The modifiable reaction appears to be the degree of conversion of 25-OHD_3 to 1,25-$(OH)_2D_3$ by kidney enzymes (*20*); the more 1,25-$(OH)_2D_3$ produced, the greater the efficiency of calcium absorption. Since the *S. malacoxylon* factor effectively bypasses (or conceivably stimulates) the controlling point, that is, the kidney hydroxylase step, calcium absorption (and probably other aspects of calcium metabolism) occurs at a high rate and essentially out of control, thus accounting for the pathological symptoms of the disease. This is supported by the finding that *S. malacoxylon* elicits no gross toxicity in chicks on a low calcium diet but is deleterious when a normal calcium diet is fed (*15*), suggesting no direct action of the plant factor alone on the soft tissues.

The *S. malacoxylon* factor is water soluble and readily extracted from the plant by aqueous or other highly polar solvents (*21*) and this has been confirmed at our laboratory. This important property tends to eliminate the factor as being vitamin D or even 1,25-$(OH)_2D_3$ per se. It could, however, be a derivative of the metabolite containing a highly polar moiety, thereby accounting for its solubility properties. Required is the isolation and characterization of the factor in order to understand the relation of the factor to the calciferol series, if any, and to aid in elucidating its mode of action. Its therapeutic potential in certain human and animal disease states remains to be determined.

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Persistence of Cadmium-Induced Metabolic Changes in Liver and Kidney

Abstract. *Daily intraperitoneal injection of cadmium chloride (1 milligram per kilogram) for 45 days enhanced gluconeogenesis as evidenced by significant increases in the activities of liver and kidney cortex pyruvate carboxylase, phosphopyruvate carboxylase, hexosediphosphatase, and glucose-6-phosphatase, the quartet of key, rate-limiting enzymes involved in the biotransformation of noncarbohydrate precursors into glucose. Whereas cadmium treatment decreased the level of hepatic glycogen, the concentration of blood glucose and urea was significantly elevated by this heavy metal. Discontinuation of the heavy metal treatment for 28 days, in rats previously injected with cadmium for 45 days, failed to restore the observed biochemical alterations in hepatic and renal carbohydrate metabolism to control values. Evidence indicates that cadmium augments the glucose-synthesizing capacity of liver and kidney cortex and that various metabolic changes persist even after a 4-week period of withdrawal from exposure to the heavy metal.*

Prolonged exposure to environmental cadmium produces cellular alterations in a variety of body tissues. Testicular atrophy (*1*), hypertension (*2*), itai-itai disease (*3*), and permanent lung damage in the form of peribronchial fibrosis (*4*) are among the toxic symptoms attributed to the widespread usage of cadmium. In addition, this heavy metal produces histological and functional damage to kidney and liver, as evidenced by renal tubular atrophy, interstitial fibrosis, proteinuria, glycosuria (*5*, *6*), and cirrhosis (*7*). A similar relation between renal and hepatic dysfunction and an increase in glucose and protein in the urine has been noted in animals treated with either methylmercury or various chlorinated hydrocarbon insecticides (*8*, *9*). Since cadmium treatment affects overall carbohydrate metabolism (*3*, *10*), and the renal and hepatic abnormalities it produces closely resemble those seen in organomercurial-treated rats, we examined the influence of cadmium on renal and hepatic gluconeogenic enzymes, hepatic glycogen, blood glucose, and serum urea. We found that daily injection of cadmium chloride for 45 days (1 mg per kilogram of body weight) significantly elevated the concentration of blood glucose and urea; enhanced the activities of renal and hepatic pyruvate carboxylase (E.C. 6.4.1.1), phosphopyruvate carboxylase (E.C. 4.1.1.32), hexosediphosphatase (E.C. 3.1.3.11), and glucose-6-phosphatase (E.C. 3.1.3.9); and reduced the concentration of liver glycogen.

Experiments were carried out in male Wistar rats weighing approximately 100 g, housed in individual cages, and having free access to Master laboratory chow and water. Cadmium chloride was dissolved in physiological saline and administered daily for 45

days by the intraperitoneal route in a dose of 1 mg/kg, control animals receiving an equal volume (0.2 ml) of the vehicle. To examine the influence of cadmium withdrawal, a group of rats which had been given the heavy metal for 45 days was maintained without any treatment for an additional 28 days. All animals were starved overnight (16 hours) before they were killed. Rats were stunned, decapitated, and freely bled, and the kidney cortices and livers were rapidly weighed. Hepatic and renal tissues were homogenized in 0.15*M* KCl, *p*H 7.4, and 5 percent homogenates and supernatant fluids were obtained as described (*9, 11*). Pyruvate carboxylase, phosphopyruvate carboxylase, and hexosediphosphatase activities were assayed in the 100,000*g* supernatant fraction, whereas glucose-6-phosphatase was determined by using the whole homogenate (*9, 11*). All enzyme assays were carried out under strictly linear kinetic conditions at 37°C and the results are expressed as specific activity per milligram of protein. Protein was determined according to the method of Lowry *et al.* (*12*). Blood glucose, serum urea, and liver glycogen were determined as described previously (*13*). All data were statistically evaluated by using Student's *t*-test, and significant differences between the means were calculated as *P* values.

