ate experiments, an increased susceptibility to the lethal effect of radiation was established for a broad series of doses ranging from 790 R to 490 R, i.e. also for moderately lethal and sublethal doses of 580 and 540 R respectively (table 2). For the whole range of doses, the increase in mortality caused by lead acetate was equivalent to an additional X-ray dose of about 100 R. The threshold between partially lethal and nonlethal doses appeared to be decreased to the same extent.

Discussion. It appears that the increased susceptibility to radiation caused by lead acetate can easily be explained in the light of the references cited in the introduction. According to those data, the blood of the irradiated animals would contain bacterial endotoxins[4] and the noxious effects of such endotoxins are enhanced by lead acetate[9,10]. This means that the lead acetate administered a few days following radiation would equally sensitize the animals against the lethal effect of intrinsic bacterial endotoxins originating from the intestine, as it sensitizes them against the lethal effect of extrinsic endotoxins parenterally administered. In accordance with this hypothesis, lead acetate proved to be ineffective in preliminary experiments if applied immediately before or after irradiation, i.e. when no intestinal damage existed.

5-Hydroxytryptamine binding to butanol extracts from myelin fragments

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Summary. The myelin fraction of rat brain stem was treated with butanol-water mixtures, and the extracted proteolipids were separated by Sephadex LH_{20} column chromatography. 2 peaks of proteolipids eluted in chloroform-methanol 4/1 showed the binding capacity for C^{14} · 5-HT. This finding suggests the necessity of the more careful investigations for the probability of proteolipids as receptor proteins in the central nervous system.

Proteolipids are a specialized group of hydrophobic proteins described by Folch-Pi and Lees[1]. They occur in brain and other tissues and have the unusual property of being soluble in chloroform-methanol[2] or butanol-water mixtures[3] but insoluble in water. In the nervous tissues, the highest concentrations of proteolipids occur in white matter, lower concentrations in grey matter and lowest in peripheral nerve[4].

Recently, several workes[5–8] have implicated the proteolipids in the binding of neurotransmitters and drugs to lipid extracts of nervous tissue. Godwin and Sneddon[9] suggested that butanol extracts from rat brain stem have a physiological function as a receptor protein to 5-HT. However, morphological examination of the specimens used there is insufficient, and thus it is uncertain whether the isolated proteolipids originate from the nerve endings. The object of the present paper is to examine the morphological features of the particulate fraction described by Godwin and Sneddon[9] and, moreover, 5-HT binding properties of the proteolipids extracted from myelin fragments will be presented.

Materials and methods. Male Wistar rats (150–200 g) were decapitated and the brains were removed in the cold. The brain stems including hypothalamus, midbrain and medulla oblongata + pons were taken and homogenized in 0.32 M sucrose (10%) with a Teflon-glass homogenizer. The myelin fraction was isolated from the homogenate by the method of Whittaker et al.[10] and examined by electron microscopy. Preparation of the particulate fraction was based on the method of Godwin and Sneddon[9], i.e., the brain stems were homogenized in 20 vol. of water and the homogenate was centrifuged at $30,000 \times g$ for 15 min. Both pellets of the myelin and particulate fraction were resuspended in 50% sucrose (2 ml/g brain stem) and extracted with 10 vol. of water-saturated butanol for 2 h at room temperature. The extraction mixture was centrifuged at $1000 \times g$ for 20 min and the butanol phase isolated. This was concentrated under N_2 at 38 °C to about one-third of its original volume (TE). Aliquots of each TE (4 ml) were treated with water (14%, v/v) to dissolve the insoluble materials, and a 3 ml sample was incubated at room temperature for 20 min with 5×10^{-7} M of C^{14} · 5-HT (48.54 mCi/mmol). After incubation, the mixtures were loaded onto a Sephadex LH_{20} column (2×30 cm). Stepwise elution was carried out with solvents of increasing polarity: 100 ml chloroform, 50 ml each of chloroform-methanol (CM) 15/1, 10/1 and 6/1, and then 120 ml of CM 4/1. Protein contents of the TE and collected fractions were assayed using the method of Lees and Paxman[11]. Lipid phosphorus was assayed by the method of Chen et al.[12]. Radioactivity of the collected fractions was counted in a tT-21 emulsion phosphor[13]. Morphological examination of the myelin and particulate fraction was performed by a Hitachi HU-12A electron microscope. The details of electron microscopy have been reported previously[14].

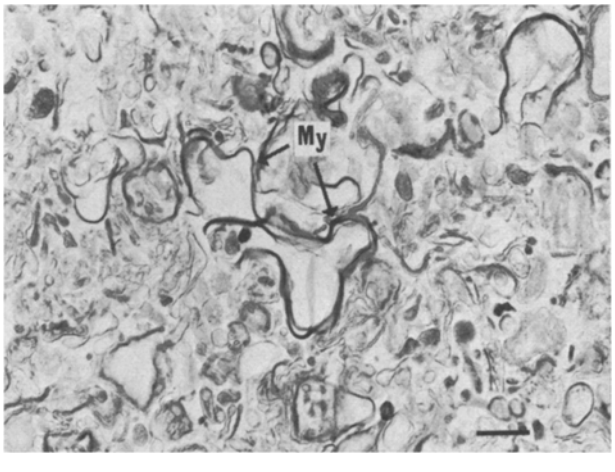

Fig. 1. Electronmicrograph of particulate fraction showing the presence of several myelin fragments (My). Bar equals 0.5 μm.

