or, if this is not possible, the action of them should be blocked by specific inhibitors when the activity of kinin is determined by the autoperfused hind-quarter of the rabbit.

During the past 10 years, biological assay methods were applied to the estimation of kinins. An isolated rat uterus and a guinea-pig ileum were widely used as the biological preparations[7]. On isolated organs, however, spontaneous contraction and relaxation are frequently observed. These movements can seldom be suppressed completely by atropine, antihistaminics, changing the concentrations of Ca in the perfusing solution, or changing the temperature of the bath fluid. Accordingly, it was difficult to obtain accurate results in these preparations.

On the contrary, the constant response to kinin was found on the above-mentioned rabbit hind-quarter preparation, because the fluctuation of the base line of the perfusion pressure was minimal under suitable anaesthetic conditions. Rabbit femoral artery was very sensitive to kinin and responded with sharp dose-dependent vasodilatation. This preparation was applicable for the assay of amounts of kinin less than 3 ng. To test the accuracy of this method, test samples of synthetic bradykinin were prepared by another investigator and estimated without knowledge of its concentrations. Only 5% experimental error was recorded. From these results, we concluded that the autoperfused hind-quarter of rabbit was an excellent organ for the estimation of a small quantity of kinin.

The only disadvantage of this method is that ATP and acetylcholine produce a similar depressor action to that of kinin. Accordingly, a contamination of these substances should be kept in mind when the activity of kinin in a crude sample is measured. However, we have developed a new extraction method for measuring the plasma kinin activity[8]. With this method, more than 95% of acetylcholine is eliminated and ATP completely removed.

Therefore, the combination of our extraction procedure with the bioassay using the autoperfused hind-quarter preparation of the rabbit seems to be very suitable for determining the activity of plasma kinin at the present stage[9,10].

Zusammenfassung. Unter Ausnützung der besonderen Empfindlichkeit der Femoralarterie des Kaninchens für Kinin wurde die autoperfusionierte Hinterextremität beobachtet. Intraarterielle Injektion von 0,5 ng synthetisiertem Bradykinin ergab eine messbare, dosisabhängige Vasodilatation in fast allen Versuchstieren. Dieses Kreislaufpräparat erweist sich zur Kinin-Bestimmung als besonders geeignet.

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Magnesium-Calcium Antagonism in the Contraction of Arterioles

Resistance vessels dilate when exposed to a high concentration of Mg in blood. The vasodilatation could be the consequence of diminished output of transmitter substance from tonically active vasoconstrictor nerve endings, or of a direct action of the Mg on the smooth muscle of arterioles. Observations reported in the literature are compatible with both interpretations[1,2].

To study further the mode of action of Mg on resistance vessels of the peripheral circulation, experiments were done on 9 anaesthetized, atropinized dogs. The right gracilis muscle was perfused with the animals arterial blood[3] using a sigma-motor pump to provide a constant flow rate. At the beginning of the experiment the blood flow was adjusted to produce a normal pressure head (90–110 mmHg). Changes of resistance were registered as changes of inflow pressure. Flow rate was monitored by a drop counter at the venous outflow, and determined from time to time by collecting blood during a measured time interval. The systemic arterial pressure was also recorded. $MgCl_2$, $CaCl_2$ and other drugs were delivered into the tubing of the perfusion pump by motor driven-syringes. Recirculation of injected Mg and Ca was not permitted. Mg and Ca concentrations were determined[4] in the plasma collected from the outflow cannula. Packed cell volume (PCV) of blood samples was also measured. The PCV was slowly decreasing throughout the experiments. The right abdominal sympathetic trunk was stimulated with shielded electrodes between L3 and L4 segments with 10 msec supramaximal pulses. The central connections of the sympathetic were severed.

In the control state stimulation of the abdominal sympathetic trunk produced powerful vasoconstrictor responses in the vessels of the perfused gracilis muscle. When $MgCl_2$ was added to the blood, the inflow pressure dropped and stimulation of the vasoconstrictor fibres became less effective. At the same time the pressor response evoked by injected noradrenaline or vasopressine also diminished (Figure 1). Mixing $CaCl_2$ with $MgCl_2$ restored the pressor effect of sympathetic stimulation and of constrictor drugs (Figures 1 and 2).

