particles. Similarly, preparations without iron gave low yields of particles having no detectable peroxidase activity. Catalytically active particles retained their activity after heating in boiling water, which is geologically reasonable. Coincidentally, the peroxidases are among the most stable of all enzymes[9]. This data is summarized in the Table. We could not demonstrate enhanced iron catalytic activity due to its presence in the microspheres.

Iron (III) readily forms complexes with chelating amine ligands[10]. The glycine-iron complex in our starting solution appears to be crucial to particle formation as well as to the catalytic effectiveness of the product. However, the analytical data indicate that only a small portion of the iron may be present in this form. Oxides and sulfides would account for the bulk of the iron.

GRANICK[11] has proposed a model of a primitive photosynthetic unit consisting of oxides and sulfides of iron. According to his model, organic compounds could form on the surfaces where hydrogen and hydroxyl ions were utilized. We observed a significant drop in pH, up to 2.5 units, after we irradiated our particle suspension in water for 1 h with the UV pen lamp. This change was reversible in several hours in the presence of the particles, but not so if the particles were removed by filtration. Boiling the supernatant did not alter the pH; thus dissolved, H_2S, for example, could not be responsible. If a large amount of hydroxyl ions were consumed at the surface, then the pH would decrease as in our system.

In order to further explore the similarities between a primitive photosynthetic system and our microspheres, we irradiated the particle suspension and then allowed to stand for several hours until the pH returned to its former value. On further irradiation, the pH decreased once again: a procedure that could be repeated several times. It is interesting to note that a simple solution of ferrous ammonium sulfate will also show a decrease of pH on irradiation, but this effect is not reversible.

The behavior of our particles suggests that we may have found a model similar to the one proposed by GRANICK[11]. In our microspheres, the iron associates with the organic complexes produced and shows strong catalytic activity as proposed by CALVIN[1]. In support of this scheme, we found that particles isolated after only 30 min of irradiation are very small. Preliminary results indicate that succinic acid, a porphyrin precursor[12] can partially replace glycine in the formation of the microspheres[13].

Résumé. L'irradiation UV d'une solution de sulfocyanate d'ammonium, glycine et fer dans l'eau de mer artificielle a produit des microsphères semblables à des cellules douées d'activité catalytique péroxidasique. La glycine a augmenté l'activité catalytique du fer, comme cela purrait s'être produit au cours de l'evolution dans la première étape de cette réaction de catalyse par le fer. On a observé des faits semblables avec un modèle à élément photosynthétique primitif.

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Intracellular Recording of Electrical Activity in Smooth Muscle of the Common Bile Duct[1]

Knowledge concerning the motility of the bile duct is still quite fragmentary and there has been no publication of intracellular measurements of the membrane potential, the most sensitive indicator of the electrical processes combined with excitation of smooth muscle cells. We therefore included the common bile duct of the guinea-pig in a programme of comparative studies on different types of spontaneously active smooth muscle preparations (taenia coli[2], stomach[3], portal vein[4] and ureter[5] of the guinea-pig).

Materials and methods. A length of 10–15 mm of the common bile duct was removed from the junction of the cystic and hepatic ducts up to approximately 2 mm before its passage into the duodenum, and the lumen was immediately cleared from the bile. One end of the preparation was pulled over a perspex cone and fixed with a thread, very similar to the technique used for measurements in the portal vein[4]. The size of the cone was selected in order that the tissue was just sufficiently stretched to prevent movements in this area, and, therefore, long-term intracellular recordings with glass microelectrodes could be performed. The greater part of the preparation was kept under normal tension conditions, stretched to its approximate length in situ. The free end was connected to a transducer for recording the tension development. The preparation was kept in an organ bath at 35 °C, perfused with Krebs solution aerated with 95% O_2 and 5% CO_2. Technical details are given more fully in preceding papers[2,4]. The described partial fixation of the bile duct preparation does not disturb the normal spontaneous activity, as could be shown by experiments where the electrical activity was recorded with extracellular electrodes without any special fixation (will be published separately).

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Results and discussion. During the normal adaptation time of about 30 min, all preparations developed spontaneous activity, which shows many similarities to that of the isolated taenia coli. The upper part of Figure 1 gives an example of the pattern of activity which was particularly evident at the beginning of the experiments. There is weak continuous activity combined with spike discharges of a frequency of 50–60/min which show, because of their regularity, that smooth muscle of the bile duct has a clear 'second-rhythm' (SR), though the potential oscillations of the SR, which trigger the spikes, are relatively small in amplitude. The fact that in the tension record small waves can be detected running synchronized with the spike discharges of the impaled cell shows that the smooth muscle cells in the bile duct are also strictly coupled and consequently act as a functional unit.

