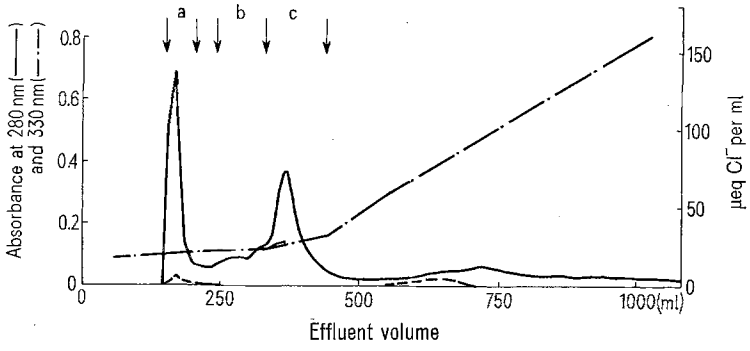

Fig. 3. Purification of the low molecular weight protein of band 4 on DEAE-cellulose column. 100 mg of protein was applied. Retinol concentrations were determined by the absorbance at 330 nm.

column (2.5 × 41 cm) with a gradient consisted of 0.01 *M* phosphate buffer, pH 7.5, and the same buffer containing 0.2 *M* NaCl. Protein concentration of fractions was estimated by measuring the absorbance at 280 nm. Disc polyacrylamide gel electrophoresis was carried out according to the method of ORNSTEIN[14] and DAVIS[15]. The samples were hydrolyzed in 6*N* HCl at 110 °C for 24 h. Amino acid analyses of hydrolysates were carried out bay the method of SPACKMAN et al.[16].

Results and discussion. Crude protein fraction precipitated by ammonium sulfate from the urine of patients was applied on Sephadex G-100 column. Figure 1 shows a typical elution pattern. Each fraction was desalted and freeze-dried. As shown in Figure 2, proteins of low molecular weight fractions in Figure 1 migrated as characteristic bands having the same mobilities on disc electrophoresis as those shown in the original urine; Albumin of band 2 was contained in fraction C, the component of band 3 mostly in fraction E, and that of band 4 mainly in fraction F but also partly in fraction E, respectively. It was estimated that fraction E contained mainly retinol-binding protein as suggested by NOMIYAMA et al.[13].

Furthermore, in order to isolate the protein of band 4, the proteins of fraction F were separated by DEAE-cellulose column. As shown in Figure 3, two major protein peaks, a) and c), were isolated. Purity of these peaks was checked by disc electrophoresis. As shown in Figure 2, peak c) migrated as a single band 4, while peak a) was still heterogenous. The amino acid compositions of the protein isolated as fraction Fc are presented in the Table. The results indicate a composition almost identical with β_2-microglobulin reported by BERGGÅRD and BEARN[5].

Thus it has been found that β_2-microglobulin is excreted remarkably in the urine of patients with Itai-itai disease and that the protein is one of the main components in proteinuria of the disease. 2 distinguishable retinol-binding proteins giving the same mobility of disc electrophoresis as that of band 3, have also been isolated preliminarily from the same urine. We might expect from these findings on urinary low molecular weight proteins that proteinuria of Itai-itai disease is similar to tubular one of chronic cadmium poisoning. However, further work on urinary levels of these proteins in this disease is required to explain the significance of this similarity.

Zusammenfassung. Im 24-h-Urin von Frauen mit der seltenen Itai-itai-Krankheit wurde das gleiche β_2-Mikroglobulin nachgewiesen, das auch bei chronischer Cadmium-Intoxikation ausgeschieden wird.

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Interference with Priming for Audiogenic Seizures by Ether and Prepriming Stimulation

Several research works have shown that audiogenic seizure susceptibility may be induced in normally seizure resistant mice by exposing (or priming) them to the sound of an electric bell during a sensitive period[1–4]. This phenomenon has been termed priming or sensitization. The effect of priming appears to be very stable. For example, it has been shown that general anesthetics, electroconvulsive shock, food deprivation, anticonvulsant agents, severe hypothermia, interference with brain protein synthesis, and drugs known to alter levels of biogenic amines in mouse brain were all ineffective in preventing priming process[4–6]. FULLER and COLLINS[2,7] have shown that onset of convulsibility can be delayed by repeated postpriming stimulation, however, it was later found that this was not due to disruption of the sensitization process but was rather a form of proactive inhibition of convulsibility[8]. This report describes a different procedure of altering the effect of priming. It was found that auditory stimulation prior to effective priming, particularly when mice were anesthetized with ether, could reduced the effect of priming.

Twenty-four BALB/c mice were exposed to the sound of an electric bell (sound level at about 97–99 db relative to 0.0002 dyne/cm^2) in a test chamber for 2 min, half at 14 days of age and half at 21 days of age. They were then tested for convulsibility at a 7-day interval with the same bell ringing for 2 min or until convulsions occurred. None of the mice in the 14-day group convulsed when tested at 21 days of age, thus giving every mouse in this group another exposure (or priming) at this age level known to be sensitive to priming[9]. When tested at 28 days of age, 5 of the 12 mice in this group convulsed whereas 11 of the 12

mice in the 21-group did so ($p < 0.025$), indicating that auditory stimulation at an insensitive period (i.e., 14 days of age) had somewhat reduced the effect of priming at 21 days.