Under the influence of excess Mg the vasoconstriction caused by injected noradrenaline was in most cases at least as much depressed as the vasoconstriction caused by sympathetic stimulation. For example in the experiment

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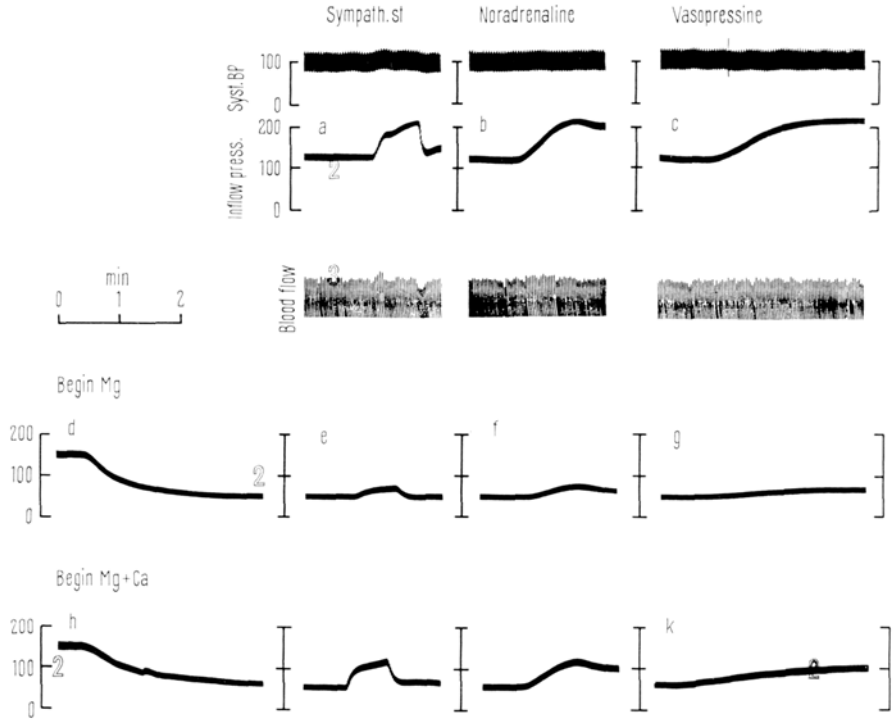

Fig. 1. Inflow pressure of perfused, atropinized gracilis muscle of a dog. On the control records (a, b and c) the systemic arterial pressure, and the rate of blood flow monitored by a drop counter, are also shown. Rate of blood flow: 2.3 ml/min. Weight of muscle: 18 g. Rate of infusion of $MgCl_2$ (records d–k): 0.045 mEq/min, plasma Mg concentration 29 mEq/l (d–g) and 27.5 mEq/l (h–k); $CaCl_2$ infusion (h–k): 0.015 mEq/min, Ca concentration during infusion: 12.4 mEq/l. Stimulation of abdominal sympathetic (a, e and i): at 3/sec for 40 sec. Noradrenaline injection (b, f and j): 2.0 μg in 1 min. Vasopressine (c, g and k): 0.007 Units in 40 sec.

illustrated on Figure 2 the injection of 0.75 μg of noradrenaline was followed by a pressor response which was slightly greater than that caused by stimulation of the sympathetic at a frequency of 4/sec in the control state, and also during the infusion of 0.06 mEq/min of $MgCl_2$; during the infusion of 0.12 mEq/min of Mg the pressor response to 0.75 μg of noradrenaline matched that of 4/sec nerve stimulation. Figure 2 shows also that during the combined perfusion of 0.04 mEq/min of Ca with 0.12 mEq/min of Mg the pressor responses equalled those obtained during perfusion of 0.03 mEq/min of Mg alone, for both, nerve stimulation and injected noradrenaline.

