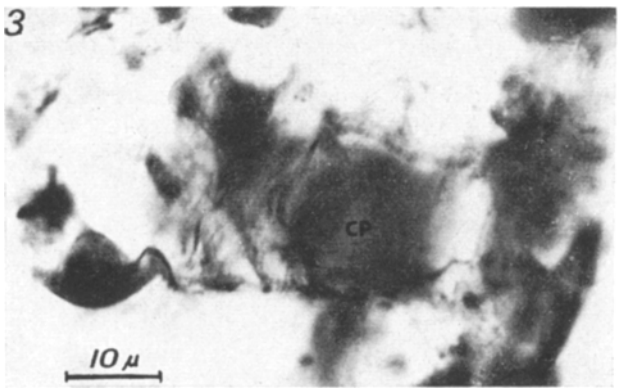

Fig. 3. Neurone dont les prolongements enserrent les cellules piliers (CP) d'un capillaire afférent (MAILLET).

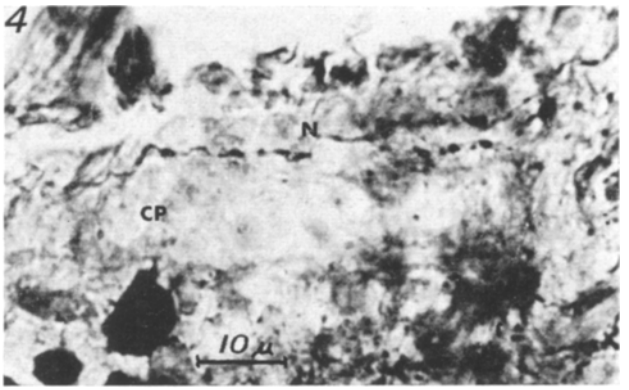

Fig. 4. Prolongement nerveux (N) associé à des cellules piliers (CP) situées à un niveau plus profond (MAILLET).

une contraction ou une relaxation des cellules du pilastre situées dans le feuillet branchial. Dans le cas particulier d'une contraction, l'irrigation des lamelles branchiales serait freinée ou détournée au profit de la circulation efférente. KEYS et BATEMAN[8], STEEN et KRUYSSE[9] ont déjà noté qu'une influence humorale modifie le flux du courant sanguin passant au travers des branchies. Ces structures nerveuses n'exclueraient nullement l'existence de l'effet humoral déjà observé et, dans cette éventualité, contribueraient à expliquer ces mécanismes contractiles et leur incidence sur la physiologie des branchies[10].

Summary. MAILLET's technique demonstrated the innervation of pillar cells of the secondary gill lamellae in *Anguilla anguilla* L. Nervous control may work in conjunction with an humoral regulation which would make clearer the understanding of the in- and outflux of blood in the respiratory organs.

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Spikes of Smooth Muscle in Calcium-Free Solution (Isolated Taenia coli of the Guinea-Pig)[1]

During the last few years, evidence has accumulated that the depolarizing current of action potentials in smooth muscle is carried by calcium ions and not by sodium ions, as in nerve and skeletal muscle of vertebrates. This concept is based mainly on the facts that spike discharge of smooth muscle (1) can continue in sodium-free solution, (2) stops in calcium-free solution, (3) is blocked by manganese ions and (4) cannot be blocked by tetrodotoxin[2–6]. Recent experiments by KEATINGE[7], however, have shown that this concept is not applicable to all types of smooth muscle, for example not to arterial smooth muscle, where the spikes seem to be 'sodium spikes'. We have now been able to record spikes in isolated intestinal smooth muscle for several hours when only K^+ and Na^+ were present as cations in the solution.

From the caecum of the guinea-pig, a piece of longitudinal muscle (taenia coli) with an in situ length of 20–30 mm was cut out and pulled into a glass capillary containing platinum wires which were used for recording the electrical activity extracellularly (Tektronix preamplifier type 122). One end of the tissue was connected to a mechano-electric transducer for recording the tension development. The glass capillary was continuously perfused with saline solution. Krebs solution aerated with 95% O_2 and 5% CO_2 was taken as normal. The system was kept at 35 °C by a thermostat.