The basic membrane potential was found at -50 to -65 mV, which means that, in connection with the limitations of the method (tip potentials of the electrodes),

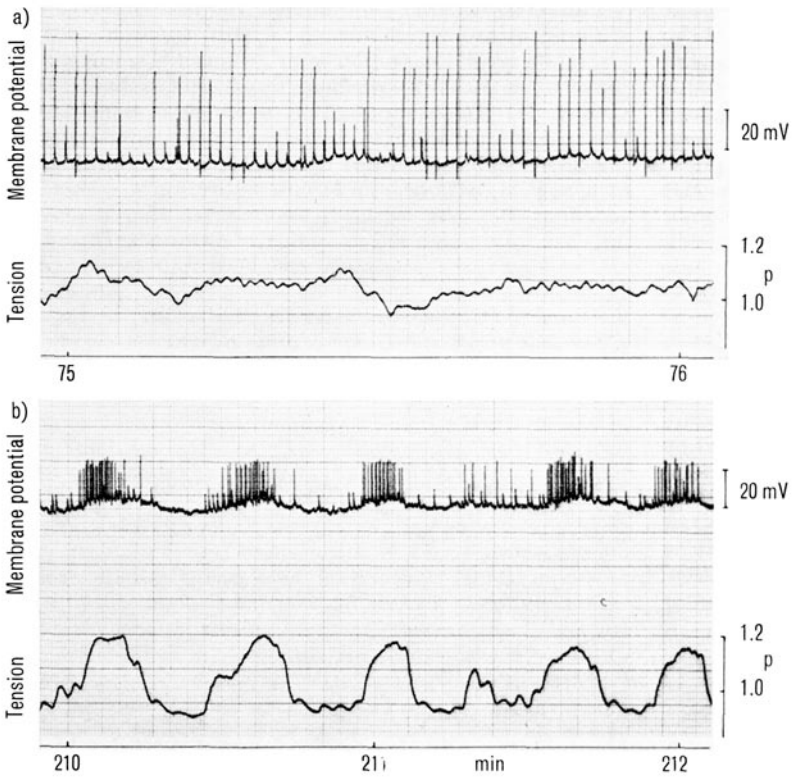

Fig. 1. Spontaneous electrical and mechanical activity of the isolated common bile duct of the guinea-pig. Time indicated in min after the beginning of the experiment; part b) recorded at lower speed. Membrane potential measured intracellularly, calibrated in mV, tension development in ponds.

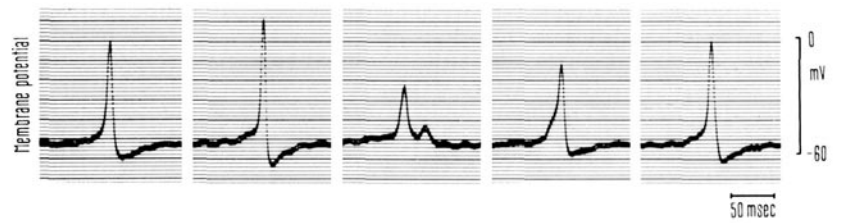

Fig. 2. Variability of spike potentials shown by 5 consecutive spikes, measured in a smooth muscle cell of the bile duct, recorded at high speed. The marked zero value gives only an approximation of the real position of the zero line because of the tip potential of the electrode. Same experiment as Figure 1.

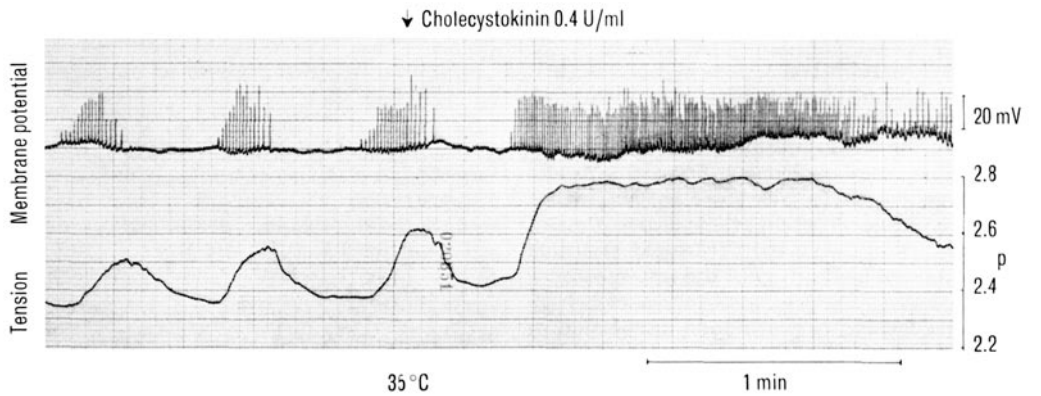

Fig. 3. Spontaneous activity of the bile duct, with marked fluctuations in the minute-rhythm (MR). Augmentation of the activity after application of cholecystokinin.

