from 2.2 to 8.8 m*M*, the same depolarization now elicited a Ca current of 5.0 μA. In a series of experiments, when these inhibitors were present the Ca current was decreased on average to 30% of its initial control value. These drug effects are also manifested by a change in the current-voltage relationship of the transient Ca current (Figure 2.) Thus it appears that both Compound D 600 and Verapamil strongly depress the Ca conductivity of the membrane. Therefore, during excitation, less Ca ions flow into the myocardial cell and so the force of contraction is weakened. But, even in the presence of the Ca-antagonistic substances, the normal Ca conductivity of the membrane can be restored by the addition of extra Ca. These effects of Verapamil and D 600 may be ascribed to a more or less complete blockade of channels or carrier mechanisms in the membrane, which are essential for the Ca influx during excitation. It is possible that these inhibitory drugs compete with Ca for a common carrier mechanism.

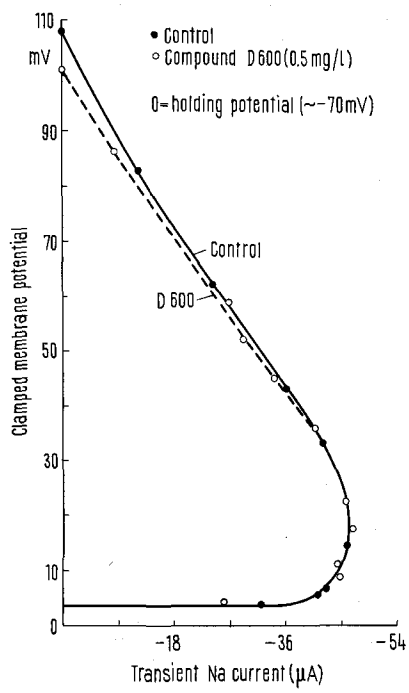

Fig. 3. Current-voltage relationships of the transient Na current. The ordinate shows the clamped (depolarized) potential; O indicates the holding potential (approximately −70 mV). Up to a depolarization of 40 mV the Na conductivity is unchanged by D 600.

The Ca conductivity of the membrane is selectively reduced by Verapamil and D 600. Concurrently the transient Na current is only marginally decreased (in average by 7%). Correspondingly in most experiments there was little change in the current-voltage relationship of the transient Na current in the presence of D 600 (Figure 3) or Verapamil. It should be stressed that D 600 does not affect the Na conductivity in that range (0–40 mV depolarization from the holding potential), within which the transient Na currents responsible for excitation are normally generated. This would account for the observation of FLECKENSTEIN et al.[2] that these Ca-antagonistic drugs do not produce any change in the upstroke velocity of the action potential.

The selective depression of Ca conductivity indicates that during excitation the influxes of Na and Ca are independent of each other. Therefore, as well as the fast Na channel in the membrane, there must also be another channel for Ca. Such a system has already been shown to be present in the excitable membrane of nerves[7,8]. In the mammalian myocardium, the existance of these two separate channels for Na and Ca is of especial functional significance because, thereby, it is possible to change the force of the cardiac contraction without any corresponding alteration in the excitability of the fibre.

Zusammenfassung. Die Ca^{++}-Leitfähigkeit der Myokardfaser-Membran wird durch Verapamil und sein Methoxyderivat D 600 teilweise bis zum vollständigen Verlust reduziert. Dieser Effekt erfolgt selektiv, da die Na^{+}-Leitfähigkeit der Membran unbeeinflusst bleibt. Die während der Erregung der Myokardfaser fliessenden transmembranären Ca^{++}- und Na^{+}-Ströme benutzen demzufolge voneinander unabhängige Membrankanäle.

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Effect of a Magnesium-Deficient Diet on the Striatal Content of Amines in the Dog

The metabolism of dopamine is involved in the pathogenesis of extrapyramidal diseases[1,2]. Despite numerous anatomical and physiological studies delineating a dopaminergic nigro-striatal pathway[3,4], and indicating that damage to this pathway will result clinically in hypokinesia, biochemically in a decreased striatal content of dopamine and of the enzymes necessary for its synthesis, and histologically in a depigmentation of the substantia nigra[5–7], we are still uncertain as to the initiating factor for the dopamine deficit; indeed other studies indicate that the defect is not limited to brain as was originally thought[8–10]. Some years ago disputed results in favour of a magnesium deficiency in Parkinson's disease were presented[11–13] but further progress along this line were limited by methodological difficulties and over-shadowed by the introduction of Levodopa into the therapeutic armentarium[14]. The opportunity to reinvestigate the possible role of magnesium in the dopamine deficit responsible for clinical hypokinesia was obtained during a study of magnesium metabolism as it relates to the thyroid C-cells and the parathyroid gland, carried out in this Institute[15,16].

