Discussion. Fatty degeneration of the viscera is a pathological abnormality of Reye-Morgan-Baral syndrome[4,5]. Lipid droplets have been reported in the liver, both in the hepatocytes and in the Kupffer cells, in this situation[3]. They have been described as having a low osmiophilic feature suggesting being formed by triglycerides[3]. In the case studied we found lipid droplets in the hepatocytes with the same morphological features, as has been described by others in Reye-Morgan-Baral syndrome. However, some lining cells of the liver sinusoids contained high osmiophilic lipid droplets. Osmiophilic-dense bodies in Reye-Morgan-Baral syndrome were previously observed only in the pericytes, near the cerebral vasculatures[6]. The high osmiophilic nature of these inclusions are generally believed to correspond to unsaturated lipids[7,8]. The different saturation level of the lipidic content of the droplets observed in the liver cells can be related with the increased mobilization of fatty acids in Reye-Morgan-Baral syndrome[8], and with a possible different response of the hepatocytes and of the lining cells of liver sinusoids to excessive circulating lipids. The removal of circulating lipids may be one of the mechanisms involved in the formation of osmiophilic-dense bodies in the lining cells of hepatic sinusoids, as it is assumed that these cells have a high endocytic activity[9,10].

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Effect of ionic environment on densities of membrane-associated particles in presynaptic membranes observed in freeze-fractured synaptosomes

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Summary. The effects of high-K and high-Ca in the incubation medium on membrane-associated particles of the presynaptic membranes were examined. There was a marked increase in the density of protoplasmic fracture face after incubation in the high-K or high-Ca medium.

Membrane-associated particles have been demonstrated on cell membranes by the freeze-fracture method[1-3]. These particles have been observed in presynaptic membranes of nerve endings of the central nervous systems in vertebrates[4-8]. These particles seem to consist of protein or glycoprotein[9,10], and especially in synapses, they may be channels for ionic movement across the membrane[7,11-14]. It has also been observed that alteration in pH, temperature[15] or calcium concentration[16,17] can induce translational movement of particles in various membranes. However, the function of membrane-associated particles is still unknown.

In the present study, the effects of potassium and calcium in the incubation medium on membrane-associated particles of the presynaptic membranes were examined. For this, pinched off nerve endings (synaptosomes) isolated from guinea-pig whole brain were incubated in potassium-rich (high-K) or calcium-rich (high-Ca) medium, and then they were fractured through the presynaptic membrane by freeze-fracture technique.

Materials and methods. Guinea-pigs, weighing 250-300 g, were decapitated, the whole brain was rapidly removed, and the synaptosomes were prepared by a slight modification of the procedure of Hajós[18]. Purified synaptosomes were incubated in control medium, or high-K, high-Ca, or Ca-free medium at 30 °C for 30 min. The control medium consisted of 140 mM NaCl, 5 mM KCl, 4 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 10 mM glucose adjusted to pH 7.6 with 20 mM tris-HCl buffer. In the high-K and high-Ca media, part of the NaCl was replaced by an equimolar amount of KCl or $CaCl_2$ to give final concentration of 55 mM high-K and 40 mM high-Ca, respectively. For the Ca-free medium, 1 mM EGTA was used. After incubation, the synaptosomes were recovered by centrifugation at 20,000 × g for 30 min and fixed in 5% glutaraldehyde with 0.1 M cacodylate buffer for 5 h. They were then washed with 0.1 M cacodylate buffer and immersed in 30% glycerol overnight.

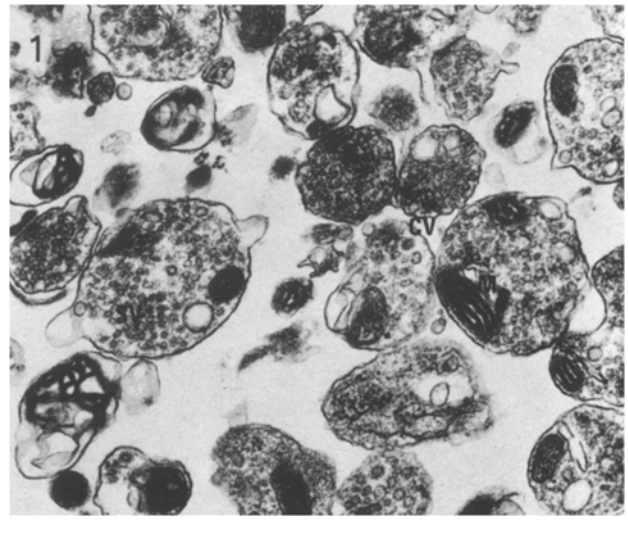

Fig. 1. Conventional thin-section of normal synaptosomes. Many uniformsized synaptic vesicles (SV), some coated vesicles (CV) and mitochondria (M) can be seen in the synaptosomes. × 24,300.

The specimens were quickly frozen in Freon 22, and kept in liquid nitrogen. The frozen specimens were fractured in a JEOL block type freeze-fracture apparatus at -110 to -140 °C under a vacuum of 3×10^{-6} torr. Pt-Pd (platinum-palladium) was then applied to the fractured surface.