From these observations we can conclude that the incompetence of resistance vessels was caused by a direct action of excess Mg on the smooth muscle of the vessels. If there was an effect on the output of transmitter from vasoconstrictor nerve endings, its contribution to the depression of the pressor response must have been quite minor compared to the incapacity of the muscle of the vessel wall to respond to chemical stimulation.

Adding $CaCl_2$ to $MgCl_2$ was not very effective in preventing the relaxation, or loss of tone of arterioles, occurring at the onset of the infusion (Figure 1d and h). Infusing $CaCl_2$ in itself had either no effect, or resulted in a small elevation of the perfusion pressure and a weak augmentation of pressor responses.

In seeking an explanation of the effect of excess Mg on arteriolar smooth muscle 3 aspects of muscle function have to be considered: excitation, excitation-contraction coupling, and the contractile mechanism. The unresponsiveness of the arterioles to neural and chemical stimulation cannot be due to a depression of excitability, because the pressor response was restored by the presence of excess Ca. Mg ions act as synergists of Ca ions on the electrical properties of smooth muscle[5], as well as of nerve fibres[6] and nerve cells[7]. But the sustained relaxation of resistance vessels could be caused in part by stabilization of the smooth muscle membrane by Mg, for excess Ca was relatively ineffective in restoring the tone of arterioles.

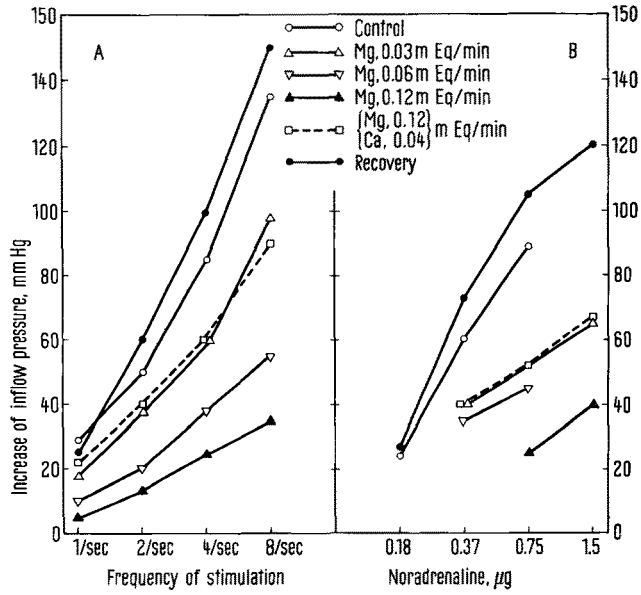

Fig. 2. The response to sympathetic stimulation (A) and to injected noradrenaline (B) of the resistance vessels of the perfused, atropinized, gracilis muscle of a dog. Weight of muscle: 36 g. Rate of flow of blood: 5.9 ml/min. Rate of infusion of $MgCl_2$ and $CaCl_2$ indicated on inset. Mg and Ca concentrations in plasma: o——o, Mg: 2.1 mEq/l, Ca: 3.9 mEq/l; △——△, Mg: 10.5 mEq/l, Ca: 4.3 mEq/l; ▽——▽, Mg: 19.0 mEq/l, Ca: 4.3 mEq/l; □——□, Mg: 30.0 mEq/l, Ca: 12.0 mEq/l. Noradrenaline doses shown are totals injected; all injections given in 90 sec. Sympathetic stimulation: 60 sec.

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It is not very probable that, under the conditions of the experiment, Mg acted on the contractile protein, or the energy yielding reactions involved in contraction. Most cells, including skeletal and cardiac muscle, normally contain a large store of Mg. Furthermore Mg activates rather than depresses glycerol-extracted ATP-treated smooth muscle and, to a lesser extent, also ATP-ase from smooth muscle[1].

The most likely explanation of the effect of Mg on the pressor response is that Mg ions prevent the entry of Ca from extracellular fluid into the muscle cells of the vessel wall, or in some other way interfere with excitation-contraction coupling. That such a competition between Mg and Ca might take place was suggested earlier by BOZLER[8], who experimented with isolated frog stomach.

