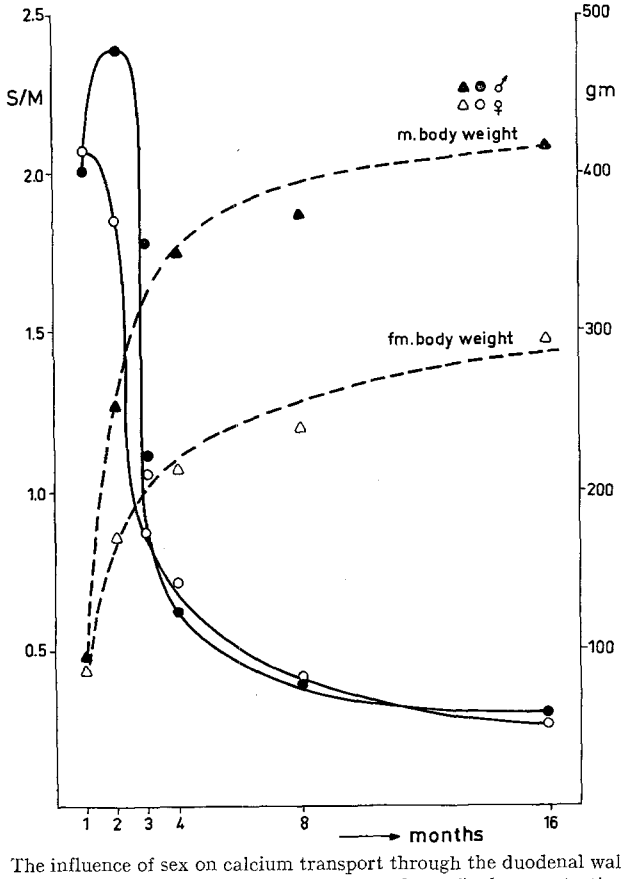
Table II. Mean body weight (± SE) of male and female rats of different age

Age (months)	♀	♂
1	86.00 ± 0.67	93.00 ± 1.33
2	170.50 ± 1.90	226.00 ± 1.94
3	210.00 ± 2.89	356.50 ± 4.22
4	214.00 ± 2.48	348.25 ± 3.56
8	239.25 ± 1.79	374.75 ± 4.78
16	295.75 ± 6.46	418.50 ± 8.15



The influence of sex on calcium transport through the duodenal wal of rats in relation to age. Data are expressed as a final concentration ratio of tracer inside the sac (serosal medium) over that outside the sac (mucosal medium), S/M. Values represent mean standard error. —, S/M ratios; ---, body weight (g).

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the mucosal (M) side of the intestinal segment with a scintillation counter (spectrometer model 3375, Packard Instrument).

Result and discussion. The results (the mean ratio of the serosal to the mucosal content of ^{45}Ca) are presented graphically in the Figure. In the 4-week-old males and females, calcium transport does not differ, but already after 8 weeks of age it is significantly ($p < 0.02$) more intense in the males. The differences in the transport of calcium between the two sexes after the 3rd month are not significant ($p > 0.05$).

The above difference between the 2 curves for S/M ratio vs. age presented in the Figure is believed to be real for the following reasons. Although the S/M data for 4-week- and 2-month-old males are not significantly different, the similarity between the male and female data for the 4-week experiment is corroborated by the body weight which is the same for both sexes at 4 weeks, too (see Figure). While the S/M data for animals older than 3 months do not differ significantly between the sexes, those obtained in the experiments with 2-month-old animals differ quite substantially for the two sexes.

Several causes of this difference between the S/M ratios for the two sexes could be envisaged. There may be some hormonal action upon the wall membrane, or a sex-dependent aging of the intestinum. However, the elucidation of the relative importance of these findings would require additional experiments.

It seems well established that a very efficient age-sensitive control of calcium transport is correlated with the rapid initial growth-phase (see the body-weight curves in the Figure), with a remarkable difference between the sexes. The latter effect seems to be timed very sharply within the life-period from 8 to 10 weeks.