In comparing different metabolic inhibitors, we noticed a strong stimulating effect of sodium fluoride. Since KLINGENBERG[8] found that NaF causes contraction of uterine smooth muscle even when the tissue is kept in Ca-free solution, we also tested these conditions in taenia coli. If the taenia is kept for a longer time in Ca-free solution (30 min or longer), NaF (5–10 m*M*) has no effect, or elicits slight electrical activity without tension development. If NaF is given simultaneously with Ca depletion, the phase of decreasing activity, which leads usually in 5–10 min to a complete stop of activity under Ca depletion alone, is prolonged. The spike discharge usually continues with smaller amplitude, or reappears, after the tension development has reached zero. In this phase an additional magnesium depletion leads to an increase

[1] This work was supported by a grant from the Deutsche Forschungsgemeinschaft (No. Go 130/7).
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of electrical activity, and the spike amplitude can reach normal values in the extracellular recording. In a first series of 28 experiments with different concentrations of sodium fluoride in different sequences and combinations with Ca- and Mg-depletion, we found the following procedure to be optimal in obtaining 'Ca-free spikes': (1) Tissue in normal Krebs solution for about 30 min; (2) Ca-free solution with NaF 5 m*M* for at least 30 min; (3) Ca- and Mg-free solution, without NaF. The sections of an experiment in Figure 1 show the normal activity (a), the silent period after transition to Ca-Mg-free solution and gradual reappearance of spike discharge (b), and 'full' electrical activity a few minutes later (c). We sometimes succeeded in recording these 'Ca-free spikes' for 2–3 h with only slight decrease of amplitude. Meanwhile we have learned to elicit Ca-free spikes without NaF. Only slight modifications of the procedure are necessary.

The calcium-free spikes produced in the manner described are different from those under normal conditions

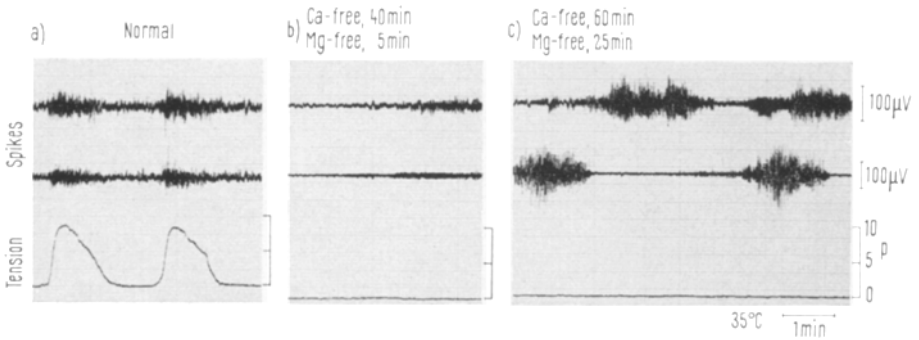

Fig. 1. Electrical and mechanical activity of guinea-pig taenia coli. Tissue length 43 mm. The spikes were recorded with 2 pairs of platinum wires with a distance of 10 mm and a grounded wire in between. (a) Spontaneous activity in normal Krebs solution; (b) pretreatment of the tissue before this record: Ca-free solution with sodium fluoride 5 m*M* (35 min), Ca-Mg-free solution without fluoride (5 min); (c) 20 min later, 'full' electrical activity without tension development, the spike amplitude is even bigger than in normal solution. After additional stretching the asynchronous phases of activity became synchronous. Calibration of the tension development in pond.

