onset (fig. 1C). The ERP amplitudes and latencies were similar in the hemisphere ipsilateral to the preferred forepaw. The local character of the dentate ERPs was confirmed by coagulation of the area of recording (anodal current, 1 mA, 20 sec). Dentate ERPs recorded 24 h after the electrolytic lesion lost the movement-related phasic components and resembled the attenuated cortical ERPs (fig. 1D).

Caudate ERPs were recorded in 8 rats. The average responses were more variable than in the cerebellum. Figure 2 shows the 4 most frequently encountered waveforms. The most prominent and consistently present component of the ERP was a negative wave (-67 ± 8 μV, $n=15$) culminating between 60 and 120 msec after reach onset. This was preceded by smaller and less regular positivity, ($+34\pm12$ μV, $n=14$), coinciding with forepaw extension. A small negative wave (about 30 μV) appeared at half of the recording sites 100–50 msec before reach detection. Later ERP components were well expressed in some rats but their irregular latencies reflected individual variability of movement duration. There were no systematic differences in the ERPs in the hemisphere ipsilateral and contralateral to the preferred forepaw.

In comparison with the reaching-triggered ERPs in the motor cortex of rats[4] the caudate ERPs were of opposite polarity and lower amplitude. At the single cell level, caudate neurons are inhibited rather than excited during reaching[5]. The positive wave corresponds to the maximum incidence of inhibitory reactions in the caudate nucleus, the subsequent negative wave to fast decay of this inhibition.

Dentate neurons react to reaching predominantly with excitation[6], the peak of which corresponds to ERP negativity. The subsequent ERP positivity coincides with termination of the excitatory reaction in most neurons. Comparison of the neuronal population response with the ERP agrees with the conventional assumption that excitation is reflected by negativity and inhibition by positivity in the monopolar macroelectrode recordings.

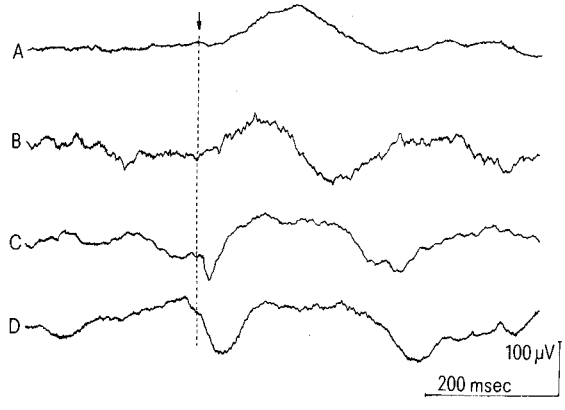

Figure 2. Examples of reach-related potentials from the contralateral caudate nucleus. Reach detection indicated by arrow. Averages of 32 response. Calibration: 200 msec, 100 μV. Negativity upward.

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Response of briefly glycerinated smooth muscle to Ca^{2+} and Mg^{2+}

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Summary. Smooth muscle, treated with 50% glycerol solution at 27 °C for 20 min, contracted on the application of Ca^{2+} or Mg^{2+}. The briefly glycerinated smooth muscle can be used as a model system of smooth muscle contraction.

Recently, it has been suggested by many investigators that the Ca^{2+}-regulatory contractile mechanism in smooth muscle is different from that in skeletal muscle[1-6]. We have shown that glycerinated smooth muscle contracted almost maximally when exposed to 15 mM Mg^{2+} and 5 mM ATP in the absence of Ca^{2+}, while glycerinated skeletal muscle needed Ca^{2+} with 15 mM Mg^{2+} and 5 mM ATP to contract[7]. The contraction of the smooth muscle without Ca^{2+} appeared slowly and was Mg^{2+}-dependent[8]. These data were obtained from muscle glycerinated in the usual way by treatment with 50% glycerol solution containing 60 mM KCl, 4 mM $MgCl_2$ and 15 mM Tris-HCl (pH 6.8) under −20 °C for 1–2 weeks. It is probable that the responses of these glycerinated muscles cannot be exactly compared to those of intact muscles. In the present study, we describe a new method of glycerol treatment, in which the period of glycerination is shortened and the temperature is kept constant at 27 °C.

