conserved in evolution. The amino acid structure of such a determinant would not necessarily be identical in placental and intestinal ALP, and such differences as exist could well account for the apparently reduced reactivities of the intestinal ALP's compared with placental ALP.

The structures of the carbohydrate moieties of the ALP's have not been determined, although some analytical data indicating differences in composition are available (*2*). If the Sp2/5 antibody is directed toward an antigenic determinant on the carbohydrate moiety of the ALP molecule, the configuration of sugars in the determinant grouping is presumably similar in the various ALP's but differs in detail, since the various ALP's show differences in reactivity with the antibody.

Our results point the way to detailed studies of the nature of the antigenic determinant at which Sp2/5 antibody is directed and in particular to the question of whether the determinant is in the protein or carbohydrate moieties of these enzyme glycoproteins. The results also illustrate the potential usefulness of such electrophoretic studies in the search for cross-reactions of monoclonal antibodies in other sets of nonallelic enzymes.

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Developmental Changes in the Biliary Excretion of Methylmercury and Glutathione

Abstract. *The long half-time for methylmercury in the neonatal rat is explained by the neonatal liver's inability to secrete the toxin into bile, which in adults is the main route of elimination. The ability to secrete mercury into bile develops between 2 and 4 weeks of age and is correlated with the increasing ability of the developing liver to secrete glutathione into bile.*

The neonatal period is an especially vulnerable time for the accumulation of many heavy metals (*1*). Methylmercury is more toxic to suckling animals and human infants (*2*) than to adults. Metals readily accumulate in young animals due to higher intestinal absorption rates and immaturity of the hepatic and renal excretory functions (*1, 3*). Neonatal mice (*4*) and rats (*5*) treated with methylmercury excrete only a small fraction of the amount administered. At 16 to 18 days of age, however, there is an abrupt increase in mercury excretion. Indirect evidence points to a similar phenomenon in human neonates (*2, 6*). The mechanisms underlying these ontogenetic differences in the excretion of methylmercury are not known. Since the rate of methylmercury elimination is a significant determinant of the body burden, data on differences in the rates of elimination at various developmental stages are important in estimating exposure risks for the fetus and neonate and in therapeutic intervention.

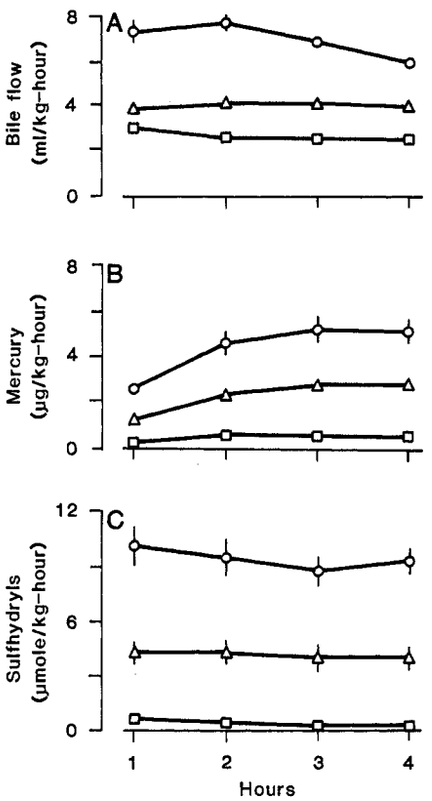

Fig. 1. Developmental changes in bile flow, mercury secretion into bile, and total reduced sulfhydryl secretion into bile. An intravenous injection of mercury (1 mg/kg) as $CH_3{}^{203}HgCl$ was given to 14-day-old (□) (N = 12), 21-day-old (△) (N = 10), and 28-day-old (○) (N = 8) rats, and bile was collected every hour for 4 hours thereafter. Values are means ± standard errors.

Fecal excretion, the main route of mercury elimination, is determined primarily by biliary secretion, and it accounts for over 80 percent of the total excretion in rodents (*7*). An important role in the regulation of the biliary secretion of methylmercury has been ascribed to glutathione (*8–10*). Glutathione, which has a concentration in bile of 1 to 4 mmole/liter, accounts for over 90 percent of the reduced sulfhydryl groups in bile (*8, 11*). Methylmercury has a high affinity for reduced sulfhydryl groups, including those of glutathione, and a methylmercury-glutathione complex has been identified as the main form of methylmercury in bile (*8*). Infusion of glutathione into rats increases the biliary secretion of methylmercury (*12*), while agents that deplete the hepatic content of glutathione inhibit the biliary secretion of methylmercury and simultaneously decrease the reduced glutathione content of bile (*9*).