even higher potential values may exist. The spike amplitude, measured from the upper peak value to the peak value of the post-hyperpolarization, reached values of up to 75 mV. As demonstrated in the example given in Figure 2, the spike potentials show a similar variability in size and shape as do those in other smooth muscle preparations[2,4], and the half duration (duration of the spike at half height) of the largest and sharpest spikes lies at 8–12 msec, similar to taenia coli[6].

The lower part of Figure 1 gives an example of another pattern where the activity is modulated by a slower 'minute-rhythm' (MR). This MR was regularly present in the isolated bile duct, either from the beginning of the experiment or, as in the example of Figure 1, developing during the first 1 or 2 h. The MR is combined with fluctuations in the basic membrane potential which lead to an inhibition of spike discharges in the phase of high polarization and stimulate spike discharges in the phase of depolarization. During the highest activity in the MR cycle, the spike frequency is increased over the preferred frequency of the SR – this again is similar to taenia coli. The period duration of 0.5 min of the MR in the bile duct is more similar to that of the portal vein than to that of taenia coli, where, under normal conditions, it is 1–3 min. But shorter period durations also occur in taenia during phases of depressed activity[7].

Following the classification of smooth muscle rhythms recently described[3], the smooth muscle of the bile duct is characterized by a spontaneous activity determined by the SR and the MR, both of which are the more general rhythms of the intestinal smooth muscle system. A pronounced 'basic organ specific rhythm' (BOR) is not usually found in the bile duct. Fluctuations with a frequency of 20–25/min, which were occasionally seen, have to be interpreted as an irradiation of the segmentation rhythm (BOR) of the duodenum to the bile duct. This assumption is supported by the fact that these fluctuations are particularly observed when the preparation of the common bile duct is extended in the distal direction into the biliary duodenal junction.

Finally, Figure 3 gives an example of augmented activity of the bile duct produced by application of cholecystokinin. A depolarization with accelerated spike discharges leads to an increase of tension under these conditions. The effect of acetylcholine is very similar.

Zusammenfassung. Im Rahmen vergleichender Untersuchungen an verschiedenen Typen spontan aktiver glatter Muskulatur wurde beim isolierten Ductus choledochus des Meerschweinchens durch intrazelluläre Messungen des Membranpotentials regelmässig eine mittelstarke Spontanaktivität, bestehend aus sekundenrhythmischen Oszillationen mit Spikes, moduliert durch langsamere Fluktuationen im Minuten-Rhythmus, gefunden.

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Relationship between Passive Permeability to Nonelectrolytes and Active Transport Activity in the Isolated Intestinal Wall[1]

It is well known that sodium concentration in the bathing mucosal fluid strongly affects glucose and amino acid transport through the isolated intestinal barrier[2–5]. This may be explained by supposing an influence of sodium on the entry of sugars[6] and of amino acids into the epithelial cells[5–7]. Actually there is a decrease in the unidirectional influx of L-alanine into the intestinal cells in the absence of sodium in the perfusing fluid[8]. According to CRANE[9], the transported substance interacts with a binding site on a carrier and this interaction is enhanced by the binding of sodium with a second site present on the same carrier.

In a recent paper[10], our aim was to investigate whether, besides the above-mentioned hypothetical specific mechanism of sodium effect, another unspecific effect occurred on the resistance of the intestinal barrier to the passage of non-actively transported molecules. From the results collected in that paper, there was some evidence that by lowering sodium concentration, the passive flux of acetamide or thiourea is decreased. To avoid criticism of our indirect method of evaluation of the test substance concentration in the intestinal sac, another set of experiments was performed using a continuous circulation of the perfusion fluid through an open and everted intestinal tract. Such a perfusion method provides a direct control of the inside and the outside concentrations of the substance tested throughout the period of the experiment[11].

Previous conclusions have been confirmed. The thermal diffusion of small molecules such as acetamide and thiourea across the intestinal barrier is reduced together with the active transport of D-glucose, when NaCl of the incubating medium is replaced by equiosmolar quantities of *Tris* Cl, Choline Cl or LiCl. Some evidence was also provided that the decrease in the passage of small molecules may be partially due to an increase in the resistance offered by the brush border. Furthermore,

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