Experimental. 12 female mongrel dogs, 6 months old, were divided into 2 equal-number groups; Group 1 animals, after an adaptation period, received a 'low magnesium test diet' (Nutritional Biochemical Co. Cleve-

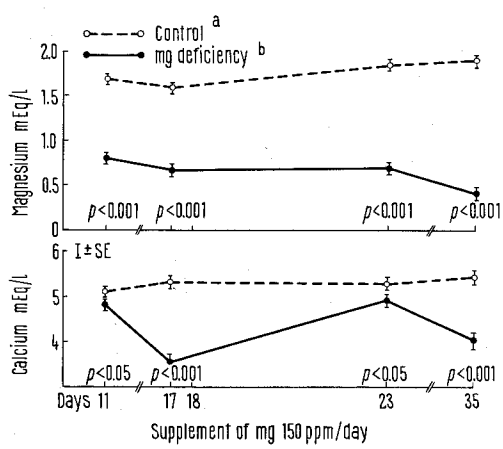
Effect of a low magnesium diet on brain amines and metabolites

	Caudate	Caudate	Caudate	Mid-brain
	Dopamine	Noradrenaline	Serotonin	Homovanilic acid
Control animals	4.93 ± 0.47	0.072 ± 0.01	1.12 ± 0.11	0.114 ± 0.021
Low Mg animals	2.68 ± 0.39	0.057 ± 0.04	1.10 ± 0.11	0.155 ± 0.004
t-value	3.56	0.97	0.06	2.04
p-value	< 0.01	N.S.	N.S.	N.S.

DOGS-μg/g tissue; 6 animals/group (mean ± S.E.M.)

land, Ohio) supplemented with 1,500 ppm of magnesium (500 ppm as $MgCl_2$; 500 ppm as MgO and 500 ppm as $MgSO_4 \cdot 7H_2O$). The test diet was found by analysis in our laboratory to contain 17 ppm Mg; Group 2 (experimental) animals were fed with the same 'low magnesium test diet'. Details of the protocol will be found in previous papers[15,16], and in the Figure. The duration of the whole experiment was 35 days. The diet was administered twice a day by means of a stomach tube. Each animal received 325 g of the diet per day and distilled water 'ad libitum'. Blood samples were collected at the end of the adaptation period and on the 11th, 17th, 23rd, and 35th day of the experiment. At the end of the 35 days the mean weight gain was 2.5 kg in group 1 and 1.0 kg in group 2. Typical magnesium deficiency symptoms similar to those observed in the literature[17,18] were recorded from the second week of the experiment. During the 4th and 5th weeks the animals from Group 2 were markedly hypokinetic and had severe hind limb ataxia. At the end of the experiment, the animals were anaesthetized with Nembutal for sacrifice. The brains were obtained rapidly and immediately kept frozen at −70°C until determination of electrolytes and monoamines with previously described methods[14,16].

Results. The 2 groups were homogeneous with respect to calcium and magnesium blood levels before the experiment. In magnesium-deficient animals, both ions decreased significantly compared to the control group (Figure 1). At the end of the experiment, the animals showed a pronounced hypomagnesemia and hypocalcemia. Sodium concentrations remained essentially unchanged. Serum potassium concentrations decreased significantly during the magnesium deficiency period to values of 3.66 ± S.E. 0.09 mEq./l at the end of the experiment ($p < 0.01$). The electrolyte concentrations in the brain of calcium and magnesium did not change significantly.



Effect of magnesium-deficient diet upon serum magnesium and calcium levels in young dogs. [a] Mg-deficient diet (17 ppm of Mg) supplemented with 1,509 ppm of Mg. [b] Mg deficient diet (17 ppm of Mg).

The analysis of brain amines revealed a significant and specific decrease in caudate dopamine concentration (Table). Other areas studied showed no significant differences and are not reported in the Table. In the caudate, noradrenaline and serotonin values did not change. Homovanillic acid was slightly but not significantly elevated in the mid-brain, while entirely normal elsewhere.