The biliary pathway of excretion is also important in the design of therapy. A nonabsorbable, thiolated resin, given orally, has been used to increase fecal excretion of methylmercury in animals and humans (*13*). The resin acts by trapping mercury secreted into bile, interrupting the enterohepatic recirculation of the metal (*7*).

In an attempt to explain the rapid increase in methylmercury excretion in young animals, we examined the developmental changes in the biliary pathway for the elimination of methylmercury in relation to the changes in glutathione secretion into bile. The bile ducts of 14-, 21-, and 28-day-old Sprague-Dawley rats were cannulated under sodium pentobarbital anesthesia. Each group had equal numbers of males and females. Labeled methylmercury chloride ($CH_3{}^{203}HgCl$) was injected into a jugular vein cannula in a nontoxic dose (mercury concentration, 1 mg/kg) in a solution of 0.9 percent

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Table 1. Concentrations of mercury and nonprotein sulfhydryl groups 4 hours after the intravenous injection of mercury (1 mg/kg) as $CH_3{}^{203}HgCl$. Values are means ± standard errors.

Age (days)	N	Body weight (g)	Mercury (μg/g)			Sulfhydryls in liver (μmole/g)
			Liver	Erythrocytes	Plasma	
14	12	30.9 ± 0.8	1.20 ± 0.02	23.5 ± 1.0	0.054 ± 0.005	4.73 ± 0.27
21	10	53.8 ± 2.5	1.29 ± 0.04	24.4 ± 1.2	0.069 ± 0.004	5.06 ± 0.25
28	8	90.1 ± 4.2	1.23 ± 0.05	22.7 ± 0.9	0.053 ± 0.002	5.38 ± 0.15

NaCl containing approximately 2 m*M* Na_2CO_3. The volume of the injection solution was 2 ml/kg and the specific activity of the mercury was approximately 10 μCi/mg. Bile was collected every hour for 4 hours into ice-chilled, tared tubes containing 200 μl of 0.20*M* disodium salt of EDTA. At the end of the experiment the portal vein was cannulated and the liver was perfused with a small volume of saline to remove the blood. The mercury content of the bile, plasma, red blood cells, and liver was then determined by gamma scintillation counting. The concentration of total reduced sulfhydryl groups in bile and the amount of nonprotein-reduced sulfhydryl groups in liver was estimated by the method of Sedlak and Lindsay (*14*). Since glutathione is the major sulfhydryl-containing compound of rat bile (*8, 11*) and of protein-free liver (*15*), sulfhydryl content was taken as an estimate of glutathione content.

The rate at which the 14-day-old rats secreted methylmercury into bile was one-tenth that of the 28-day-old rats (Fig. 1B) (*16*). Bile flow was also less in the 14-day-old rats, but only by a factor of 2 (Fig. 1A). Thus the smaller biliary secretion of methylmercury in the 14-day-old rats was mainly attributable to a lower concentration of methylmercury in bile. The ability to secrete glutathione into bile was also significantly less in the 14-day-old rats (Fig. 1C). Development of the ability to secrete glutathione paralleled development of the ability to secrete mercury. Indeed, the biliary concentration of glutathione was correlated with the methylmercury concentration in all three groups (Fig. 2).

The concentrations of mercury and nonprotein sulfhydryl groups in the liver, plasma, and blood cells were similar in the three groups (Table 1). However, the younger rats were unable to secrete methylmercury and glutathione in bile. This indicates that the neonatal liver is able to take up mercury, but that its capacity to secrete methylmercury and glutathione into bile develops only gradually, reaching adult levels in 28 days.

Our results provide further evidence of the importance of glutathione in determining the biliary secretion of methylmercury (*8–10*). They also suggest the presence of a biliary transport system for glutathione. As with other biliary transport systems, including those for anions (*17*) and neutral compounds (*18*), this system is immature in sucklings but develops at weaning. Further evidence of a biliary transport system for glutathione is provided by some of our recent studies on the biliary transport of methylmercury (*10*). We found that indocyanine green and sulfobromophthalein, but not a complex of sulfobromophthalein and glutathione, inhibited the biliary secretion of methylmercury in adult rats. This inhibition was accompanied by a parallel inhibition of reduced glutathione secretion into bile and occurred without any changes in liver glutathione levels. Also, when bile flow was altered with dehydrocholate, hypertonic sucrose infusion, or hypothermia, the absolute rates of methylmercury and glutathione secretion into bile were not affected despite parallel changes in the biliary concentration of methylmercury and glutathione. Because of the high affinity of glutathione for methylmercury [($K_1 = 10^{15.8}$) (*19*)], the proposed biliary transport system for glutathione may determine the biliary secretion of methylmercury.

