effects that are evident are an increased slope conductance at very hyperpolarized membrane potentials and a significant decrease in the NSR. Although some of the reduction of NSR may result from a competing potassium conductance, both of these effects can be produced independently by varying stimulus parameters.

Our results are most consistent with a cyclic AMP mediation of those inputs that act mainly via an increased slope conductance, presumed to be potassium-mediated. A more mechanistic analysis of the mediation of these long-lasting synaptic events in R15 awaits a more detailed description of the conductance changes evoked by individual fibers within the branchial nerve. Bursting pacemaker activity is an important feature of normal neuronal activity; it is also an important feature of abnormal activity, possibly manifested in disorders such as epilepsy. The finding that the conductances underlying such activity may be under synaptic control suggests that complex output patterns may be produced or altered by relatively short input regimes.

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Lactose Facilitates the Intestinal Absorption of Lead in Weanling Rats

Abstract. *The milk sugar lactose is known to facilitate calcium absorption and has been shown to enhance the uptake of essential trace metals from the intestines as well. Its physiological role as the major carbohydrate source for suckling mammals is thus complemented by its ability to facilitate the absorption of necessary minerals. The studies reported here show that the intestinal absorption of lead and its uptake into blood, liver, kidney, and bone are also increased by lactose in young weanling rats. These data extend the known range of lactose facilitation of mineral absorption to a nonessential, toxic element, confirming the nonspecificity of its action on the gut. In addition, they suggest an explanation for some of the conflicting evidence regarding the prophylactic efficacy of milk in lead poisoning.*

It is well known that children are more susceptible than adults to lead poisoning (*1*). This sensitivity to the toxicity of lead results in part from increased absorption and retention of ingested lead (*2*). Enhanced absorption of lead by the young is probably a general characteristic of mammals and has been documented in nonhuman primates (*3*) and in rodents (*4*). The reasons for the subsequent decline in lead retention with age are not completely understood but may include the development of more selective intestinal absorptive processes, more efficient biliary and renal excretion of absorbed lead, and changes in the diet.

Dietary changes may be particularly significant in mammals, since the preweaning diet under normal circumstances consists almost exclusively of mother's milk, whereas the postweaning diet contains little or no milk. Milk diets have been shown to increase the retention of ingested lead in experimental animals (*5*), probably by facilitating intestinal absorption of the metal. The question of the prophylactic efficacy of milk in lead poisoning in humans has been debated for years but not resolved (*6*).

One difficulty associated with analyzing the metabolic effects of milk is its chemical complexity. Hamilton (*7*) has argued, for example, that the facilitation of lead retention observed in rodents fed a milk diet (*5*) is an indirect result of the low iron content of milk, since iron deficiency has been shown repeatedly to increase the absorption and retention of ingested lead (*7, 8*). However, milk contains other materials that directly influence lead metabolism, including calcium, phosphorus, vitamin D, fat, and protein. Another constituent, unique to milk, is the milk sugar lactose, which also has profound effects on mineral metabolism in mammals. The absorption and retention of many minerals, including calcium (*9*), iron (*10*), zinc (*11*), manganese (*12*), cobalt (*13*), magnesium, strontium, barium, and rubidium (*14*), are enhanced by dietary lactose. To our knowledge, however, the effects of lactose on the absorption and retention of lead or other toxic metals have not been investigated. We report here that lactose, in physiological quantities, facilitates the intestinal absorption of orally administered lead in weanling rats and thus increases its uptake by tissues.

Male weanling rats (Holtzman Company, Madison, Wisconsin) were housed

Table 1. The effects of glucose or lactose on the absorption and tissue uptake of lead. Rats which were 26 days old and which had fasted for 24 hours were intubated with 1.0 ml of a dosing solution containing 0, 1, 3, or 6 mg of sugar per gram of body weight and 4.0 μCi of ^{210}Pb. The rats were killed 18 to 22 hours later. Tissues were assayed for radioactivity as described in the text. Values shown are the mean (± the standard error) percent of administered dose absorbed from the gastrointestinal tract (percentage absorption) or the mean percent of administered dose per gram of tissue (percentage uptake).

