changes in the discontinuous ECoG. Photic driving was substantially augmented by GHB in adult and 25-day-old rats (fig. 2); in 15-day-old animals it was absent before as well as after GHB injection.

Discussion. Pattern of the GHB-induced ECoG activity registered in adult rats in our acute experiments is identical with that described by Godschalk at al.[3] in rats with chronically implanted electrodes. This identity is important for the developmental study – it could be performed under conditions of acute experiments and we thus avoided the serious difficulties of chronic implantation of electrodes in immature rats.

The appearence of discernible GHB-induced activity between the 9th and 12th days is in agreement with the development of spike-and-wave cortical self-sustained after-discharge (SSAD) elicited by rhythmic stimulation of the thalamus. This type of SSAD could be evoked for the first time in 12-day-old rats and its shape matured until the 18th postnatal day[6].

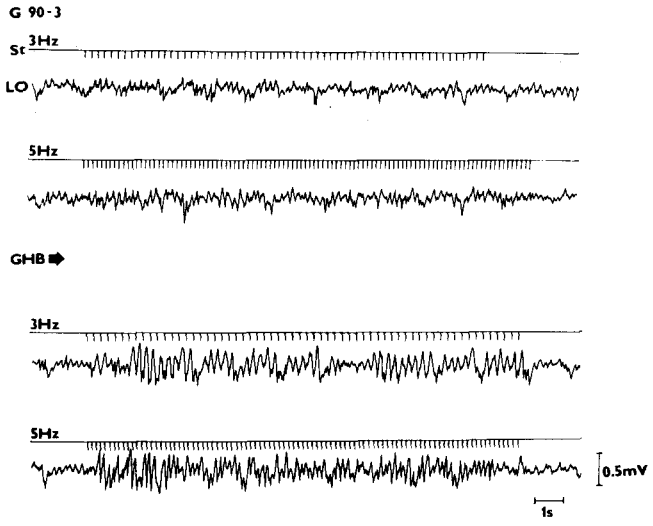


Figure 2. Photic driving elicited by rhythmic photostimulation of the right eye in adult rat. Upper part before, lower part 30 min after the administration of GHB. In each section St = marks of stimuli (frequencies 3 and/or 5 Hz), LO = monopolar recording from the left occipital, i.e. primary visual area. Other details as in figure 1.

Comparing adult rats with young ones the difference in localization of GHB-induced activity becomes obvious: diffuse incidence of this activity in adult rats is in contrast to clear-cut predominance of frontal activity in comparison with the occipital one in all immature rats. In animals aged 15 days or less the same holds true for spontaneous ECoG activity without drug pretreatment, but 18- and 25-day-old rats exhibit well-expressed spontaneous ECoG in all cortical regions[7]. This distribution strongly resembled a central maximum of spike-and-wave episodes in children with classical absences – the rat's frontal activity is formed by motor and somatosensory areas and is thus analogous to human central region.

The hypothesis that GHB-induced activity could be a model for petit mal epilepsy was formulated by Godschalk et al.[2,3] as well as by Snead[8]. Our results could be taken as additional evidence supporting this hypothesis – especially in relation to results of pharmacological studies in adult rats[2] and monkeys[8-10] as well as in 25-day-old rats[11,12]. An increased sensitivity to rhythmic photostimulation found in our experiments fits into the current concept of petit mal epilepsy[13].

1 H. Laborit, J.M. Jouany, J. Gerard and F. Fabiani, Agressologie *1*, 397 (1960).
2 M. Godschalk, M.R. Dzoljic and I.L. Bonta, Neurosci. Lett. *3*, 145 (1976).
3 M. Godschalk, M.R. Dzoljic and I.L. Bonta, Eur. J. Pharmac. *44*, 105 (1977).
4 A. Matthes, Epilepsie Fibel. Thieme, Stuttgart 1969.
5 R. Rokyta, P. Mareš and P. Sobotka, Activitas nerv. sup. *21*, 178 (1979).
6 D. Staudacherová, P. Mareš, J. Fischer and S. Trojan, Physiol. bohemoslov. *27*, 277 (1978).
7 P. Mareš, A. Zouhar and G. Brožek, Activitas nerv. sup. *21*, 218 (1979).
8 O.C. Snead, Neurology *28*, 636 (1978).
9 O.C. Sneed, Neurology *28*, 643 (1978).
10 O.C. Snead, Neurology *28*, 1173 (1978).
11 R. Rokyta, P. Mareš and A. Ruzickova, Plzeň. lék. Sb., in press (1981).
12 R. Rokyta, M. Suchý and M. Menzelová, Plzeň. lék. Sb. *41*, 13 (1974).
13 M. Myslobodsky, Petit mal epilepsy. Academic Press, New York 1976.

