indeed, asphyxia, ischemia and or autolisis, do produce this type of appositions in the brain of the rat[5]. Moreover, these 'labile appositions' can be demonstrated despite the fixative hypertonicity, which indicates that, had the interneuronal appositions we had described[1] developed during the blood-washing period preceding the perfusion of the hypertonic fixative, they should not be expected to reverse because of the fixative hypertonicity. It was therefore important to find out whether or not some interneuronal tight junctions would persist after the perfusion of both a hypertonic washing solution and a hypertonic fixative. Thus, the 12 *M* formaldehyde method of fixation[1] has been applied to young adult normal albino rats, but, in addition to the usual components, the washing solution contained 1.8% NaCl.

At the electron microscope, the ECS ranged from moderately to extremely enlarged, but bouton-bouton attachments were present in all instances. Moreover, it was possible to detect neuron-neuronal quintilinear complexes in which the middle line was thinner than the outer ones (Figure). Such images indicate that neuron-neuronal quintilinear complexes do not necessarily result from mere apposition of contiguous outer leaflets belonging to independent unit membranes.

No hypothesis about the possible functional significance of these axo-axonic close appositions shall be formulated at this moment. It will be, however, mentioned that, considering the conspicuous synaptic vesicles which appear at these complexes, the implication of electrical synapses is not favored.

Zusammenfassung. Eine quintilineare neuronale Verbindung, die nicht durch hypertonische Spül- und Fixationsmittel zu sprengen ist, wird beschrieben.

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Feeding Elicited by Injections of Ca^{++} and Mg^{++} into the Third Ventricle of Sheep

The ionic composition of cerebrospinal fluid plays an important role in the function of central mechanisms involved in behavior[1]. While Ca^{++} and Mg^{++} decrease the excitability of neurons, Na^{+} and K^{+} increase it[1–3], thus regulating neuroconductivity. Ca^{++} injections into the lateral ventricles of rats[4] and perfusions of the ventromedial hypothalamus of cats[5] elicit feeding and often ataxia and a sleep-like conditon. Perfusions of the posterior hypothalamus of cats with excess Na^{+} produce arousal and hyperexcitability[3]. A specific role has been attributed to the Ca^{++}/Na^{+} ratio in the hypothalamus for temperature[6] and energy balance regulation[4] in cats, monkeys and rats. An increased ratio results in hypothermia and feeding, while a decreased ratio results in hyperthermia[6]. Although these responses might be predicted from the known effects of Ca^{++} and Na^{+} on neuro-excitability and the effect on feeding and temperature of CNS active drugs, e.g. barbiturates[7–10], neither Mg^{++} nor K^{+} were effective in these tests, in spite of their similar neural effects[4–6].

In the present experiments our objective was to determine if: 1) 'sated' sheep eat following injections of Ca^{++} into the cerebrospinal fluid (CSF), 2) Mg^{++}, which also decreases neuro-conductivity, elicits feeding, and 3) body temperature changes as a result of injecting Ca^{++} and Mg^{++} into CSF.

Ten sheep (40–45 kg wethers) were surgically implanted with third ventricular guides[11]. For temperature probes, a silastic tube closed at the proximal end was located near the dorsal portion of the liver and held in place with a dacron mesh skirt sewn subcutaneously. The animals were fed ad libitium, the daily ration given 1 h before the injection. Water was available at all times. One half ml of

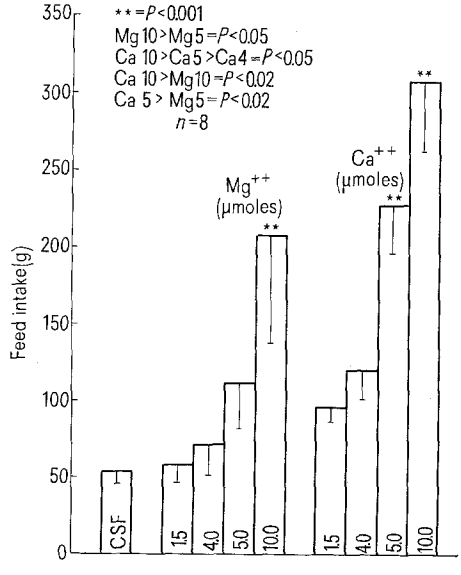

Fig. 1. Average 60 min feed intake of sheep as affected by injections of Ca^{++} and Mg^{++} into the third ventricle. xx = Denotes that those treatments were different from the control.