Treatment	Dose (mg/g)	*N*	Absorption (%)	Uptake by			
				Femur (%)	Kidneys (%)	Liver (%)	Blood (%)
Water (control)	0	10	38.7 ± 5.1	3.40 ± 0.59	3.89 ± 0.65	0.76 ± 0.13	0.52 ± 0.09
Glucose	1	12	40.8 ± 7.2	3.35 ± 0.62		0.84 ± 0.15	0.55 ± 0.10
Glucose	3	10	44.5 ± 2.7	4.13 ± 0.32	4.68 ± 0.41	0.85 ± 0.06	0.60 ± 0.05
Glucose	6	11	42.7 ± 3.6	2.83 ± 0.28	3.70 ± 0.24	0.77 ± 0.05	0.56 ± 0.04
Lactose	1	12	40.0 ± 5.2	3.35 ± 0.52		0.87 ± 0.14	0.61 ± 0.09
Lactose	3	9	74.7 ± 3.6*	6.74 ± 0.35*	7.31 ± 0.47*	1.52 ± 0.16*	0.90 ± 0.06*
Lactose	6	12	69.1 ± 3.0*	5.88 ± 0.33*	7.78 ± 0.91*	1.36 ± 0.10*	1.09 ± 0.08*

*Values that differ from the water control at $P < .01$.

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Table 2. A comparison of the effects of glucose, galactose, maltose, and lactose (3 mg/g) on the absorption and tissue uptake of ^{210}Pb. All lactose values differ from those for glucose ($P < .05$), whereas those for galactose and maltose do not. See Table 1 legend and text for procedures.

Treatment	N	Absorption (%)	Uptake by			
			Femur (%)	Kidneys (%)	Liver (%)	Blood (%)
Glucose	5	45.8 ± 4.9	4.26 ± 0.61	4.73 ± 0.77	0.80 ± 0.11	0.57 ± 0.09
Galactose	6	48.9 ± 6.3	4.30 ± 0.68	5.23 ± 0.81	0.94 ± 0.16	0.51 ± 0.07
Maltose	6	40.9 ± 9.9	3.79 ± 0.95	4.77 ± 1.25	0.81 ± 0.23	0.50 ± 0.14
Lactose	5	70.6 ± 4.9	6.96 ± 0.61	7.32 ± 0.73	1.41 ± 0.10	0.91 ± 0.09

singly in suspended wire cages for 5 days beginning on day 21 after birth and were maintained on freely available distilled water and a vitamin-enriched semi-purified diet containing 0.47 percent calcium and 0.3 percent phosphorus (*15*). Food, but not water, was removed from the cages 24 hours prior to treatment.

Treatment consisted of a single intubation of a test solution on day 26 after birth followed 18 to 22 hours later by tissue assay. Under ether anesthesia, a 1.0-ml dosing solution containing 4.0 μCi of ^{210}Pb, 0.10 ml of 0.01 m*M* lead acetate carrier, and a test dose of 0, 1, 3, or 6 mg of glucose or lactose per gram of body weight (*16*) was administered by gavage. After dosing, animals fasted overnight and were killed the next day by ether overdose. Blood was obtained by cardiac puncture; the liver, kidneys, femur, the entire gastrointestinal tract from esophagus to rectum, and all feces were collected, weighed, and placed in 20-ml plastic scintillation vials.

Tissues were assayed for radioactivity by gamma-counting at the 47-KeV emission peak of ^{210}Pb with a scintillation spectrometer (Packard Auto-Gamma, model 5220). To assess lead uptake by specific organs, counts were converted to counts per minute (cpm) per gram of tissue for each sample and compared to a standard composed of the initial dosing solution diluted to about 2 ml in distilled water. The percentage absorption from the gut was calculated by difference according to Smith *et al.* (*17*) as follows:

$$\% \text{ absorption} = 100\left(\frac{\text{cpm}_{\text{std}} - \text{cpm}_{\text{gut + feces}}}{\text{cpm}_{\text{std}}}\right)$$

Group mean differences were tested for significance by an analysis of variance and by Dunnett's test for comparison of experimental means to a control (*18*).