Specific metal binding sites on calcified concretions in epithelial cells of the clam kidney

N.G. Carmichael and S.C. Bondy

Department of Histopathology, Royal Postgraduate Medical School, DuCane Road, London W12 OHS (England), and Laboratory of Behavioural and Neurological Toxicology, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park (North Carolina 27709, USA), 22 September 1980

Summary. Calcified granules (concretions) in the kidneys of bivalve molluscs are able to absorb metals from solution and this ability is the result of the existence of saturable, medium affinity metal binding sites on the concretions. In addition different metals may compete for the same sites so that some metals will be accumulated preferentially over others.

Marine bivalve molluscs possess a remarkable ability to concentrate metals and other pollutants from seawater and there are currently extensive investigations into their properties as environmental monitoring organisms[1]. Until recently little was known about the process by which high tissue levels of metals were produced from environmental metal levels below the detection limit of analytical instruments. It has now been shown that certain organs, particularly the kidney have high concentrative capacity[2], and that this is due in part to metal binding proteins in this organ and in part to subcellular calcium-phosphorus concretions[3]. These concretions in the bivalve kidney contain such metals as zinc, manganese, copper, cadmium etc. but little is known of their formation or function. This study was

Binding site density and dissociation constants for the absorption of cadmium and manganese to calcareous concretions of clam kidney

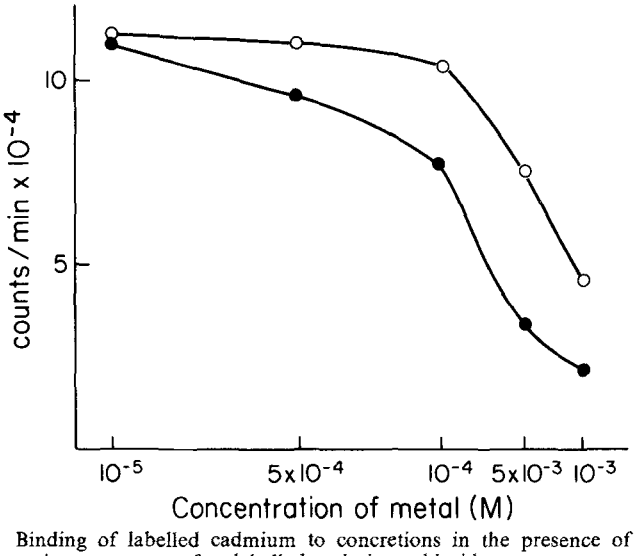
	B_{max} (nmoles metal bound/mg concretion)	K_D
Cadmium	15.1	7.7×10^{-5} M
Manganese	14.9	11.2×10^{-5} M

designed to find out whether isolated concretions had any tendency to bind metal ions in an in vitro situation and if so, to characterize this property.

Concretions were prepared from fresh clam kidneys by a method that has been described previously[3]. Briefly the procedure involves sedimentation of these particles through high density (2.5 M) sucrose (3,000 g ave $\times 15$ min). Concretions are purified by resuspending twice in deionized water and resedimentation. The final pellet when examined by electron microscopy consists very largely of particles of varying size (10–100 µm in diameter) with very little contamination by cell membranes.