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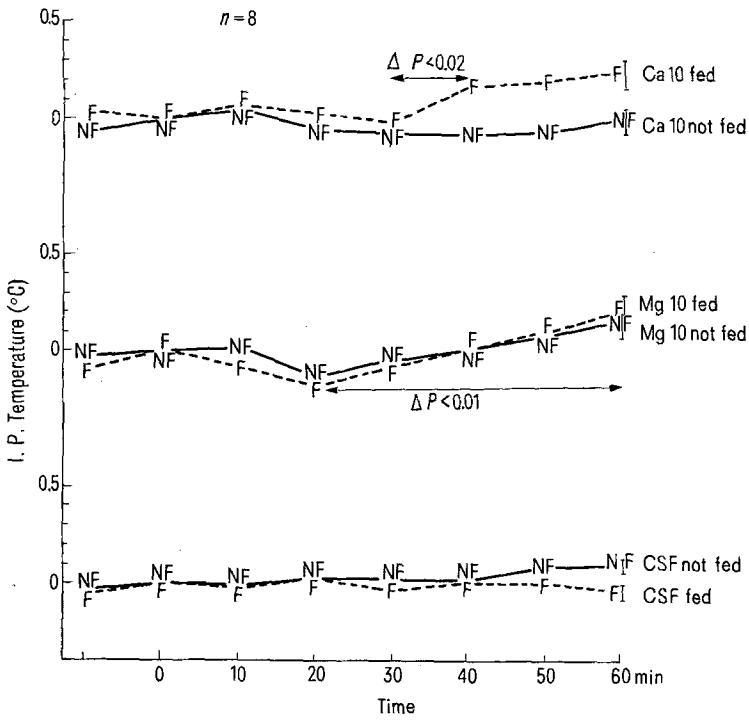

Fig. 2. Effects of injections (10 μmoles) of Ca^{++} and Mg^{++} into the third ventricle of sheep on intraperitoneal (IP) temperature. Not fed = Feed was withheld during 1 h postinjection. Sheep were injected at time 0 min.

either control (synthetic CSF[12]) or test solutions (Mg^{++} and Ca^{++} at doses of 1.5, 4.0, 5.0 and 10.0 μmoles per sheep for each ion) was passed through a millipore filter and injected at a rate of approximately 0.19 ml/min.

Both Ca^{++} and Mg^{++} elicited a marked dose-dependent feeding response, Figure 1. There was a latency of about 2 min after the initiation of injection before feeding occurred. Feeding lasted up to 60 min with the higher doses tested. At all doses, the response elicited by Ca^{++} was larger than that of equimolar doses of Mg^{++}.

Figure 2 shows the effects of 10 μmole injections of Ca^{++} and Mg^{++} on the intraperitoneal temperature of sheep either fed or fasted for 1 h after the injection. An initial trend towards a decrease in temperature was observed after the injection of Mg^{++}. This was followed by an increase ($P < 0.01$) for the period of 20 to 60 min post-injection to values 0.2 °C higher than those at injection time. This change can not be attributed to the feeding response, since the temperature change was similar when feed was withheld from the animals. A small increase ($P < 0.02$) in temperature was observed after the injection of 10 μmoles of Ca^{++} from 30 to 40 min post-injection when the animals had feed available. This change appeared to be related to the feeding elicited, since the temperature was not changed when feed was withheld after the injection. There were no significant temperature changes following injections of synthetic CSF (control). Neither of the ions caused any apparent ataxia in these tests.

The feeding responses observed are possibly explained by the neurodepressant action of Ca^{++} and Mg^{++} on inhibitory fibers acting on the lateral hypothalamus. Known neuro-depressant drugs elicit similar responses when injected into the third ventricle[8]. The larger response due to Ca^{++} may be related to its specific action in norepinephrine release[13], since norepinephrine injected into the medial hypothalamus of sheep also elicits a dose dependent feeding response[14]. The significance of these findings in the control of feed intake is questionable, since there is probably little variation in the ionic concentrations of cerebrospinal fluid[15]. However, injections of as little as 1.5 μmoles of either cation elicited a feeding response within 2 min after the initiation of the injection and lasted for about 15 min (41 and 67 g for Mg^{++} and Ca^{++} respectively versus 18 g for control, $P < 0.10$ and $P < 0.01$). Although the Ca^{++} required to elicit feeding in sheep relative to brain size is probably similar to that required for rats[4], the Mg^{++} required was much less than that shown to be effective in cats, i.e. 5 vs 200 μmoles[16].

From our results it is apparent that the mechanisms by which the hypothalamus controls feed intake are sensitive to small changes in ionic cencentrations. Sheep, unlike monkeys[17] are not hypothermic but remain normothermic following Ca^{++} injections into the CSF. Mg^{++}, however, caused a small decrease in body temperature followed by an increase independent of the feeding response.

Résumé. Une augmentation de la prise de nourriture s'observe après injection cérébro-ventriculaire du Ca^{++} et du Mg^{++} en doses de 1.5 à 10 μmoles, chez les brebis. La température de la cavité abdominale n'en est pas affectée sauf une légère augmentation due probablement de la prise de nourriture.

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