The absorption of lead from the gut and the uptake by femur, kidneys, liver, and blood in 26-day-old weanling rats are shown in Table 1, for three dosages of sugar. Controls given water absorbed 38.7 percent of the administered lead, a level consistent with those reported for animals close to weaning and food-deprived for nearly 48 hours (*5, 19*). Equivalent amounts of lead were absorbed by all groups given glucose and by the group given 1 mg of lactose per gram. At the higher doses of lactose, however, the absorption of lead was nearly doubled, reaching 74.7 percent of the administered dose (193 percent of the control) at 3 mg/g ($P < .01$). A similar pattern was obtained for tissue lead uptake values, which were approximately doubled by lactose doses of 3 and 6 mg/g ($P < .01$).

In a second experiment, we tested the specificity of the effect for lactose by comparing the lead absorption and tissue uptake when glucose, galactose, maltose, and lactose were used. All sugars were given at concentrations of 3 mg/g to 26-day-old weanling rats. Table 2 shows that galactose and maltose produced lead absorption and uptake values comparable to that for glucose, whereas lactose, as before, increased lead absorption ($P < .05$). Thus, neither galactose, the monosaccharide paired with glucose to form lactose, nor maltose, a disaccharide composed of two glucose moieties, was able to enhance the absorption and uptake of lead by weanling rats, as was lactose.

These data show that lactose enhances the absorption of lead from the intestines, but this result does not rule out the possibility of further, extra-alimentary interaction. Such an interaction in bone has been proposed (*20*) to account for the facilitation of calcium absorption by lactose. However, Lengemann (*14*) demonstrated that parenteral lactose had no effect on the femoral uptake of orally administered calcium; in fact, calcium uptake was enhanced only when lactose and calcium were administered simultaneously into the same gut segment. This result suggests an immediate effect of lactose on intestinal transport processes.

Kello and Kostial (*5*) showed that diets containing milk markedly increased the retention of orally administered lead (30- to 60-fold increases over the control) and had only minor effects on parenterally administered lead (1.2 to 1.3 times the control). These effects have been attributed to a secondary effect of iron deficiency induced by the low iron content of the milk diets used (*5, 7*). In light of the data presented here, however, an explanation of these results must now consider the possibility that lactose in the milk diets facilitated directly the absorption of orally administered lead.

These findings suggest that the increases in tissue lead concentrations in the present experiments are the result of the enhanced absorption of lead from the intestine. Little is known about this process; however, it may be argued that, because lactose acts so nonselectively in promoting the absorption of minerals (*9-14*), lead is treated similarly in this respect. If so, then the mechanisms proposed to explain lactose facilitation of calcium absorption should apply to lead as well. These mechanisms involve facilitation of passive ion diffusion in the distal small intestine and are distinct from the vitamin D-dependent active transport system in the duodenum. In this model, lactose nonselectively increases the permeability of the intestinal membrane to divalent cations, probably by altering a sodium-dependent electrical potential across the intestinal wall which normally opposes passive cationic diffusion to the serosa (*21*).

In vitro studies have demonstrated that other sugars facilitate ileal (but not duodenal) absorption of calcium in adult rats (*22*). These effects are not apparent in vivo because of the rapid hydrolysis and absorption of these sugars in the upper small intestine (*23*). Lactose, which is hydrolyzed and absorbed much more slowly, thus passes into the ileum where it can influence the absorption of metals present. The rapid hydrolysis of maltose into glucose, and the rapid absorption of glucose and galactose, can account for the lower levels of lead absorption associated with these sugars, as compared to lactose.

Milk in the diet of the young mammal plays a complex role in determining the sensitivity to lead poisoning. On the one hand, its lactose content would enhance absorption of lead, as shown here, whereas its high concentrations of calcium, phoshorus, zinc, and protein would be expected to reduce the retention of lead (*24*). The fact that milk contains such "protective" species suggests that it should protect against lead poisoning. However, for such prophylaxis to be fully realized, lead (and possibly other toxic heavy metals including mercury and cadmium) must be absent from the ingested milk and, in fact, removed from the milk meal to prevent the immediate effects of lactose on the intestinal trans-

port of metals. Because of this complexity, it is not surprising that earlier experiments with lead and milk should have yielded conflicting results (*6*).