From the practical point of view, regarding the methodology in experiments involving calcium transport in animals 8- to 10-week-old, it seems to be of prime importance 1. to separate the animals by sex and 2. to have the age under strict control.

Zusammenfassung. Nachweis, dass bei umgestülpten Duodenalabschnitten junger Ratten (1–16 Monate) nur bei den ca. 8wöchigen männlichen Tieren der ^{45}Ca-Transport wesentlich gesteigert war; die übrigen Altersgruppen zeigten keinen Geschlechtsdimorphismus.

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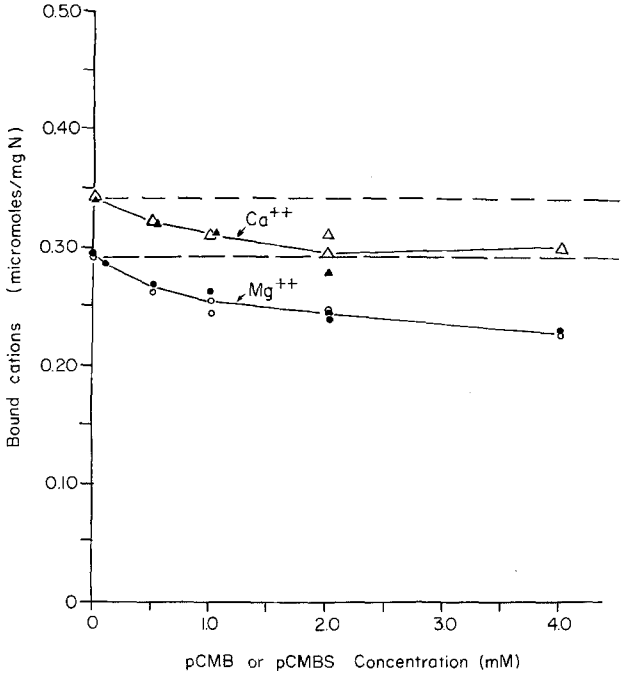
The Effect of Sulfhydryl Reagents on Cation Binding by Membrane Fragments

The concept that sulfhydryl (-SH) reactive agents may affect membrane permeability by displacement of bound divalent cations suggests an important role for membrane -SH groups in cation binding and transport. This possible role is supported by the results of Tolberg et al.[1,2] who reported a close correlation between displacement of tightly bound erythrocyte membrane calcium by the -SH reagent, *p*-chloromercuribenzoate (pCMB), and the resultant increased loss of cellular potassium. Forstner and Manery[3], however, suggested that the observed loss of calcium is explainable in terms of the solubilization of membrane protein by pCMB.

Sulfhydryl reagents have also been reported to affect cation binding by mitochondria[4] and sacroplasmic

reticulum[5], but the interpretation of these results is complicated by the presence of both binding and transport in these systems. Thus, although the effect of -SH reagents on cation binding has been reported for several different membranous preparations, the results are inconclusive. The present report describes the displacement by mercurials of divalent cations bound to rat liver cell microsomal material for which extensive binding data are available and which exhibits cation exchange properties similar to those of erythrocyte ghosts[6,7].

Method. The microsomal fraction was isolated in 0.25 *M* sucrose solution from homogenates of perfused rat liver by differential centrifugation at 0–4 °C. Aliquots of the washed fraction were equilibrated for 12 h at 0–4 °C in media containing approximately 95 m*M* NaCl, 95 m*M* KCl, 2.0 m*M* $MgCl_2$ and 1.8 m*M* $CaCl_2$, comparable binding of the 4 cations being obtained in control samples under these conditions at pH 7. In order to study the effects of -SH reagents, different concentrations of $HgCl_2$, pCMB or *p*-chloromercuribenzene sulfonate (pCMBS) were added to the equilibration media. Appropriate adjustments were made to maintain the same equilibrium free cation concentrations in all samples. Following equilibration, samples were centrifuged for 1.5 h at 102,750 *g* and washed twice with 0.25 *M* sucrose solution by resuspension and centrifugation as previously described[6]. Equilibrium cation concentrations were measured in duplicate in all equilibration supernatants, and microsome-associated cations were measured by atomic absorption spectrophotometry in activated oxygen ashed microsomal samples[8]. Total nitrogen was measured by a micro Kjeldahl procedure routinely employed in this laboratory.