Daily treatment with cadmium for 45 days produced no apparent sign of toxicity and there was no significant difference in body weight gain compared to controls. Table 1 shows the hepatic and renal gluconeogenic enzyme activities in control rats, rats injected with cadmium for 45 days (treated group), and rats treated and then withdrawn for 28 days (withdrawal group). Long-term administration of cadmium produced significant increases in the activity of liver pyruvate carboxylase, phosphopyruvate carboxylase, hexosediphosphatase, and glucose-6-phosphatase compared with the control values. Withdrawal for 28 days failed to restore the enzyme activities to the control values. In fact, the activities of hepatic phosphopyruvate carboxylase, hexosediphosphatase, and glucose-6-phosphatase were somewhat higher in the withdrawal group than in the treated group. Prolonged cadmium treatment enhanced the activities of all four renal gluconeogenic enzymes, and the observed increases persisted 28 days after discontinuation of treatment. Cadmium treatment produced no significant change in the protein content of hepatic and renal cortex homogenates, and similar changes in gluconeogenic enzyme activities were seen when they were calculated per milligram of tissue (wet weight).

Long-term administration of cadmium resulted in an increase in blood glucose and urea and the effect persisted after withdrawal for 28 days (Table 2). In contrast, the amount of liver glycogen was markedly decreased in treated rats, and the glycogenolysis persisted after withdrawal for 28 days.

Even though cadmium exerts a variety of effects on mammalian tissues, little is known about the biochemical basis of these effects. Our study demonstrates that daily administration of cadmium for 45 days augmented the activities of four enzymes known to play a key, rate-limiting role in the synthesis of glucose from noncarbohydrate sources. Prolonged treatment reduced liver glycogen and elevated the concentration of blood glucose and urea. After withdrawal from treatment for 28 days, animals previously given cadmium for 45 days still showed similar biochemical alterations in kidney and liver. Smaller doses of cadmium (0.25 mg kg^{-1} day^{-1}) also decreased hepatic glycogen content, augmented blood glucose and urea, and significantly increased the activities of gluconeogenic enzymes of both tissues (*14*). In addition, heavy metal treatment produced a rise in the excretion of protein and glucose above control as indicated by the Combistix and Dextrostix tapes, respectively (Ames Company of Canada Ltd., Toronto, Ontario), and the observed changes persisted in the withdrawal group to about the same extent. The presence of glycosuria and proteinuria in cad-

Table 1. Effect of 28-day withdrawal after 45 days of cadmium treatment on gluconeogenic enzymes in liver and kidney. Means ± standard error of the mean represent four to five animals in each group. Cadmium chloride was administered to ten rats daily for 45 days by the intraperitoneal route. Half of these rats, the withdrawal group, were maintained without any treatment for an additional 28 days. Abbreviations are PC, pyruvate carboxylase; PPC, phosphopyruvate carboxylase; HDP, hexosediphosphatase; and G6P, glucose-6-phosphatase. Enzyme activities are calculated as micromoles of substrate metabolized per hour per milligram of protein. Data are also given in percentages, with the values for control animals taken as 100 percent.

Enzyme	Enzyme activity					
	Control		Treated		Withdrawal	
	Micromoles per hour per milligram	Percent	Micromoles per hour per milligram	Percent*	Micromoles per hour per milligram	Percent*
			Liver			
PC	226 ± 21	100	318 ± 18	141	310 ± 10	138
PPC	15 ± 0	100	24 ± 1	160	27 ± 1	181†
HDP	4.7 ± 0.2	100	5.9 ± 0.1	126	6.6 ± 0.5	140
G6P	2.8 ± 0.3	100	4.7 ± 0.2	169	6.0 ± 0.1	215†
			Kidney cortex			
PC	74 ± 3	100	160 ± 7	216	196 ± 9	265†
PPC	25 ± 2	100	34 ± 1	136	38 ± 1	152
HDP	7.7 ± 0.5	100	11.9 ± 0.3	155	9.9 ± 0.1	129
G6P	10 ± 1	100	13 ± 0	130	14 ± 1	140

* All values were statistically significant compared with the values for control rats ($P < .05$). † Statistically significant compared with the values for rats given cadmium for 45 days ($P < .05$).

Table 2. Alterations in blood glucose, urea, and liver glycogen after long-term cadmium treatment and subsequent withdrawal. Means ± standard error of the mean represent four or five animals in each group. Cadmium chloride (1 mg/kg) was injected intraperitoneally to ten rats daily for 45 days. In half of these rats, treatment was withdrawn for 28 days. Data are also given in percentages, with the values for control animals taken as 100 percent.