Since it is known that anesthesia does not prevent sensitization, a second experiment was run to determine if this protective effect could be obtained when mice were anesthetized during prepriming auditory exposure. Negative outcome would suggest that conscious state during exposure was necessary. 39 BALB/c mice were exposed to the sound of the bell (sound level at 98–100 db) at 14 days (30 mice) or 21 days (9 mice) of age for 2 min and were then tested for seizures at a 7-day interval. Half of the mice in the 14-day group were ether anesthetized immediately before being exposed to the ringing bell (Group 14A) while the other half were not (Group 14NA). 1 mouse in Group 14NA convulsed on the very first exposure and was thus excluded; none of the remaining 14 mice convulsed when reexposed to the same sound at 21 days of age. 2 of the 15 mice in Group 14A convulsed (1 of them died) on test at 21 days of age. It is of interest to note that these 2 mice were deeply anesthetized during the prepriming exposure as too much ether was used on that day. All the other mice began to move around 4–5 min after application of ether but these 2 mice took more than 15 min to recover.

When tested at 28 days of age, 8 of the 9 mice (89%) in Group 21 convulsed but only 3 of the 13 mice (23%) in Group 14A and 8 of the 14 mice (57%) in Group 14NA did so. The difference in incidence of convulsions between Group 14A and Group 21 was significant statistically ($p < 0.005$), but the difference between Group 14NA and Group 21 did not attain the conventional level of significance ($p = 0.124$), probably due to the small sample size of Group 21. Thus prepriming auditory stimulation under ether anesthesia at 14 days of age appears to have a very strong interference effect on the priming process at 21 days of age. Since there was an interval of 14 days between the original exposure and testing, it is very unlikely that the reduced seizure rate of Group 14A was due to the residual effect of ether itself. Split litter technique was used throughout. Thus the observed effect could not be attributed to the uncontrolled environmental factors such as early rearing conditions, systematic change in the tonal characteristics of the sound.

Ether anesthesia is not the primary factor for the preventive effect. Nine 14-day old BALB/c mice were exposed to the loud sound for 2 min when anesthetized while the other 9 mice were anesthetized but sham exposed. At 21 days of age, all mice were again exposed to the same loud sound for 2 min during which none of them showed seizure reactions. When tested for seizures at 28 days of age, all mice in the sham exposed group convulsed but only 2 of the 9 mice in the exposed group did so ($p < 0.005$), indicating that ether anesthesia alone is not the main factor for the preventive effect. This finding strongly suggests that prepriming auditory stimulation is a necessary condition for the protective effect to occur and that ether may just serve to potentiate the action of prepriming stimulation.

It is possible that exposure to the intense sound at 14 days of age may cause physical damage to the receptor system impairing the sensitivity of hearing, and consequently reduced the effect of priming. However, the following finding suggested that this might not be the case. 20 BALB/c mice were first exposed to a 108–110-db sound at 14 days and again at 21 days of age for 2 min (Group 14), while the other 20 mice were sham exposed at 14 days but were exposed to the same sound for the same duration at 21 days of age (Group 21). 3 mice in Group 14 convulsed on the second exposure to the loud sound and were thus excluded. When tested for audiogenic seizures at 28 days of age, 89% of the remaining 17 mice in Group 14 and 90% of the 20 mice in Group 21 convulsed, suggesting that the effect of prepriming stimulation was a negative function of the sound intensity used. A similar result was obtained even when mice were anesthetized during exposure at 14 days of age. 30 BALB/c mice were exposed to the 108–110-db sound first at 14 days of age and again at 21 days for 2 min, half being ether anesthetized during the first exposure. When test for seizures at 28 days of age, 13 of the 15 mice in the anesthetized group and 14 of the 15 mice in the non-anesthetized group convulsed. Thus the prepriming effect could not be due to the impairment of hearing sensitivity itself, as the higher intensity sound was expected to cause severer damages to the receptor and thus increase the magnitude of the interference effect. One possible hypothesis is that prepriming stimulation may affect the animal in such a way that it facilitates development of an active inhibitory process on the second exposure resulting in reduction of seizure rate on subsequent test.

These results clearly show that the reactions of a mouse to an intense noise depend not only on whether it has been exposed to this stimulus or not, but also on when and what physiological state this stimulation occurred. The intensity of the noise was also found to be very important Priming and prepriming procedure may provide a useful experimental model for studying the dynamic effect of early stimulation on the development of the function of the auditory system[10].

Résumé. Une crise audiogène peut être provoquée chez les souris BALB/c génétiquement non influencées agées de 21 jours, en les exposant à un bruit fort (sensibilisation). Cependant, cet effect de sensibilisation pourrait être considérablement réduit si les souris préalablement anesthésiées à l'éther, sont d'abord exposées au même bruit à l'âge de 14 jours.

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