Results and discussion. For the studying of 5-HT binding to butanol extracts of rat brain stem, Godwin and Sneddon[9] used the particulate fraction instead of the synaptic membranes fractions, and thus it is very easy to imagine the presence of other subcellular organelles in addition to the synaptic membranes. In fact, electron microscopic examinations revealed that the particulate fraction contained lots of myelin fragments (figure 1). Since proteolipids are the main protein component of myelin[15,16], it should be examined whether or not a TE from myelin fraction has the capacity to bind 5-HT.

The chromatographic pattern of a TE from the myelin fraction is shown in figure 2. Initial protein peaks, which accounted for 44.5% of the protein recovered, were eluted with chloroform, together with 72.2% of the lipid phosphorus. Other peaks of protein and phosphorus were eluted with the more polar solvent mixtures, CM 6/1 and 4/1. These chromatographic patterns are in good agreement with those of the particulate fraction described elsewhere[9]. Total recovery of protein and phosphorus from the column were 12.4 and 95.9%, respectively. At present we have no explanation for the lower recovery of protein. The yield of proteolipid from myelin fraction was 2.4 mg/g fresh brain stem.

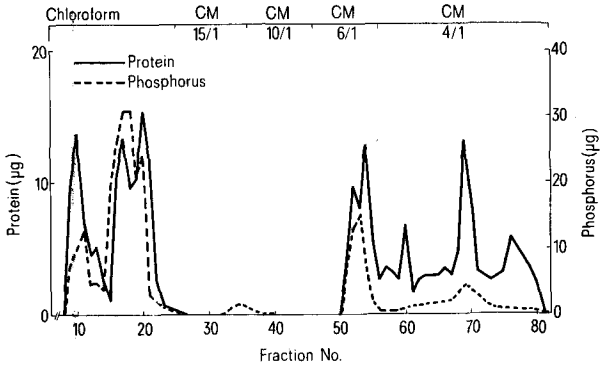

Fig. 2. Elution pattern of protein and phosphorus of a butanol extract (TE) from myelin fraction. TE was passed through a Sephadex LH_{20} column and eluted with the following solvents: 100 ml of chloroform, 50 ml each of chloroform-methanol (CM) 15/1, 10/1 and 6/1, and then 120 ml of CM 4/1. Aliquots of 4 ml were collected in each fraction.

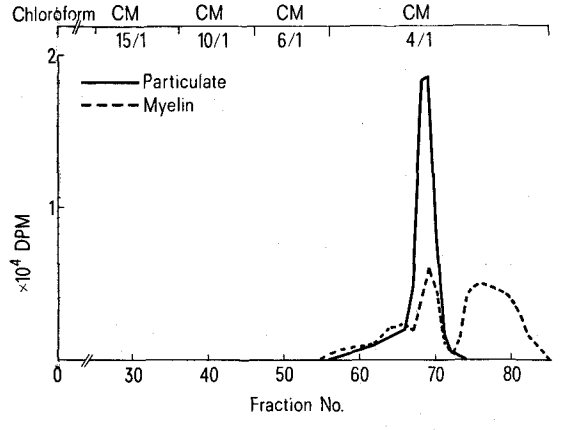

Fig. 3. Elution patterns of radioactivity of butanol extracts from particulate and myelin fraction. Both extracts were incubated with C^{14} · 5-HT (5×10^{-7} M). The discontinuous elution system described in figure 2 was used. Aliquots of 4 ml were collected in each fraction.

The elution patterns of radioactivity which were obtained after incubation of TEs from the particulate and myelin fraction with C^{14} · 5-HT can be seen in figure 3. In the particulate fraction, radioactivity appeared as a single peak at the solvent mixture of CM 4/1. On the other hand, in the myelin fraction 2 peaks of radioactivity were eluted in the same solvent. These 2 peaks of radioactivity coincide with the peaks of protein in CM 4/1. At 5×10^{-7} M of C^{14} · 5-HT, 24.9 nmoles of 5-HT/mg proteolipid of the particulate fraction were bound, and for the myelin fraction 5-HT binding was 23.4 and 102 nmoles, respectively. The recovery of radioactivity from the column was 80% in both cases. As a control experiment, C^{14} · 5-HT was run without proteolipids, in which case a little radioactivity appeared in CM 4/1 (11.7%). However, the remaining portion of radioactivity was still retained on the column until eluted with the methanol. These findings show that the proteolipids extracted from myelin have a binding affinity to 5-HT.

Marchbanks[17] reported that the high affinity binding components for 5-HT were extracted from nerve endings with butanol, but the detailed results were not indicated. In addition, we could not obtain butanol extracts from the synaptic membranes of rat brain stem, which possessed a binding capacity for C^{14} · 5-HT[18]. In a butanol-water extraction from cat brain, Fiszer and De Robertis[19] indicated the existence of the proteolipids which represent a 5-HT binding affinity. However, their materials were also the total particulate fraction prepared by a centrifugation of $10^5\times$g for 30 min. These findings show the necessity of more careful investigations for the probability of the proteolipids isolated from central nervous system as receptor proteins.

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