For conventional thin-sections, the preparations were fixed as described above, washed with 0.1 M cacodylate buffer, and fixed in 1.5% OsO_4 for 2 h. After dehydration by passage through an ethanol series, specimens were transferred to propylene oxide and finally embedded in epoxy resin.

Results and discussion. In conventional thin-sections of fairly pure preparations, most synaptosomes appeared roughly circular (about 0.7–1.2 µm in diameter) and were generally filled with synaptic vesicles (450–500 Å in diameter), although occasional large dense cored vesicles were seen (figure 1). When precipitated synaptosomes were fractured by the freeze-fracture method, many membrane-associated particles of 90–125 Å diameter were seen on the protoplasmic fracture face (PF-face)[19] of fractured presynaptic membranes, and few on the exoplasmic fracture face (EF-face)[19] (figure 2). Occasionally the synaptosomes were cleaved cross-wise, and in these many synaptic vesicles could be seen as thin-sections.

To study the effects of high-K and high-Ca media on membrane-associated particles, we incubated the synaptosomes in control medium, in Ca-free medium with 1 mM EGTA, and in 55 mM (high-K) and 40 mM (high-Ca) media at 30 °C for 30 min. When synaptosomes incubated in high-K medium were fractured through presynaptic membranes, the density of membrane-associated particles on the PF-face was significantly higher than that in control synaptosomes (figure 2, table). There were very few membrane-associated particles on the EF-face, and their number were not affected by the incubation medium.

Similar results were obtained after incubation in high-Ca medium; the density of particles on the PF-face after incubation in high-Ca medium was significantly more than that after incubation in control medium or Ca-free medium (figure 2, table). Moreover the particles on the PF-face tended to be aggregated in synaptosomes incubated in high-Ca medium. No changes were detected in the particles on the EF-face after incubation in these media.

Our freeze-fractured replicas showed that the density of membrane-associated particles in presynaptic membranes of potassium depolarized synaptosomes from the brain was much higher than that of control synaptosomes (figure 2,

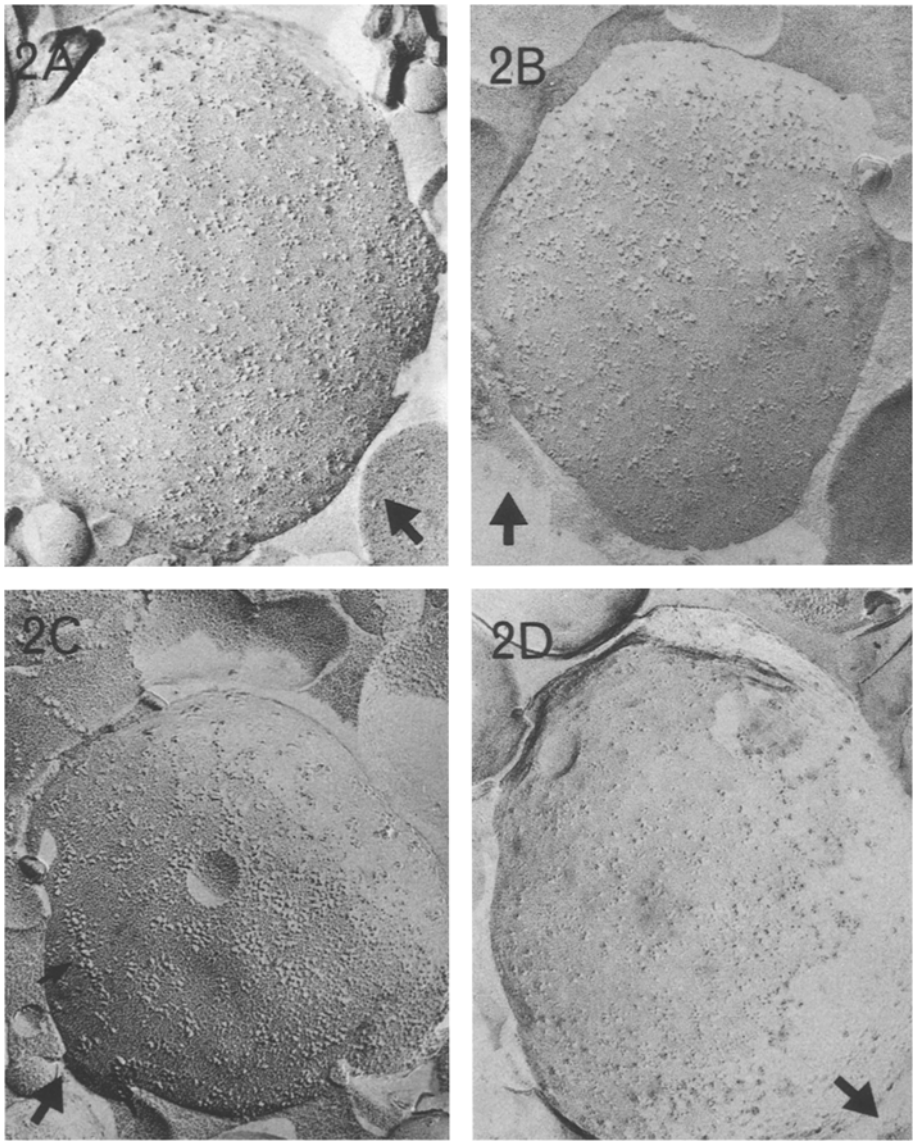

Fig. 2. Differences in distribution of membrane-associated particles in freeze-fractured synaptosomes. *A* Synaptosomes incubated in 55 mM high-K medium; *B* Synaptosomes incubated in control medium; *C* Synaptosomes incubated in 40 mM high-Ca medium. Small arrow shows the aggregation of particles. *D* Synaptosomes incubated in Ca-free medium with 1 mM EGTA. all × 58,000.