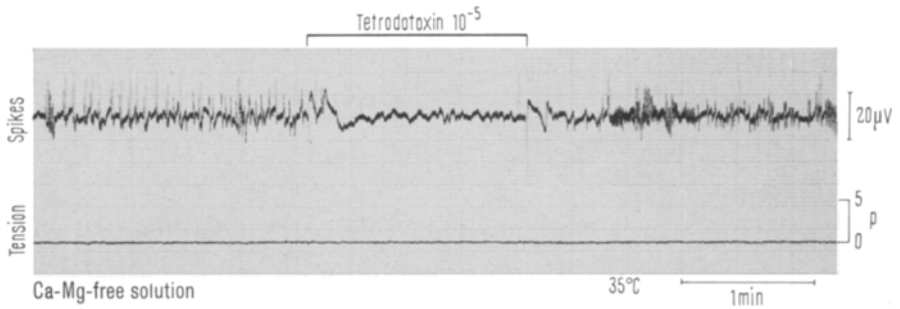

Fig. 2. 'Ca-free spikes' of guinea-pig taenia coli. The spike discharge is blocked by tetrodotoxin 10^{-5} g/ml. Pretreatment of the tissue: Ca-free solution with NaF 5 m*M* (40 min), Ca-Mg-free solution (80 min).

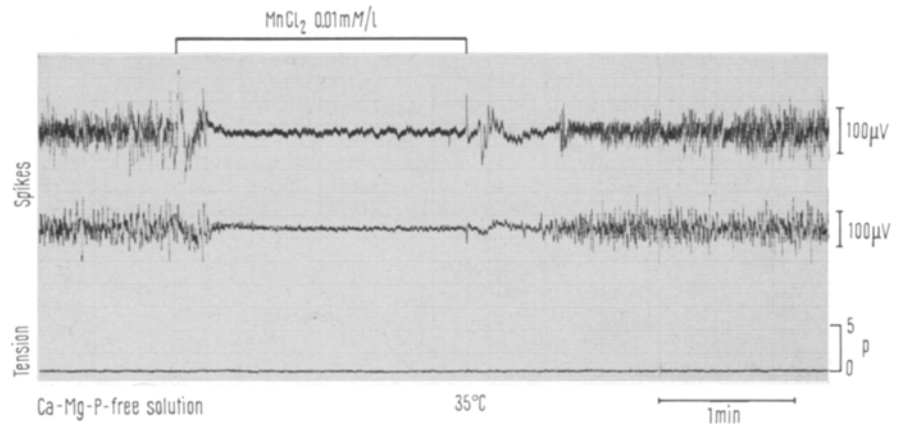

Fig. 3. 'Ca-free spikes' of guinea-pig taenia coli. The spike discharge is blocked by manganese ions in a concentration of only 0.01 m*M*. Pretreatment of the tissue: Ca-free solution with NaF 5 m*M* (25 min), Ca-Mg-P-free solution (20 min). Phosphate had to be left out in order to avoid precipitation with manganese. Electrical recording as in Figure 1. Tissue length 45 mm.

in 2 important respects: (1) Tetrodotoxin (10^{-5}), which is ineffective in blocking the normal spikes of taenia coli, does block the Ca-free spikes completely (Figure 2). (2) Manganese ions, which block the normal activity in concentrations of 0.5–1 mM [4–6], inhibit the Ca-free spikes in a concentration of only 0.01 mM (Figure 3).

On the basis of the ionic theory, the only explanation of the Ca-free spikes seems to be that sodium ions are the charge carriers producing the depolarizing current of the spike. The positive tetrodotoxin effect on these spikes is consistent with this view [9,10]. The fact that the Ca-free spikes are about 100 times more sensitive to manganese than are the normal spikes of taenia coli indicates that manganese does not inhibit the spikes by competition with calcium in its function as the charge carrier of the spike. It cannot be decided whether the manganese inhibition under 'Ca-free' conditions, when small amounts of Ca are still present in the tissue, is independent of Ca, or perhaps a competition with Ca in another function. The inhibitory effect of manganese ions on spikes can no longer be interpreted as an indication that calcium is the charge carrier of these spikes.

