2-methyl fluorohydrocortisone, a potent Na-K active steroid in high concentration, did not alter the measured currents in squid axon. Similarly, carbohydrate-active steroids significantly alter the manner by which nerve conduction takes place in man[1,5]. In patients in whom excessive endogenous secretion of carbohydrate-active steroids occur, e.g., in patients with Cushing's syndrome or with an adrenal cortical adenoma or carcinoma, peripheral nerve conduction velocity is commonly slower than normal[10]. However, a potent carbohydrate-active steroid in high concentration did not alter the measured currents in squid axon. In man and in animals in the absence of carbohydrate active steroids and in the presence of high concentrations of ACTH peripheral conduction velocity is commonly faster than normal[1,5]. However, ACTH in high concentration did not alter the measured currents in squid axon.

In order to carry out these experiments in squid axon the hormones employed had to be administered at low temperature and over relatively short time intervals. These factors could contribute to the lack of effects observed. Similarly, one may argue that the Schwann cell layer surrounding the axon could act as a barrier for these compounds and that the 10 to 30 min. time period over which they were present in the external solution might not be expected to produce any effect. However, diphenylhydantoin[11] and tetrodotoxin[12,13], substances of similar molecular weight and size to those used in the present study do alter currents in the squid giant axon within this time period. Hence, it does not appear likely that the ineffectiveness of these drugs is due to a significant Schwann cell barrier.

Since the present experiments were carried out over short time intervals any changes produced by these hormones in the Na-K pumping mechanism would not have had sufficient time for the effects to produce any change in the ionic gradients. This can be demonstrated by the results shown in both Figures 1 and 2, in that the potential at which the Na current is equal to zero is the same indicating that the Na-ionic gradient is not changed during the experiment. In other experiments, however, these hormones could possibly effect the Na-K ionic gradients thereby altering the driving force and producing subsequent changes in conduction velocity[5,6]. The present results indicate only that adrenocorticosteroids, with or without ACTH, do not alter the ionic currents responsible for the action potential.

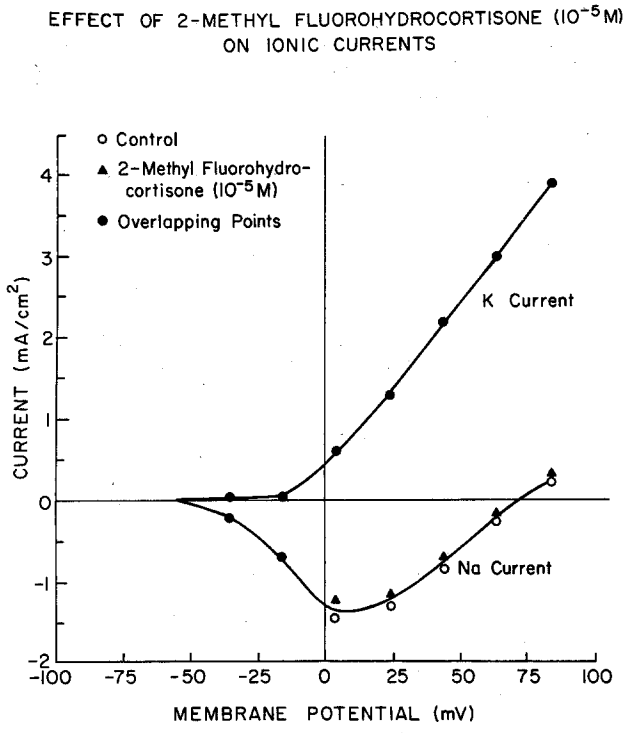

Fig. 2. Effect of 2-methyl fluorohydrocortisone on Na-K currents of squid giant axon.

Zusammenfassung. Adrenocorticosteroide und Adrenocorticotropin bewirkten in hohen Konzentrationen keine Änderungen der Na-K Ströme in Tintenfischriesenaxon; das weist darauf hin, dass die Ionenbegebenheiten, die das Axenpotential hervorrufen, durch diese Hormone nicht beeinflusst sind.

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Isolation of β_2-Microglobulin from the Urine of Patients With Itai-Itai (Ouch-Ouch) Disease

Several investigations have shown that chronic cadmium intoxication in man causes proteinuria with low molecular weight proteins[1-4]. Recently BERGGÅRD and his coworkers have isolated some low molecular weight proteins, such as β_2-microglobulin[5], free light chains[6] and retinol-binding protein[7], from the urine of patients with chronic cadmium poisoning. Moreover, the excess excretion of β_2-microglobulin[8] and free light chains[9] has been characterized in renal tubular damage with tubular proteinuria resulting from chronic cadmium poisoning.