Effects of pCMB and pCMBS on magnesium and calcium binding by rat liver cell microsomes. Aliquots of microsomal material were equilibrated for 12 h at 0–4 °C in media containing different concentrations of either pCMB or pCMBS. Equilibrated samples were centrifuged and washed twice with 0.25 *M* sucrose solution. Equilibration supernatants contained 95.6 ± 1.9 m*M* Na^+; 94.3 ± 1.3 m*M* K^+; 2.00 ± 0.05 m*M* Mg^{++}; 1.74 ± 0.04 m*M* Ca^{++} at pH 6.98 ± 0.09 (mean ± S.D.). Each sample contained 7.75 ± 0.08 mg total nitrogen which provided approximately 1 m*M* of cation binding sites in the 10 ml equilibration system. The solid symbols represent data for pCMB and the open symbols data for pCMBS. The curves represent averages of the plotted data for each cation.

Results and discussion. Mercuric chloride produced the most pronounced effect on cation binding, with total cations being reduced by over 70% at 2 m*M* $HgCl_2$. The organic mercurials pCMB and pCMBS both produced smaller but significant decreases in cation binding, the total for 4 cations being decreased 18% at 4 m*M* pCMBS. Of particular interest was the fact that bound magnesium and calcium decreased most markedly up to 1 m*M* organic mercurial concentration and then tended to level off at higher concentrations (Figure). This tendency to level off suggests an effect on a fixed number of specific sites and provides evidence of the greater specificity of action of the organic mercurials as compared to inorganic mercury[9] which produced a 50–60% decrease in bound divalent cations at 2 m*M* $HgCl_2$.

In the present experiments, there was no significant loss of total membrane nitrogen in the presence of up to 4 m*M* pCMB, recoveries ranging from 99.4% to 104.4% of control values. This is in contrast to reports of loss of protein[2,3] amounting to as much as 30 to 40%[10,11] on exposure of erythrocyte membranes to these mercurials. One possible explanation for the difference in behavior is the presence of magnesium in the equilibration media in the present experiments, this cation being reported to reverse the loss of protein produced by mercurials in erythrocyte membranes[11]. Whatever the reason, the decrease in microsomal cations in the present experiments is not associated with a loss of protein and therefore probably represents displacement from binding sites on the microsomes.

There are several possibilities as to the mechanism of action of the mercurials; pCMB and pCMBS could directly displace magnesium or calcium bound to -SH groups. Alternatively, mercaptide formation[9] could produce conformational and/or charge density changes in the membrane, thereby affecting cation binding to other groups indirectly. In view of evidence that organic mercurials are not completely specific for -SH groups[9,12], further investigations are required to elucidate the mechanisms involved in the effects of these reagents on membrane cation binding. Nevertheless, the present study provides evidence of a displacement of approximately 20% of microsome bound divalent cations by pCMB and pCMBS. The effects of pCMB on microsomal calcium are similar to those reported for erythrocyte ghosts[2] in terms of bound calcium displaced and the effective pCMB concentration range. The results demonstrate that pCMB also displaces bound magnesium, and that pCMBS affects

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divalent cation binding in a manner similar to that for pCMB[13].

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Zusammenfassung. Nachweis an Lebermikrosomen der Ratte, dass Sulfhydryl Reagenzien *p*-Chloromercuribenzoat (pCMB) und *p*-Chloromercuribenzol Sulfonat (pCMBS) die Bindung von Magnesium und Calcium ungefähr um 20% vermindern.

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An Ultrastructural Study of the Synaptic Glomeruli in the Intermediolateral Nucleus of the Rat

After the early studies of the intermedio-lateral nucleus (ILN) by conventional light microscopy (see RÉTHELYI[1] for references), major contributions were made by RAMÓN and CAJAL[2] using the Golgi silver impregnation technique and later by DAHLSTRÖM and FUXE[3] by means of fluorescence microscopy. But studies of the ILN at the ultrastructural level appears to be very limited. The ultrastructure of the ILN of the cat has been reported by RÉTHELYI[1] and that of rats after 6-hydroxydopamine induced experimental degeneration by WONG and TAN[4]. It was during the latter study that the axons and dendrites in the ILN of rat were noted to be organized into large numbers of synaptic complexes. This paper describes the organization of these complexes and discusses the significance of the findings.