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Binding and Mobility of the Cell Surface Receptors for 3,3′,5-Triiodo-L-Thyronine

Abstract. *A fluorescent derivative of the thyroid hormone 3,3′,5-triiodo-L-thyronine binds to cultured mouse fibroblasts; such binding is saturable. Video intensification fluorescence microscopy indicates that binding occurs at the plasma membrane. Diffusion coefficients, obtained by fluorescence photobleaching recovery, are consistent with binding to a protein receptor on the cell surface.*

Although much evidence suggests that thyroid hormone action is initiated by binding of 3,3′,5-triiodo-L-thyronine (T_3) to nuclear receptors (*1*), the mechanism for delivery of T_3 to the nucleus is not fully understood. It was long thought that T_3 enters cells by passive diffusion through the plasma membrane (*2*), but recent studies with [^{125}I]T_3 suggest that there are cell-surface receptors for T_3 and that entry of T_3 is, at least in part, energy-dependent (*3*). We have synthesized a rhodamine derivative of T_3 (Rho-T_3) that binds specifically to the nuclear receptor for T_3 with a dissociation constant (K_d) of 20 n*M* (*4*), and in this report we show that Rho-T_3 also binds to a membrane component that has a mobility in the plasma membrane similar to the mobility of polypeptide hormone receptors.

When 3T3 fibroblasts from Swiss albino mice are incubated with Rho-T_3 for 20 minutes at 23° or 37°C, fluorescence is rapidly localized in endocytic vesicles that appear as bright points of light (Fig. 1E) (*5*). Only background fluorescence is observed in cells incubated with rhodamine-thyronine (Rho-T_0). In double-labeling experiments with fluorescein-labeled α_2-macroglobulin and Rho-T_3, we found that these endocytic vesicles are the same as those which take up insulin, epidermal growth factor, and the serum protein α_2-macroglobulin (*5*). We demonstrated previously (*6*) that α_2-macroglobulin-occupied receptors cluster over coated pits before they are internalized and that the clustering can be inhibited by primary alkylamines. Figure 1 shows that methylamine also affects the uptake of Rho-T_3. On a flat cell (Fig. 1A) the fluorescence appears diffusely distributed after 20 minutes of incubation with Rho-T_3 at 23°C in the presence of 20 m*M* methylamine. By focusing up and down through the cell we found that the fluorescence was located at or near the upper and lower surfaces of the cell, as would be expected for binding to the plasma membrane. When grown at high density the cells become somewhat rounded, and this allows one to see clear evidence for membrane fluorescence because the optical path at the boundary between cells contains more of the plasma membrane than the optical path away from the boundary. Whereas bright fluorescence occurs at the boundary between two cells after incubation with Rho-T_3 in the presence of 20 m*M* methylamine, such incubation in the absence of methylamine leads to fluorescence appearing in endocytic vesicles but not, at least in quantity, at the plasma membrane (Fig. 1, C and E).

To show that binding was saturable, we used a microscope fluorescence spectrophotometer to quantify the fluorescence intensity observed after incubating the cells with Rho-T_3 and methylamine in the presence or absence of unlabeled T_3 (20 μ*M*) (see legend to Fig. 1). Fluorescence intensities were measured on 20 randomly selected cells in each dish (*7*). Untreated cells gave an average fluorescence intensity of 33 arbitrary units ± 7 (standard deviation); this intensity was due to cellular autofluorescence. Cells treated with Rho-T_3 alone had an intensity of 103 ± 42 units and cells incubated with Rho-T_3 and excess unlabeled T_3 had an intensity of 54 ± 13 units. Thus, unlabeled T_3 competed for 70 percent of the Rho-T_3 binding. The standard deviations in these measurements are an indication of cellular heterogeneity. Duplicate dishes gave the same average values within ± 10 percent.

Since Rho-T_3 remains associated with the plasma membrane in the presence of methylamine, we were able to measure the diffusion coefficient, *D*, in the plane of the plasma membrane using fluorescence photobleaching recovery (FPR). In this method (*8*), fluorescence from a small region (approximately 1 μm in diameter) on the plasma membrane is partially bleached by a brief exposure to laser light focused on the membrane. The diffusion coefficient is determined from the rate at which fluorescent molecules diffuse into the bleached area. In general, the diffusion coefficients of membrane proteins are 5×10^{-10} cm^2/sec or less, whereas the diffusion coefficients of lipids and lipid probes are about 10^{-8} cm^2/sec (*9*). By using FPR we could determine whether Rho-T_3 behaves as if it is simply dissolved in the membrane lipid phase and diffuses like a lipid probe, or whether its diffusion is more characteristic of a membrane protein. As shown in

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