Discussion. One of the major difficulties in early investigations of the individual symptoms of basal ganglia damage was the lack of good experimental models. Such a model for tremor is obtained in monkeys with brain-stem lesions[5,7,8]. Recently, NEFF, BARRETT and COSTA[19] produced both an extrapyramidal syndrome and a caudate deficiency in dopamine and serotonin in squirrel monkeys after chronic manganese dioxide administration. The

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present experiments indicate that the magnesium deficient young dog is also a good potential model for the experimental study of hypokinesia. After a few weeks of deficient diet the animals became markedly hypokinetic without any tremor. The amine deficit was then limited to dopamine and, in the brain, located mainly in the caudate nucleus.

It is well known that calcium and magnesium metabolism are closely related but not always in parallel as shown by SHILS[18]. The magnesium deficient rat is usually hypercalcemic while deficient puppies were shown by BUNCE et al.[17] to develop hypocalcemia. We confirm this finding in the present study. Moreover the low magnesium dogs also had hypokaliemia at the end of the experiment. This last point is of interest in view of the recent observation by PARKES[20] that total body potassium content is decreased in parkinsonian patients. However, control studies recently carried out in this laboratory on potassium deficient dogs (with Drs. G. CANTEN, R. BOUCHER and J. GENEST) failed to reveal any changes in the concentration of dopamine, noradrenaline or serotonin in the caudate nucleus, hypothalamus, thalamus, hippocampus or brain stem of these animals.

Finally in support of our findings it is interesting to note that STACHURA and PEARSE[21] recently demonstrated a decreased storage of dopamine in the thyroid C-cells of magnesium-deficient rats. This may thus indicated that the effect of a magnesium deficiency on dopamine may not be limited to the brain, but also involve other dopamine containing areas of the body[22].

Résumé. Une diète déficiente en magnésium administrée à de jeunes chiennes pendant 35 jours est suffisante pour produire une diminution significative de la concentration en dopamine du noyau caudé, mais non des autres amines mesurées. Cliniquement les animaux étaient hypokinétiques, mais ne tremblaient pas. Cette approche expérimentale pourrait s'avérer utile dans l'étude de la pathophysiologie de l'hypokinésie.

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Cortical Acetylcholinesterase and 'Handedness' in Rats

The possibility of training rats to use one forelimb to reach for food has been reported by PETERSON[1]. The important characteristics of the 'handedness' is that it is controlled by the cells localized in a small region (0.6 to 1.8 mm³) of the motor cortex in the frontal lobe[2, 3]. Such a concentration of the cortical projection might suggest a relatively high proportion of the cells probably engaged in the plastic change. This learning model appears to be particularly suitable for the biochemical studies, since the control of handedness is completely lateralized, allowing both experimental and control tissue to be taken from the same animal. The present report concerns acetylcholinesterase (AChE) activity determination in the area of the motor cortex controlling handedness (MC) under different conditons of the handedness experiments.

Material and methods. Adult albino rats of the Wistar strain were used. Before starting the learning procedure the animals were food-deprived with free access to water for 48 h. 4 kinds of experiments were performed: 1. The first group of animals was trained to reach with 1 forelimb for spheric pellets (5 mm in diameter) of Larsen's diet put 2 cm deep into a horizontal glass tube 1.4 cm in diameter. 20 trials per day were made. A 90% criterion (18 consecutive reachings made by the same limb) was usually reached in 2 to 3 days. On the next day another 20 trials were performed and the rats were killed by immersion into liquid nitrogen (spontaneous handedness). 2. Enforced use of 1 extremity was induced by denervation of the opposite one by crushing its ipilateral plexus brachialis[4]. The animals were given at least 7 days of postoperative recovery. One group of the operated animals was given the same procedure as in the first experiment, while remaining rats served for checking the possible effect of denervation alone (denervation control, enforced handedness). 3. Third experimental group of animals was forced to develop more effort during learining. First, hand preference was tested as in the first experiment. The preferred forepaw was then immobilized by denervation. After 7 days of recovery the rats were trained to use the previously non-preferred, now intact limb to get food from the tube. 3 to 4 days were necessary for the animals to relearn (handedness transfer). 4. In the last experiment (overtrained handedness) the rats were subjected to the same procedure as in the first experiment except that additional training (20 trials per day) continued on 4 consecutive days after the criterion had been reached. AChE activity was determined colorimetrically in approximately 1.8 mm³ of the tissue corresponding to MC (coordinates for the central point of the sample: 2.7 mm lateral, 1.6 mm rostral from bregma, 1.7 mm below the surface of the cortex[3]) dissected from the frozen frontal slab 1.3 mm thick. (For details see[5].)

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