The immaturity of this transport system in the neonatal rodent may explain the long half-times for methylmercury in sucklings (*4, 5*). Factors such as diet (*20*) and gastrointestinal flora (*21*) influence methylmercury excretion in adult animals. Investigations of their role in the neonate must take into account the large ontogenetic changes in the biliary excretion of methylmercury and glutathione. Moreover, since bile is the main excretory route for many heavy metals (*22*) and since most of these metals have a high affinity for reduced sulfhydryl groups, the proposed glutathione-dependent secretion mechanism may explain the high retention of other heavy metals by neonates (*1, 3*).

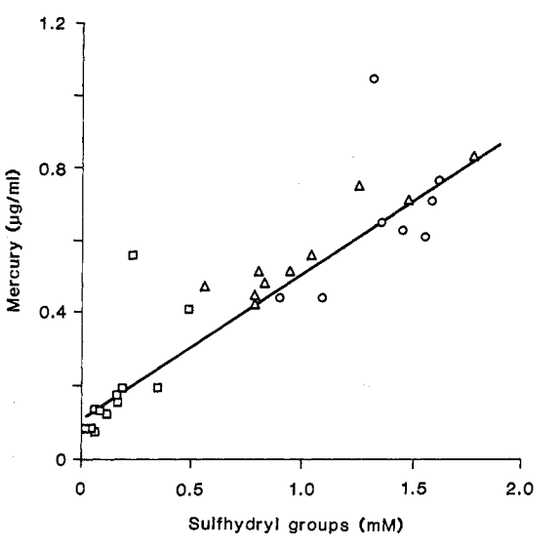

Fig. 2. Relation between the concentrations of mercury and sulfhydryl groups in the bile of 14-, 21-, and 28-day-old rats. Each point is the average concentration of mercury and sulfhydryls during the 4-hour bile collection period following the intravenous dose of $CH_3{}^{203}HgCl$. The equation for the linear regression, excluding the two outlying points, is $y = 0.40x + 0.11$; $r = .96$, $P < .001$. (When all data are included, $y = 0.40x + 0.13$; $r = .90$, $P < .001$.)

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Mutual Flocculation of Algae and Clay: Evidence and Implications

Abstract. *Algae-clay aggregates were formed when algal and clay suspensions were mixed in the presence of an electrolyte. The maximum ratio of clay to algae in the aggregates was 1.7, 0.2, and 0.03 milligrams of clay per milligram of algae (wet weight) for* Anabaena, Chlamydomonas, *and* Chlorella *sp., respectively. The aggregates formed at* Ca^{2+} *concentrations higher than* 5×10^{-4}M *or* Na^{+} *concentrations higher than* 2×10^{-2}M. *The mutual flocculation and subsequent sedimentation have many practical and ecological implications for bodies of water.*

The two materials most commonly found suspended in most lakes and impoundments are algal cells and clay particles. Aggregation of these particles would lead to the formation and sedimentation of larger particles and thus to the clarification of the water. These interactions may profoundly affect the ecological structure of bodies of water in terms of the temporal and spatial distribution of organisms, the concentration of suspended inorganic particles and nutrients, nutrient cycling, substrate utilization by filter-feeding organisms, and other processes.

Considerable information exists on microbial or microbial-clay aggregation in soils, water treatment plants, dental plaque, and other systems (*1*). Such aggregation is attributed to the presence of extracellular polymers, especially polysaccharides. Algae excrete large amounts of polysaccharides and other polymers. The amounts released may represent from 15 to 60 percent of the photoassimilated carbon (*2*). Thus, an effective aggregation of algae and clay particles could be expected; however, little work on this topic has been published. The aggregation of *Anabaena* filaments, attributed to the excretion of mucilage, was demonstrated by Walsby (*3*). Altman (*4*) suggested that muddy fish ponds be clarified through the addition of fertilizers, barnyard manure, hay, or other organic materials. Kimmel and Lehman (*5*) observed that the introduction of high-turbidity runoff to experimental water columns resulted in the removal of algae from the columns; the effect was apparently associated with clay-particle flocculation and sedimentation. The addition of less turbid runoff water did not remove algae from the water columns. Avnimelech (*6*) suggested that algae-clay aggregation leads to the sedimentation of the suspended load in tributaries of the Jordan River. Zur (*7*) isolated a blue-green alga, *Phormidium* sp., from the Jordan. This alga, and its filtrate, has a marked flocculation activity toward clay.

