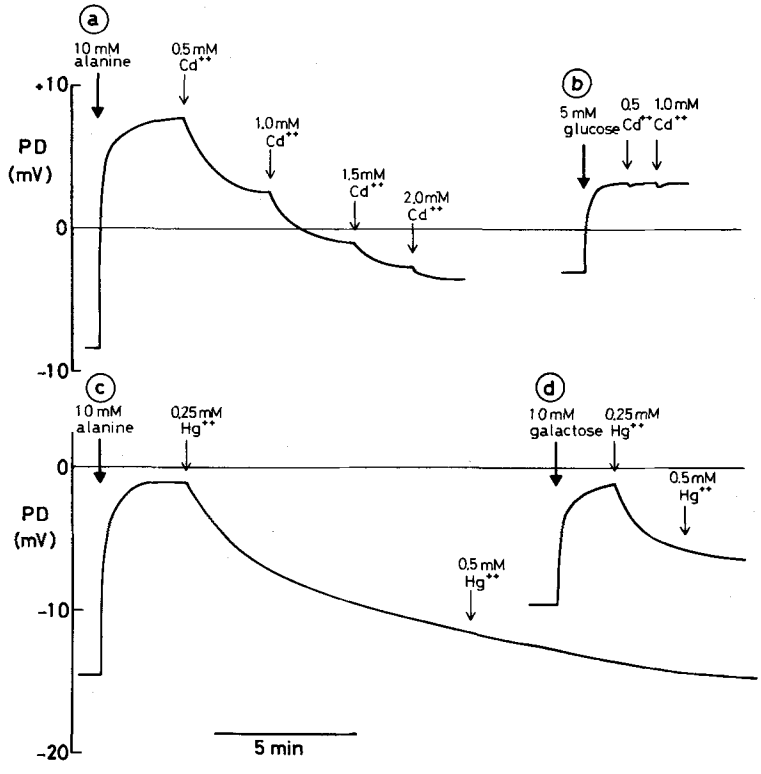

Figure 1. Effects of Cd^{2+} (a,b) and Hg^{2+} (c,d) applied to the mucosal fluid on the amino acid- and sugar-evoked potentials in everted bullfrog small intestines.

potential was also observed by the application of ruthenium red, which is a popular dye with some specificity for the carbohydrate-containing compounds such as mucopolysaccharides and glycoproteins. The Lineweaver-Burk plot (fig. 2, a) indicates that the inhibition by Cd^{2+} or ruthenium red can be classified as competitive.

In contrast to Cd^{2+}, mercuric chloride inhibited, in a noncompetitive fashion (fig. 2, b), not only amino acid- (fig. 1, c) but also sugar-evoked potentials (fig. 1, d) in the bullfrog small intestine. These results are in good agreement with those obtained in the rabbit ileum[12]. As shown in figure 1, c and d, these evoked potentials were inhibited by the exposure to more than 0.25 mM of $HgCl_2$. This inhibition was irreversible and the full effect was slowly attained. In addition, the inhibitory action was nongraded or 'all-or-none' in nature. Since the presence of sulfhydryl groups is essential for the intestinal transport mechanisms of both amino acids[12-15] and sugars[12,16-18], the inhibitory effect of inorganic mercuric ions may be due to their interactions with some sulfhydryl groups associated with the transport sites. Supporting this hypothesis, a similar non-graded, irreversible and non-competitive (fig. 2, b) inhibition was observed in the presence of another sulfhydryl blocking agent, N-ethylmaleimide (NEM), although its potency was much less than $HgCl_2$ (fig. 2, b).

Because the Cd^{2+} effect was entirely different from that of the sulfhydryl-seeking reagents, sulfhydryl groups do not seem to be implicated in the effect of Cd^{2+}. It is known that Cd^{2+} is one of the specific inhibitors for Ca^{2+} channel[19] and that ruthenium red blocks, under a certain condition, the Ca^{2+}-binding site at the plasma cell membrane[20,21]. As illustrated in figure 2, b, however, the transports of both sugar and amino acid were non-competitively inhibited by the deprivation of external Ca^{2+} ions with EDTA. This behavior is in contrast with the competitive inhibition by Cd^{2+} or ruthenium red (fig. 2, a). Therefore, the effects of Cd^{2+} and ruthenium red cannot be ascribed to the block of the Ca^{2+}-transport or Ca^{2+}-binding sites. Thus, it is sug-