Materials and methods. Canine intestinal smooth muscle (jejunal portion), free from mucosa, was separated into longitudinal and circular muscles. The circular muscle (about 0.5 mm diameter and about 5 mm length) was suspended in an organ bath (1 ml) containing Tyrode's solution at 27 °C, bubbled with a mixture of 95% O_2 and 5% CO_2. After 1 h the bathing medium was changed to the relaxing solution, the composition of which was: 80 mM KCl, 4 mM ethylene glycol bis (2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 5 mM adenosine tri-

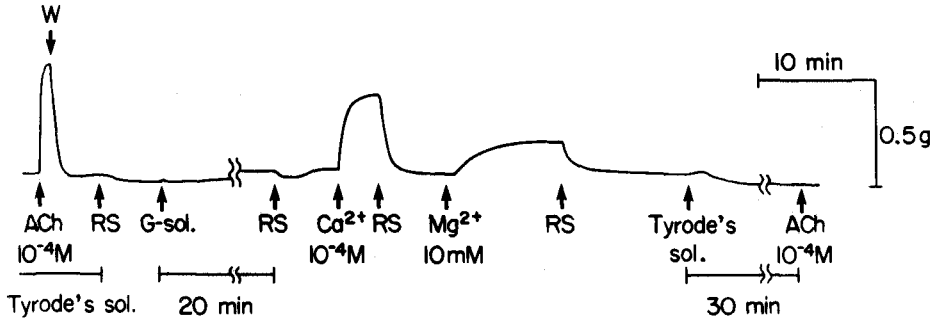

Figure 1. The response of a briefly glycerinated smooth muscle to Ca^{2+} and Mg^{2+}. Bath temperature was kept constant at 27 °C during the experiment. ACh (10^{-4} M) contracted the muscle in Tyrode's solution, bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the solution was (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.6. Tyrode's solution was changed to relaxing solution (RS) without the bubbling of a mixture of 95% O_2 and 5% CO_2. Next, the muscle was treated with glycerol solution (G-sol) for 20 min, and was washed by RS. Application of Ca^{2+} (10^{-4} M free ion by adding 3.98 mM $CaCl_2$ to RS containing 4 mM EGTA) developed a rapid tension, while that of Mg^{2+} (10 mM free ion by adding 15 mM $MgSO_4$ to RS containing 5 mM ATP) developed a slow tension. After changing to Tyrode's solution, the response to ACh (10^{-4} M) was compared with that before glycerol treatment.

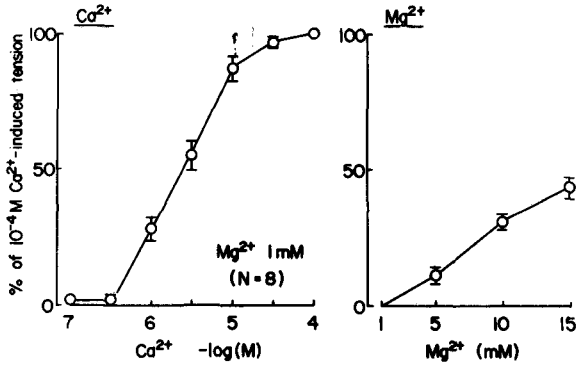

Figure 2. Dose-response relationships for Ca^{2+}-induced and Mg^{2+}-induced tensions. Left: the effect of Ca^{2+} in the presence of 1 mM Mg^{2+}. Right: the effect of Mg^{2+} in the absence of Ca^{2+}. Abscissa: concentration of Ca^{2+} (M) or Mg^{2+} (mM). Ordinate: percent of the contractile response to 10^{-4} M Ca^{2+}. Ca^{2+} and Mg^{2+} concentrations are shown as free ions (see text). Each point represents the mean value from 8 observations with ± SE as a vertical line.