We undertook to study the interaction of clay and algae by using several representative algal species, to establish methods for such a study, and to provide some quantitative data on this interaction. *Euglena gracilis*, *Anabaena* sp., *Chlamydomonas* sp., and *Chlorella* sp. were grown in Bold's medium (*8*) enriched with nutrient broth. Algae were separated from the medium before each experiment by centrifugation and redispersed in a 0.2 percent sucrose solution, isotonic to the growth medium yet devoid of electrolytes, or in $10^{-3}M$ $CaCl_2$. Wyoming bentonite saturated with Na^{+}, having a particle size smaller than 0.2 μm, was prepared by high-speed centrifugation.

We determined the algal concentrations by using in vivo fluorescence (*9*) calibrated against microscopic counting. We determined the clay concentrations by measuring the light scattering at 420 nm. Scanning electron micrographs were taken after fixation of the samples with 2 percent osmium tetroxide.

Chlorella-bentonite suspensions flocculated only above a critical concentration of $CaCl_2$ or NaCl (Fig. 1a). The flocculation value was $5 \times 10^{-4}M$ for $CaCl_2$ and $2 \times 10^{-2}M$ for NaCl. Suspensions of algae without clay were not flocculated by either NaCl or $CaCl_2$ within the concentration ranges studied.

Algal suspensions were mixed with

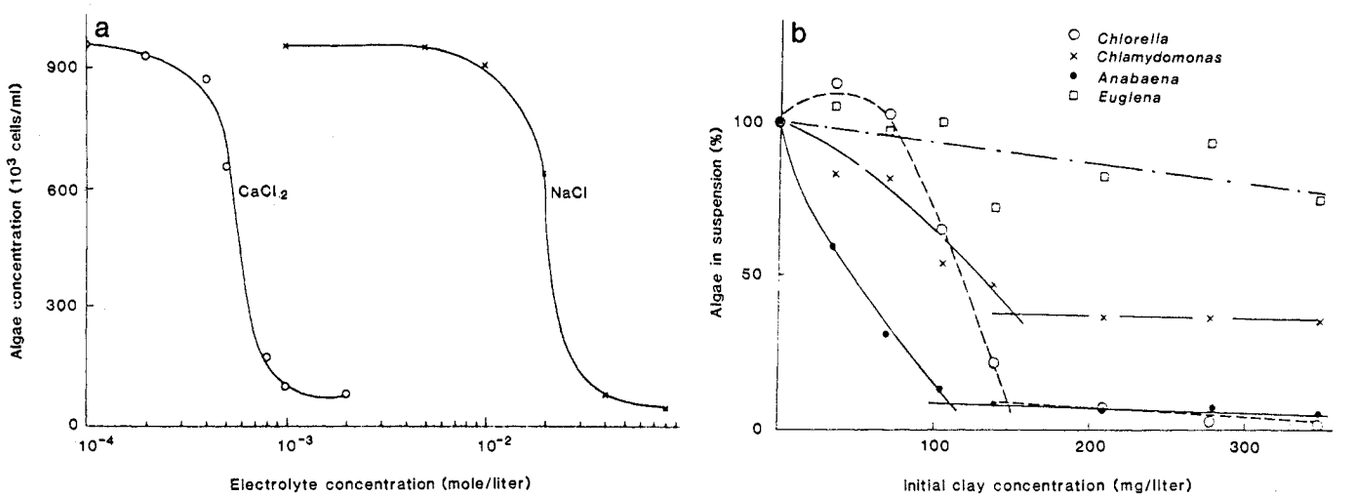

Fig. 1. (a) Flocculation of bentonite clay and *Chlorella* suspensions as a function of NaCl and $CaCl_2$ concentrations. (b) Algal concentration in the top 6 cm of the suspension, after 120 minutes of sedimentation time, as a function of the initial clay concentration.

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