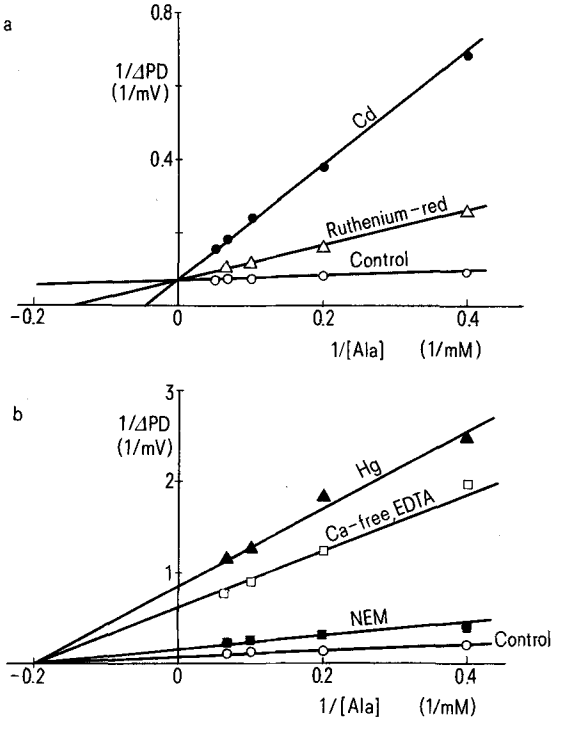

Figure 2. Double reciprocal plots for the alanine-evoked potentials (△PD) against the alanine concentrations ([Ala]) in the mucosal fluid. ○, Mean values obtained in the basic solution (n=2 in a, n=3 in b); ●, values obtained in a representative experiment with 2 mM Cd^{2+} (after 2-5 min); △, values obtained in a representative experiment with 0.5 mM ruthenium red (after 2-5 min); ▲, values obtained in a representative experiment with 0.25 mM Hg^{2+} (after 5-10 min); □, values obtained in a representative experiment in the Ca^{2+}-free, 2 mM EDTA solution (after 15-37 min); ■, values obtained in a representative experiment with 0.5 mM NEM (after 6-10 min).

gested that the Cd^{2+} effect results from its interaction with some metal-binding ligands, other than sulfhydryl groups, which are presumably present in the carbohydrate-containing components at the brush border membranes.

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The effect of aging on rat liver regeneration[1]

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Summary. The effect of age on hepatocyte mensuration and mitotic activity 48 h after partial hepatectomy was investigated in rats. Both age and partial hepatectomy had significant effects upon hepatocyte counts per microscopic field. The number of hepatocytes per microscopic field declined with age in the control groups of different advancing ages and in the experimental groups of advancing ages. There was essentially no mitotic activity in the livers of the control groups. However, mitotic counts were greatly increased in livers from those animals that were partially hepatectomized; the increase in mitotic activity in the 13-month-old animals was double over that observed in both the very young and the very old.

It has been reported that the rate of liver regeneration in mice at 7 days decreased with age and was slowest in 18-month-old mice[3]. In partial hepatectomy studies of rats, the peak rate of DNA synthesis was delayed 5–9 h in 12–15-month-old rats when compared to 4-month-old rats[4]. This delay in peak rate of labeling occurred after the removal of only 9% of the liver. At least 68% of the liver needed to be excised in the younger rats to stimulate liver regeneration[5]. 14 days after partial hepatectomy, 1–2-month-old rats had a greater percentage of hepatic and binucleate cells[6] and a higher percentage of liver restored than 21–30-month-old rats[6,7]. Fine structural analysis of the organelles of the regenerated hepatocytes in the older rats showed that the cell components were changed in regenerated liver cells in the same manner and degree as in unoperated aged livers[8]. Our experiment used hepatocyte counts and mitotic activity to test the effect of partial hepatectomy in a more acute phase (at 48 h post surgery) of liver regeneration in rats of 3 different ages.

Methods. 3 groups of female Wistar rats were obtained from the Gerontology Research Center (NIA), Baltimore, Maryland. The 3 groups were 3 months old, 13 months old and 24 months old. 6 rats from each group were weighed and then partially hepatectomized (70%) under ether anesthesia[9]. The left lateral and median lobes of the liver were removed[9] and weighed. 48 h after surgery the rats were weighed, lightly anesthetized with ether and killed by decapitation. The remaining liver was weighed, and tissue samples were similarly prepared for microscopy. Mitotic and hepatocyte counts were performed on coded samples of liver at a magnification of $\times 450$. The results were expressed as the number of mitotic figures per 60 high power fields (HPF) and number of hepatocytes per high power field. Alternating fields equivalent to 2000–3000 nuclei were searched for mitotic figures. 3 additional rats from each age group served as controls. Significant differences were determined by Student's t-test.

Results. Liver weights. The average amounts of liver to be removed at partial hepatectomy increased with age; 4.9 g of liver in 3-month-old rats, 6.5 g of liver in 13-month-old rats, and 7.6 g of liver in 24-month-old rats (table 1). As expected, at autopsy, 48 h after partial hepatectomy, the amount of liver remaining increased with age (see table 1).

Hepatocyte mensuration and mitotic activity. Light microscopic examination of the liver tissue revealed hepatocyte changes and mitotic activity that were dependent upon both the partial hepatectomy and upon the age of the rats. Livers from the 3-month-old control rats showed 71.1 hepatocytes per HPF. Livers from the 13-month-old control rats had 58.3 hepatocytes per HPF, an 18% ($p < 0.01$) decrease in number, while the livers of the 24-month-old control rats had 49.2 hepatocytes per HPF, a 16% ($p < 0.01$) decrease (table 2). This steady age-dependent decrease of hepatocytes in control rats was paralleled in regenerating livers, but at a considerably lower hepatocyte count. The regenerating liver in the 3-month-old rat had 49.5 hepatocytes per HPF, while that of the 13-month-old rat had 43.5 hepatocytes per HPF, a 12% ($p < 0.01$) decrease, and that of the 24-month-rat had 35.5 hepatocytes per HPF, an 18% ($p < 0.01$) decrease (table 2). There were fewer hepatocytes