Group	Blood glucose		Serum urea		Liver glycogen	
	Milligrams per 100 ml	Percent	Milligrams per 100 ml	Percent	Grams per 100 g	Percent
Control	81 ± 2	100	26 ± 1	100	2.2 ± 0	100
Treated	150 ± 2	185*	34 ± 0	131*	0.9 ± 0.1	41*
Withdrawal	137 ± 7	169*	43 ± 1	165*†	0.7 ± 0.09	32

* Statistically significant compared with the values for control rats ($P < .05$). † Statistically significant compared with the values for rats given cadmium for 45 days ($P < .05$).

mium-treated rats agrees with the results of Nomiyama *et al.* (*6*), who also suggested that the increased excretion of protein and glucose in rabbits continuously injected with cadmium might be associated with renal tubular dysfunction. Treatment with cadmium has also been shown to produce liver damage and a rise in the glycogen-degrading enzyme glycogen phosphorylase, as well as a significant decrease in the activity of hepatic aldolase (*7*, *10*). Our data on the stimulation of the gluconeogenic pathway by cadmium provide an enzymatic basis for the increase in urinary protein and glucose, as suggested in the case of methylmercury and several organochlorine pesticides (*8*, *9*). It is also possible that the cadmium-induced glycosuria might be related to a decreased sugar reabsorption resulting from renal failure (*6*). Our data suggest that the presence of cadmium in the environment could lead to an impairment of hepatic and renal carbohydrate metabolism, and this may account for its environmental toxicity.

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Ecto-Enzyme of Granulocytes: 5′-Nucleotidase

Abstract. *The 5′-nucleotidase of guinea pig polymorphonuclear leukocytes is localized exclusively on the plasma membrane of intact cells. The active site of this enzyme faces the external medium, not the cytoplasm.*

Although it is now generally accepted that the 5′-nucleotidase of mammalian cells is localized on their plasma membranes, the evidence is hardly conclusive. Histochemical techniques present many difficulties (*1*), one of which is the possibility that substrate is not penetrating into cells or organelles. Thus the histochemical approach does not rule out the existence of an intracellular 5′-nucleotidase. In biochemical studies, preparations of plasma membrane from rat liver exhibit specific activities of 5′-nucleotidase about 20 times that of the original homogenate (*2*). However, recoveries of this enzyme in the plasma membrane fraction are 10 percent (or substantially less) that of the original homogenate (*3*), so that most of the activity may still be associated with another organelle.

We have proposed an approach to the selection of markers for the plasma membrane (*4*). This approach is based on the circumstance that the plasma membrane is the one cellular structure that can be studied without disrupting the cell. With a suspension of intact cells in vitro the outer surface of the membrane is accessible to biochemical investigation and chemical alteration. We now report our use of intact cells to determine the localization of 5′-nucleotidase in guinea pig polymorphonuclear leukocytes.

Cells were harvested from the peritoneal cavities of guinea pigs as described (*5*). The enzyme (5′-nucleotidase) was assayed in Krebs-Ringer phosphate buffer (KRP), *p*H 7.4, with the use of 1 m*M* adenosine [^{32}P]-monophosphate and 1 m*M* *p*-nitrophenyl phosphate.

The *p*-nitrophenyl phosphate was shown by competition studies to prevent the hydrolysis of adenosine monophosphate (AMP) by nonspecific phosphatases present on the surface of intact cells and in disrupted cell preparations. Further, we showed that, when levamisole, an inhibitor of nonspecific phosphatases (*6*), was included in the reaction mixture, *p*-nitrophenyl phosphatase was inhibited virtually completely, while adenosine monophosphatase activity was identical to that measured as described above. The assay mixture was incubated at 37°C, and the reaction was terminated by the addition of an equal volume of 10 percent (weight to volume) suspension of acid-washed Norit in 10 percent (weight to volume) trichloroacetic acid. The charcoal, which binds AMP but not inorganic phosphate (P_i), was removed by filtration through Whatman No. 1 filter paper, and $^{32}P_i$ in a portion of the filtrate was counted. This assay was found to be linear for at least 30 minutes and over the range of 1×10^6 to 8×10^6 cells per assay vessel. It was used successfully not only with intact or disrupted cells in suspension, but also with cells that had formed a monolayer on a small plastic dish. The diazonium salt of sulfanilic acid was synthesized by the indirect method (*7*). Radioactivity was determined by counting in a toluene-ethanol–based scintillation fluid (*8*). When necessary, cellular fragments were dissolved in NCS solvent (Amersham/Searle) at 37°C.

Intact guinea pig polymorphonuclear leukocytes hydrolyzed AMP added to the medium at the rate of 316 nmole per 10^7 cells in 15 minutes. We showed that this activity was due neither to leakage of enzyme from the cells nor to broken cells; that the adenosine monophosphatase of intact cells was associated with the polymorphonuclear leukocytes and was not attributable to contaminant cell types (monocytes, lymphocytes, eosinophils, and erythrocytes); and that washing the cells ten times with KRP or sucrose did not affect the hydrolysis of AMP by intact cells.

The first experiment to determine whether the intact cell adenosine monophosphatase is an ecto-enzyme—that is, localized exclusively on the plasma membrane, with the active site oriented to the medium—was based on the reasoning that, if a protein reagent that does not penetrate into the intact cells can inhibit the activity of the enzyme, the protein involved must be located on the plasma membrane. Cell monolayers were treated with the diazonium