Diameter and densities of membrane-associated particles from brain synaptosomes incubated in various media

	Diameter Å ± SD (n)		Densities particles/μm^2 ± SD (N)	
	PF-face	EF-face	PF-face	EF-face
Control (5 mM-K^+, 4 mM-Ca^{2+})	104 ± 19 (66)	99 ± 17 (21)	1145 ± 163 (11)	353 ± 58 (7)
Ca^{2+}-free (5 mM-K^+, 1 mM-EGTA)	104 ± 21 (47)	98 ± 11 (14)	1072 ± 165 (12)	397 ± 52 (10)
High-K^+ (55 mM-K^+, 4 mM-Ca^{2+})	103 ± 17 (92)	101 ± 17 (29)	1493 ± 177 (14)[a,c]	387 ± 78 (15)
High-Ca^{2+} (5 mM-K^+, 40 mM-Ca^{2+})	116 ± 20 (75)	100 ± 22 (27)	1349 ± 192 (11)[b,d]	365 ± 63 (7)

n: number of particles, N: number of fields, significantly different from control [a] $p < 0.001$, [b] $p < 0.05$, significantly different from Ca^{2+}-free, [c] $p < 0.001$, [d] $p < 0.01$.

table). Similary membrane-associated particles in the active zones of presynaptic membranes of rat spinal cord were reported to be more abundant in unanesthetized rats than in the anesthetized rats[7].

The density of particles increased on incubation of the synaptosomes in high-Ca medium. These particles also tended to be aggregated after calcium treatment (figure 2). Calcium-induced aggregation has also been observed in chromaffin granules from bovine adrenal glands[16].

These findings suggest that membrane-associated particles of the presynaptic membranes take part in neurotransmitter release in some way; for instance, as channels for ionic movement across the membrane. Further studies are required on this possibility.

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The 'mauve factor' of schizophrenia and porphyria, 5-hydroxyhaemopyrrole lactam, has low pharmacological potency on guinea-pig ileum

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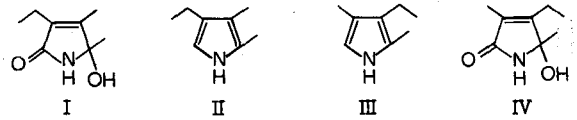
Summary. 5-Hydroxyhaemopyrrole lactam, the 'mauve factor' reported in the urine of schizophrenics and porphyrics was found to inhibit electrically-stimulated contractions of guinea-pig ileum only at high concentrations ($ID_{50} = 8.5$ mM). This low potency makes it unlikely that the compound can account for neurotoxic effects in human porphyria.

Reports of the excretion in urine of schizophrenics[2,3] and porphyrics[4-6] of a 'mauve factor', identified recently as the α-hydroxy-pyrrole-α'-lactam[5,7] (HPL), **I**, of haemopyrrole, 4-ethyl-2,3-dimethylpyrrole, **II**, have led us to examine some biological properties of this compound in an attempt to assess its possible clinical pathological role in these disorders. Whilst krytopyrrole, **III**, the β-side chain isomer of haemopyrrole, originally thought to be the 'mauve factor'[8,9], is highly toxic to mice and has muscle-relaxant[10] and behavioural effects[11], oxygen-free alkylpyrroles, **II** and **III**, cannot be detected in urine[12,13], and because of their high chemical reactivity with water and oxygen are unlikely to occur in other biological fluids. It has been postulated therefore that the lactams may themselves be responsible for any pharmacological actions associated with these pyrroles[5], and 5-hydroxykryptopyrrole lactam, **IV**, has been shown to increase the urinary excretion of porphyrins in rats[14] and to decrease their liver haem and cytochrome P450[15].



I II III IV

In previous studies[16], we compared the effects of kryptopyrrole, **III**, on nerve conduction in rat and on electrically-stimulated contraction of isolated guinea-pig ileum with those of its 5-hydroxy-lactam (KPL), **IV**. The lactam was found to have no effect on nerve conduction at up to 10 mM and to be approximately 100 times less active than its precursor in inhibiting the gut. Although these results made it unlikely that the lactam (KPL) had clinically relevant neurotoxic properties, it was felt necessary to test its β-side chain isomer (HPL) also, in case their structural differences were associated with major differences in their biological properties.

4,5-Dimethyl-3-ethyl-5-hydroxy-3-pyrrolin-2-one (HPL), **I**, was synthesized[17], purified by recrystallization and tested