Meanwhile, it has been considered that Itai-itai (Ouch-ouch) disease, of which main symptoms are a kind of osteomalasia and proteinuria with low molecular proteins, is caused by chronic cadmium exposure[10-12] weight. NOMIYAMA et al.[13] have suggested the presence of retinalbinding protein and β_2-microglobulin as the main

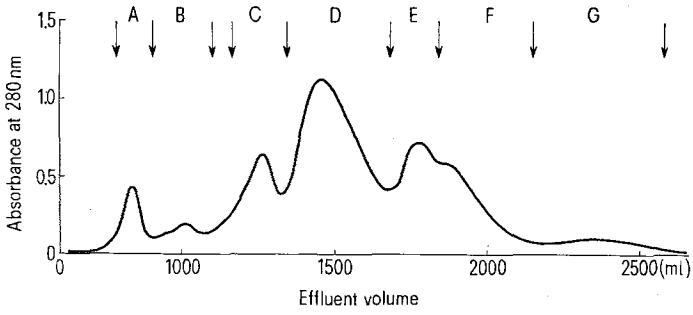

Fig. 1. Gel filtration on Sephadex G-100 of urinary protein precipitated by ammonium sulfate. The column was equilibrated with 0.02 *M Tris*-HCl buffer, pH 8.0, containing 1.0 *M* NaCl. The applied sample contained 638 mg of protein.

components in proteinuria of Itai-itai disease. The present study shows that β_2-microglobulin can be isolated from the urine of patients with this disease and that it is characterized as one of the main components in the proteinuria.

Amino acid composition of fraction Fc isolated from the urine of Itai-itai disease

Amino acid	To nearest integer Fc[a] (residues/molecule)	β_2-microglobulin[b]
Lysine	8	8
Histidine	4	4
Ammonia	11	9
Arginine	5	5
Aspartic acid	12–13	12
Threonine	5	5
Serine	8	10
Glutamic acid	11–12	11
Proline	3	5
Glysine	3–4	3
Alanine	2–3	2
Half-cystine	trace	2
Valine	6–7	7
Methionine	1	1
Isoleucine	4–5	5
Leucine	7	7
Tyrosine	5–6	6
Phenylalanine	5	5
Tryptophan	N.D.[c]	2

[a] All tables are values from 24 h hydrolysis. [b] Values by BERGGÅRD and BEARN[5]. [c] Not determined.

Materials and methods. Twenty-four-h urine specimens were collected from female patients with Itai-itai disease. Urinary protein was determined by the biuret method according to Piscartor's procedure[2]. Urinary protein varied between 0.7 to 1.4 g per 24 h. The protein was precipitated by 50 w/v% ammonium sulfate and dissolved with distilled water. After centrifugation at 12,000 $\times g$ for 1 h, the supernatant was concentrated with the ultrafiltration use of Diafilter G-0.5T (ULVAC Corp.) The specimen was gel-filtrated on Sephadex G-100 column (5.0 $\times$ 131 cm) with 0.1 *M Tris*-HCl buffer, pH 8.0, containing 1.0 *M* NaCl. Subsequently, low molecular fractions were concentrated with the ultrafiltration by use of Diaflo UM-2 (Amicon) were separated on DEAE-cellulose

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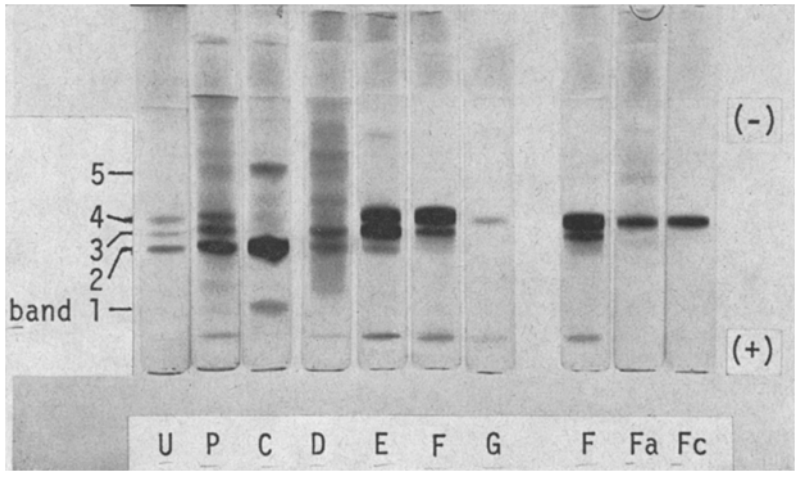

Fig. 2. Disc electrophoresis of low molecular weight fractions. U, original urine; P, protein fraction precipitated by ammonium sulfate; C–G, pooled fractions after gel filtration; Fa and Fc, pooled fractions after DEAE-cellulose chromatography.

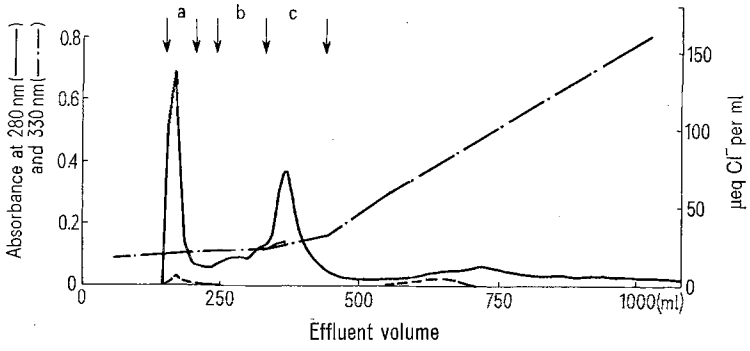

Fig. 3. Purification of the low molecular weight protein of band 4 on DEAE-cellulose column. 100 mg of protein was applied. Retinol concentrations were determined by the absorbance at 330 nm.

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