Male adult albino rats which were apparently healthy and weighing between 200–250 g were anaesthetized with ether and perfused through the left cardiac ventricle with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer[4]. The fixative also contained 0.5 mg/l $CaCl_2$. After 20 min of perfusion, the thoracic cord was dissected out and thin slices were cut with razor blade and immersed in similar fixative for a further 2 h or overnight at 4 °C. The tissues were post-fixed in osmium tetroxide[5] for $1^1/_2$–2 h, after which they were dehydrated in a graded series of acetone and embedded in Araldite. Semi-thin sections of 0.5 µm thick were cut with glass knives and stained with methylene blue. The ILN was identified and the block was retrimmed. Ultra-thin sections of silver-gray interference colours were cut in the frontal plane with a Porter-Blum ultramicrotome and double-stained on grid with uranyl acetate[6] and lead citrate[7]. The sections were viewed through an Hitachi HS-8 electron microscope.

The axon terminals in the ILN of rat appear as large, medium sized or small boutons; they may contain either round, flattened, ellipsoidal or pleomorphic vesicles.

Large boutons are observed to contain only round vesicles (LR boutons). Large boutons with flattened vesicles have so far not been observed to be present. Medium-sized and small boutons, however, may contain either round or flattened vesicles. Some of these profiles with round vesicles may possibly be part of an LR bouton.

The LR boutons are usually invaginated and contacted by dendritic shafts and spines or small axon terminals (Figure 1). Their vesicles, which are spherical and agranular, are distributed throughout the bouton but are more closely packed adjacent to the presynaptic membrane. Large granular vesicles (LGV) have not been observed in the LR boutons. Sometimes, however, the contour of the LR bouton may be more regular and not so heavily indented by postsynaptic profiles (Figure 5).

The medium-sized (Figures 2 and 4) and small boutons (Figures 2 and 4) have more regular contours and may be somewhat rounded, oval or crescent in shape. Their vesicles may be round or flattened. Some of these terminals may contain a few LGV (Figure 3).

The LR boutons form Gray Type 1 synapses[8] with a primary dendrite or with a dendritic spine (Figure 1). No spine apparatus, however, has been observed in the latter. In addition, the LR boutons are often contacted by small axonal profiles forming axo-axonal synapses (Figures 1 and 2). When such synapses are present, the smaller profiles are always presynaptic to the LR bouton, while the latter is in turn presynaptic to a dendrite. The small presynaptic axon terminal usually contains round vesicles (Figures 1 and 2).

The neural elements which establish synaptic contact with each other are often enclosed in a prominent glial lamina (Figures 1, 2, 4 and 5) to form synaptic glomeruli. The number of elements in each glomerulus varies. These glomeruli may be tentatively classified into 3 types. Type 1 (Figure 1), in which there is a prominent central LR bouton surrounded and contacted by dendrites and small axon terminals. Type 2 (Figure 4), in which the prominent central profile is a large dendrite surrounded and contacted by medium-sized and small axon terminals. Sometimes other dendritic profiles may lie adjacent to the large dendrite, but no synapse has been observed between them although desmosomal types of contact may be present. Type 3 (Figure 5), in which the central profiles consist of an LR bouton contacted by a large dendrite. They are surrounded and contacted by other axons and dendrites.

The present study has shown that glial encapsulated synaptic glomeruli constitute a prominent feature in the ILN of rat, although RÉTHELYI[1] did not describe them in the cat. Such synaptic complexes are now a well-established feature in many areas of the central nervous system e.g. lateral geniculate nucleus[9,10], medial geniculate nucleus[11,12], pulvinar[13,14], ventrobasal nucleus of the thalamus[15,16], cuneate nucleus[17], nucleus gracilis[18], and

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