Since anti-excitation-contraction-coupling is a cumbersome phrase, we suggest the use of the term 'disengagement' or 'disengaging action' to describe this effect. The expression 'uncoupling' already has another technical meaning[9].

Zusammenfassung. Erhöhung des Mg-Spiegels im Blut hemmt Tonus und Kontraktilität der Widerstandsgefässe im Muskel. Durch Noradrenalin hervorgerufene Verengerung wird gleich stark gehemmt wie die durch Reizung sympathischer Nerven verursachte Kontraktion. Ein Überschuss an Ca^{++} verhindert die blockierende Wirkung des Mg^{++} auf die Kontraktilität, der Tonusverlust der Gefässe wird durch Ca^{++} jedoch nicht rückgängig gemacht. Die antagonistischen Wirkungen von Mg^{++} und Ca^{++} beruhen wahrscheinlich auf deren gegensätzlicher Wirkung auf die elektromechanische Kopplung im Gefässmuskel.

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Evolution of Ovarian Grafts in Male Guinea-Pigs Castrated the First Day of Life

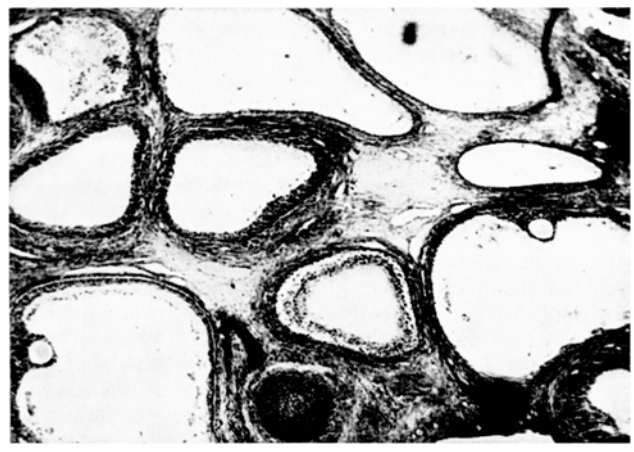
It has been shown by several authors that ovaries grafted into male rats castrated within the first 3 days of life, develop corpora lutea. In contrast to this, when the graft is made in males castrated after the fifth day of life or in adult age, only polycystic ovaries without luteinization are produced. On the other hand, it is well known that administration of testosterone in female rats before the fifth day of life induces in those animals polycystic ovaries, permanent oestrus and infertility. From these and other experiments it has been concluded that in the rat the sexual differentiation of the hypothalamic control of the gonadotrophin secretion is attained after birth and that circulating androgens secreted by the testis are responsible for that differentiation[1-3].

In the guinea-pig, conditions are diverse. We have shown in former papers[4,5] that testosterone injected in high doses in the new-born female guinea-pig, or even in the intrauterine period during the last 2 weeks of pregnancy, does not prevent normal puberty, normal oestrous cycles, both ovulation and corpora lutea, or normal pregnancy and delivery.

What we want to investigate now is whether, in the male guinea-pig, the pattern of gonadotrophic secretion is already established before birth or if, on the contrary, the presence of the testis is necessary in order to obtain that differentiation.

Material and methods. New-born male guinea-pigs were castrated, under ether anaesthesia, by a single escrotal incision within the first 24 h of life. At the age of 60 days, 9 of them were grafted with an ovary of a prepuber female guinea-pig (between 20 and 30 days of age), and 4 were grafted with adult ovaries taken on the eleventh day of the oestrous cycle. The grafts were made in the muscles of the anterior wall of the abdomen. Six control adult males were castrated, and 60 days afterwards an ovarian graft was performed in the same way as in the males castrated at birth.

The animals were kept in separate cages and fed with alfalfa and barley, and tap water was available ad libitum. After 50–60 days the animals were killed by bleeding under ether anaesthesia. The grafts were dissected, fixed in Bouin, embedded in paraffin, serially sectioned, and



Ovarian graft of immature guinea-pig into mature male castrated at birth. Well developed follicles; no corpora lutea were observed.

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