phosphate sodium salts (ATP), 6 mM $MgSO_4$ and 20 mM Trismaleate (pH 6.8). The activating solution was made by adding $CaCl_2$ or $MgSO_4$ to the relaxing solution as shown in figure 1. A variable free Ca^{2+} concentration was obtained by using an EGTA-CaEGTA buffer system[9]. Decrease in pH, which is due to the release of H^+ from EGTA when $CaCl_2$ is added, was adjusted using 10% KOH. Free Mg^{2+} concentration was calculated as the difference between ATP concentration and $MgSO_4$ concentration, assuming that ATP was strongly associated with Mg^{2+}[10]. The bath was stirred with a magnetic stirrer, and the medium exchanged by pouring in an excess of fluid and allowing it to overflow. The glycerol solution was composed of 50% glycerol and the same chemicals as in the relaxing solution. The tension developed by the muscle was recorded isometrically with a force displacement transducer (SB-1T, Nihon Kohden) and a carrier amplifier (RP-3, Nihon Kohden).

Results and discussion. Figure 1 shows the response of a briefly glycerinated smooth muscle to Ca^{2+} and Mg^{2+}, and the comparison of the responses to acetylcholine (ACh) before and after the glycerination. After a glycerol treatment of only 20 min, the smooth muscle contracted on application of Ca^{2+} (10^{-4} M), but not with ACh (10^{-4} M). The Ca^{2+} (10^{-4} M)-induced tension was 70.5 ± 8.36% (n = 8) of the ACh (10^{-4} M)-induced tension obtained before the glycerol treatment. The muscle also contracted on application of Mg^{2+} (10 mM) in the absence of Ca^{2+}. Therefore, these preparations produced by brief glycerination could provide a contractile model for smooth muscle. Skeletal muscles treated with glycerol by the same procedure responded only slightly, or not at all to the addition of Ca^{2+}, indicating that the method was not suitable for producing preparations of skeletal muscle. However, Julian[11] showed that briefly glycerinated skeletal muscle contracted on the addition of Ca^{2+}. The difference of his results from ours may depend on the procedure; Julian reported that the muscle was treated with a detergent in addition to glycerol. Figure 2 shows the Ca^{2+} dose-response and Mg^{2+} dose-response curves of briefly glycerinated smooth muscles. ED_{50} value of Ca^{2+} was 2.8×10^{-6} M (n = 8). This Ca^{2+} dose-response curve is similar to that obtained in other preparations of chemically skinned smooth muscles[12-14]. Ca^{2+}-independent tension was observed by the increase in Mg^{2+} concentration from 1 mM to 15 mM, as similar to previous studies[8]. The Mg^{2+}-dependent, Ca^{2+}-independent tension of the chemically skinned smooth muscle has also been reported by some investigators[12-15]. It is suggested that the Mg^{2+}-dependent tension is a unique property of smooth muscle, because it was not observed in preparations of skeletal muscle[8]. Increase in Mg^{2+} concentration did not produce a maximal tension in the briefly glycerinated smooth muscle (fig. 2). Ca^{2+}-induced tension was always observed in the presence of a high concentration of Mg^{2+}. However, the muscle glycerinated in the usual way scarcely responded to Ca^{2+} after the exposure to a high concentration of Mg^{2+}[8]. These preparations may show an inactivation of the contractile proteins. More recently, it was reported that the contractile response of smooth muscle to a hyperosmotic solution was dependent on Mg^{2+} but not Ca^{2+}[16]. There is a possibility that smooth muscle has 2 contractile mechanisms, one Ca^{2+}-dependent and the other Mg^{2+}-dependent.