The binding of $^{109}Cd\ Cl_2$ or $^{54}Mn\ Cl_2$ was assayed in a 1-ml incubation volume containing 3.5% saline, 0.3–1.2 mg concretions and 0.1–0.4 µCi of the radioactive metal in the presence of various amounts of unlabelled $Cd\ Cl_2$ or $Mn\ Cl_2$. Incubation was at 22 °C for 3 h with intermittent shaking. At the end of this period, the mixture was filtered through nitrocellulose filters (0.45 µm pore size). These filters themselves were found to bind virtually no labelled material. The concretions trapped by the filters were then washed 3 times, each time with 4 ml saline. Filter discs were then counted in a γ-counter (Biogamma II, Beckman Instruments). Aliquots of the original isotope were also counted so as to determine the original radioactivity in the incubation mixture. Each assay point presented was derived from the mean of triplicate determinations. Calcium content of the granules was approximately 300 µg/mg as determined by atomic absorption spectroscopy.

The extent of binding of labelled cadmium atoms was reduced in the presence of increasing amounts of the nonradioactive metal (fig.). This indicated a saturable and thus limited number of receptor sites within the concretions. Cadmium binding was also reduced in the presence of added nonradioactive manganese although this competition was less pronounced than that obtained with the homologous non-radioactive metal (fig.). This implied that both cadmium and manganese can bind to the same sites within or on the calcareous concretions. In order to determine whether a homogenous binding site population existed, scatchard plots were constructed[4] for both cadmium and manganese binding. After preliminary surveys over a wide concentration range, the appropriate range measured in detail in the final determination was $10^{-2}M$–$10^{-4}M$. From a regression analysis based on these determinations, a clear linear relation was obtained suggesting a specific and homogenous family of binding sites. The statistical correlation coefficients for cadmium and manganese were 0.98 and 0.93 respectively. Kinetic parameters for this interaction are shown in the table. The maximum number of receptor sites was virtually identical for both metals studied, being around 15 nmoles/mg concretion. Since there was considerable competition between metals (fig.), it is likely that these sites are identical. This is substantiated by the lower dissociation constant of cadmium (table) which may account for the lower ability of manganese to compete with cadmium for absorption to the concretions.



Binding of labelled cadmium to concretions in the presence of various amounts of unlabelled cadmium chloride or manganese chloride. Each value represents the mean of triplicate determinations. Each incubation tube contained 0.56 mg concretions in 1 ml volume. Incubation was for 3 h at 22 °C. The original incubation mixture contained 218,000 dpm/ml. ●, $Cd\ Cl_2$; ○, $Mn\ Cl_2$.

Calcium granules of one form or another are widespread in invertebrate tissues and most likely perform a variety of functions. It is thought that many of these granules perform an excretory function[5] and this may well be the role of these renal concretions in bivalves. It was previously shown that these concretions accumulate metals in vivo[3] and this study demonstrates that this is a property of the concretions per se. The relationship between the soluble metal binding proteins in the kidney and the concretions is unclear and it is not known whether the in vitro binding reported here is a property of the organic or inorganic matrix of the concretions. While it is thought that all invertebrate calcification requires a protein substrate; highly specific sites have been described for absorption of amino acids to minerals devoid of an organic matrix[6].

In either case it seems clear that molluscan renal concretions have the capacity to chelate cytotoxic heavy metal ions. Although the concentration range within which this reaction occurs is much greater than ambient levels in seawater, tissue levels of most metals in bivalves are several orders of magnitude higher than seawater. This is therefore clearly not the mechanism by which bivalves concentrate metals from seawater but is more likely a means of controlling the animal's internal environment. In this way the concretions may provide a means of detoxifying metals by selectively removing them from intracellular solution into an inaccessible and possibly excretable compartment. Furthermore it appears that cadmium, which normally exists in estuarine conditions at much lower concentrations than manganese, binds to these concretions more competitively resulting in the more toxic metal being more efficiently chelated.

1 E.D. Goldberg, V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Scheider and E. Gamble, Envir. Conservation *5*, 101 (1978).
2 N.G. Carmichael, K.S. Squibb and B.A. Fowler, J. Fish. Res.Bd.Can. *39* 1149 (1979).
3 N.G. Carmichael, K.S. Squibb,, D.W. Engel and B.A. Fowler, Comp. Biochem. Physiol. *65A*, 203 (1980).
4 G. Scatchard, Ann.N.Y. Acad.Sci. *51*, 660 (1949).
5 K. Simkiss, Calcium in biological systems; exp. Biol. Symp. *30*, 423 (1976).
6 S.C. Bondy and M.E. Harrington, Science *203*, 1243 (1979).