With these results, some questions about the spike mechanism in intestinal smooth muscle are again moot. The fact that sodium spikes can be elicited relatively easily seems to indicate that sodium fluxes may also be involved in the spike generation under normal conditions. The most interesting aspect of our results seems to be that the differences in spike mechanism of various smooth muscles (see KEATINGE [7]) need not be interpreted as fundamental differences, since in one tissue 'calcium spikes' can be converted to 'sodium spikes' by simple procedures.

Zusammenfassung. An der glatten Darmmuskulatur (Taenia coli) des Meerschweinchens konnten nach völligem Entzug von Kalzium (und Magnesium) aus der Nährlösung über lange Zeit Spikes gemessen werden. Diese 'kalziumfreien Spikes' werden, im Gegensatz zu den Spikes unter normalen Bedingungen, durch Tetrodotoxin 10^{-5} blockiert, und sie werden wie die normalen Spikes mit 100fach niedrigerer Schwelle durch Manganionen blockiert. Es wird angenommen, daß Na-Ionen die für diese Spikes verantwortlichen Ladungsträger sind.

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Physiologisches Institut der Universität, 355 Marburg/Lahn (Germany), 14 October 1968.

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Degeneration of the Peripheral and Central Nervous System in Vitamin-B_{12}-Deficient Monkeys

Following on the report of OXNARD and SMITH [1] on reduced serum-vitamin-B_{12} levels and neurological degeneration in captive monkeys, further qualitative and quantitative investigations have been carried out, with particular reference to the peripheral nerve lesions and to the effect of treatment.

Materials and methods. 43 monkeys in the colony of the Anatomy Department, University of Birmingham, were studied. There were 40 rhesus monkeys (*Macaca mulatta*), 2 patas monkeys (*Erythrocebus patas*), and 1 baboon (*Papio anubis*); 2 were males and 41 were females of which 10 were pregnant. The animals were grouped according to duration of captivity and diet: group I comprised 12 monkeys kept in captivity from 11 months to 10 years on a standard vegetarian diet; group II comprised 14 monkeys captive from 11 months to almost 19 years, originally fed vegetarian diets but subsequently given a series of injections of vitamin B_{12} followed by a normal diet for periods that varied from 6 months to 4 years; and control group III comprised 17 recently captive monkeys given vitamin B_{12} since arrival in the colony, 14 animals having been in captivity for less than 1 month, and the other 3 animals for 6, 7 and 15 months respectively. The amounts of vitamin B_{12} in the serum were estimated in 38 cases by the bioassay technique with *Euglena gracilis*; OXNARD [2] found the mean total level to be 271 µg/ml in recently captive monkeys. In group I 10 animals had deficient levels of the vitamin in the serum (mean total level 79 µg/ml); in group II 12 animals had high serum levels (mean total > 900 µg/ml); and in group III the serum levels were normal, except for 2 pregnant monkeys that had low readings.

Results. The results are given in the Table.

A high incidence of histological lesions was found in animals of groups I and II and 5 animals showed overt paralysis (see Table). It should be noted that this does not represent the true incidence of paralysis in deficient animals, which is much lower: paralysed animals were selected because of the paralysis and in order to try and assess the effect of treatment. In the spinal cord the changes resembled those of human subacute combined degeneration, and were more severe in the paralysed monkeys; cerebral lesions were found in 5 animals. Frozen sections of the sciatic and popliteal nerves showed sudanophilic degeneration in 21 animals (the median and ulnar nerves were normal in all 43 cases). Segmental demyelination, usually with remyelination, was seen in teased peripheral nerve fibres in every animal that showed sudanophilic degeneration (see Figure); in a few animals there was also axonal (wallerian) degeneration.

In group III minimal lesions were found in 4 animals. Two of these were pregnant and had low serum-vitamin-B_{12} levels (pregnancy depresses the serum vitamin B_{12} in monkeys: OXNARD [2]) and a third animal had a low-normal level; though the previous dietary history of these 3 animals was unknown, their nervous systems could have been affected before they arrived in captivity. The fourth animal had been captive for 15 months (more than twice as long as any other animal in group III) and

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