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The effect of hexamethonium on the secretion induced by sodium deoxycholate in the rat jejunum[1]

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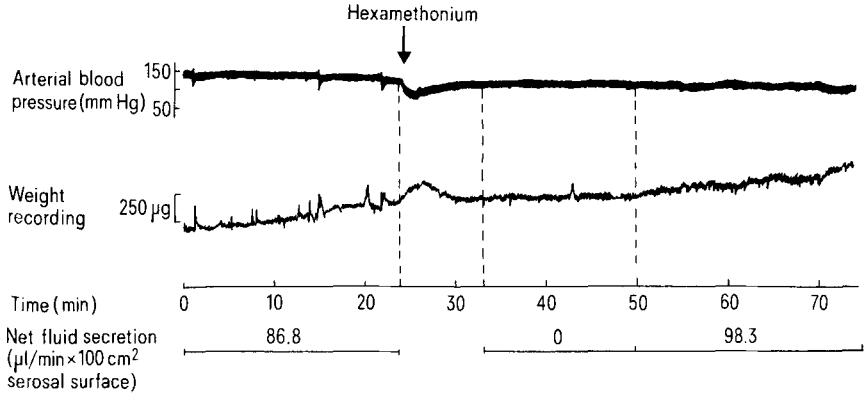
Summary. The i.v. administration of hexamethonium reduces or abolishes net water secretion induced by sodium deoxycholate in the denervated rat jejunum. The findings suggest that a local nervous reflex may be involved in bile salt-induced intestinal secretion.

The mucosa of the gastrointestinal tract is heavily innervated by the 'enteric nervous system'[2], but little is known about its functional importance for the control of intestinal fluid transport. Vasoactive intestinal polypeptide, considered by most authors to be localized only in nervous tissue in the gut[3] and thus probably a transmitter of the enteric nervous system[3], has been found in the stools of cholera patients[4]. Furthermore, Cassuto et al.[5] recently provided experimental evidence for the proposal that cholera toxin induces intestinal secretion in part via intramural nervous reflexes. One of the drugs used by Cassuto and co-workers was hexamethonium, a cholinergic ganglionic blocking agent, which proved to be very efficient in inhibiting choleraic secretion in rats. It was, therefore, considered to be of interest to study if the well-known bile salt-induced intestinal secretion might also be influenced by the administration of hexamethonium.

Methods. Male Sprague-Dawley rats, weighing 250–350 g, were used. Anesthesia was induced with nembutal 50 mg/kg i.p. and maintained by repeated small i.v. injections (5 mg every 2 h). Arterial blood pressure was recorded from the right femoral artery by a pressure transducer (Statham P 23DC). The nerves surrounding the superior mesenteric artery were divided. In all experiments a 10 cm segment of the proximal jejunum with an intact vascular supply was perfused in a recirculating system with a modified Krebs-Henseleit solution containing per l 30 mmoles mannitol and 8 mmoles Na-deoxycholate. The perfusion rate was 0.2 ml/min. Net intestinal transport of fluid was continuously recorded in 4 experiments with the method described by Jodal et al.[7] for the cat, adapted for rats. In the remaining experiments a weight method was used, continuously monitoring weight changes of the intestinal segment. This method made it possible to measure net fluid transport rates in the face of intestinal peristaltic movements[5,8,9]. All recordings were made on a Grass polygraph. Hexamethonium (20 mg/kg) was given i.v. after 30 min of constant intestinal secretion. This hexamethonium dose has in other experiments been shown to abolish the vagal influence on heart rate[9].

Results. During bile salt perfusion, there was a net intestinal secretion in all experiments (n = 8). After giving hexamethonium, the secretion was reduced to about 16% of the initial value (see table). In one experiment the secretion turned into absorption and in 3 other experiments the secretion was totally abolished. The effect lasted for 15–50 min after which secretion gradually returned. A representative experiment is illustrated in the figure.

Discussion. The results of this study indicate a possible nervous reflex involvement in bile acid-induced intestinal secretion by showing that hexamethonium, a cholinergic ganglionic receptor blocker, markedly inhibited the intestinal secretion produced by sodium deoxycholate. The nervous reflex is probably confined to the intestinal wall since the extrinsic nerves of the intestine, surrounding the superior mesenteric artery, had been sectioned in all ex-



The effect of hexamethonium on blood pressure and sodium deoxycholate induced intestinal secretion. Secretion was deduced from the continuous recording of the intestinal weight.