so obtained was used for the assay of cMDH. The pellets were suspended in sucrose (0.25 *M*) and used for the assay of mMDH.

Spectrophotometric assay of MDH. The method of assay for MDH was the same as that of OCHOA[7] with a little modification. The assay mixture included 2.49 ml of water, 0.3 ml of phosphate buffer (0.1 *M*, pH 7.4), 0.01 ml of NADH, 0.1 ml of supernatant and 0.1 ml of oxaloacetate (0.25 m*M* final concentration). The total volume of the assay mixture was 3.0 ml. The concentration of NADH was so adjusted that 0.01 ml gave the O.D. of 0.5 at 340 nm. The reaction was started by adding the oxaloacetate and the decrease in O.D. was recorded at 340 nm in SP-500 spectrophotometer. The percentage inhibition of cMDH and mMDH activities were studied by using citrate concentration of 6.66 μM/ml. This concentration of citrate was decided upon from the inhibition data of preliminary experiments producing the inhibition between 30–50%. The total volume of assay mixture was adjusted by decreasing the volume of water. The activity of MDH was expressed as units/g wet wt. of the tissue. The level of significance between the two sets of data were calculated according to SIEGEL[8].

Results and discussion. The Table shows that the activity of both the isoenzymic forms of malate dehydrogenase, cMDH and mMDH, is significantly greater in the brain and heart of 22-week-old rats as compared to that of the 96-week-old rats. Also, the activity of cMDH is higher in both the tissues than that of the mMDH at both the ages. The activity of mitochondrial malate dehydrogenase was inhibited by citrate to a greater extent than that of the cMDH. However, there was no change in the percentage inhibition of mMDH activity in the brain and heart of 22- and 96-week-old rats. The activity of cMDH was inhibited by citrate at both the ages, but the percentage of inhibition was significantly greater in the brain of 96-week-old rats than that of the 22-week-old rats. There was no significant difference in the percent inhibition of the activity of cMDH of the heart of the 22- and 96-week-old rats.

The greater activity of the cMDH in both the aerobic tissues, brain and heart, indicates that they have efficient glycolytic pathway and that these tissues can tolerate the absence of oxygen during young adulthood (22-week-old) as compared to that of the older rats (96-week-old). This finding is consistent with the earlier observations[9,10]. The activity of mitochondrial malate dehydrogenase is highly inhibited by citrate as compared to that of the cMDH at both the ages of rats. This may suggest that the accumulation of citrate within the cell, which is an intermediate of the Krebs cycle, has some controlling mechanism in the operation of the cycle[11].

Zusammenfassung. Es wurde die cytoplasmatische und mitochondrische Malate Dehydrogenase im Gehirn und Herz junger und alter Ratten spektrophotometrisch untersucht und signifikante Aktivitätsunterschiede gemessen.

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The Effect of Maltose on the Aldosterone Activated Sodium Transport of the Frog Bladder

Little attention has been focused on the metabolism of circulating disaccharides, while the absorption of dietary disaccharides has been studied by many reseachers. Recently some studies of metabolism of circulating disaccharides, especially maltose, were reported[1,2]. They suggested that circulating maltose, unlike lactose and sucrose, might be hydrolyzed by extraintestinal maltases and subsequently metabolized. In the present study, the possibility that maltose was utilized as a substrate after permeating across a cell membrane is discussed.

Materials and methods. The urinary bladder of the frog, *Rana catesbiana*, was used as a model. Frogs were kept at 8°C to keep them in a steady state. After double pithing of the frogs, the bladders were excised and halved. Each hemibladder was mounted in a lucite chamber and incubated for 14 h in Ringer's solution containing penicillin G (1 mg/ml) and streptomycin (1.6 mg/ml) to make the bladder steroid-free. After this overnight incubation, tissue 11-OHCS decreased to approximately 40% of the control[3]. The experiments were carried out at room temperature. The area of the chamber orifice was 3.16 cm^2. To prevent mechanical distortion of the bladder membrane, both orifices of the chambers were covered by nylon mesh and the bladder was sandwiched between 2 discs of nylon mesh. The short-circuit current (SCC) was measured by the method of USSING and ZERAHN[4]. The composition of the Ringer's solution was: NaCl, 111 m*M*; KCl, 3.5 m*M*; $CaCl_2$, 0.9 m*M*; $MgCl_2$, 1.5 m*M*; NaH_2PO_4, 1.9 m*M*; and Na_2HPO_4, 8.1 m*M*. The osmolality was 232 mOsm/L and the pH was 7.4.

After 14 h incubation in glucose-free, antibiotic-containing Ringer's solution, the chambers were washed 3 times with fresh Ringer's solution and then maltose was added to one chamber at the concentration of 10 m*M* as a substrate, while glucose was added to the paired chamber at the concentration of 10 m*M* as the control. After the SCC reached a plateau, D-aldosterone (Sigma Co. Ltd.) was added to the chambers at the concentration of $1 \times 10^{-6} M$.

Before carring out the present experiment, the substrate-dependency of the aldosterone action on the sodium transport was checked. As shown in the Figure A, D-aldosterone increased the SCC when glucose was used as a substrate, but showed no effect on the SCC when the substrate-free Ringer's solution was used. This result is consistent with the data of EDELMAN[5] who used *Bufo marinus*, and indicates that the aldosterone effect on the sodium transport is a substrate dependent action in both species.

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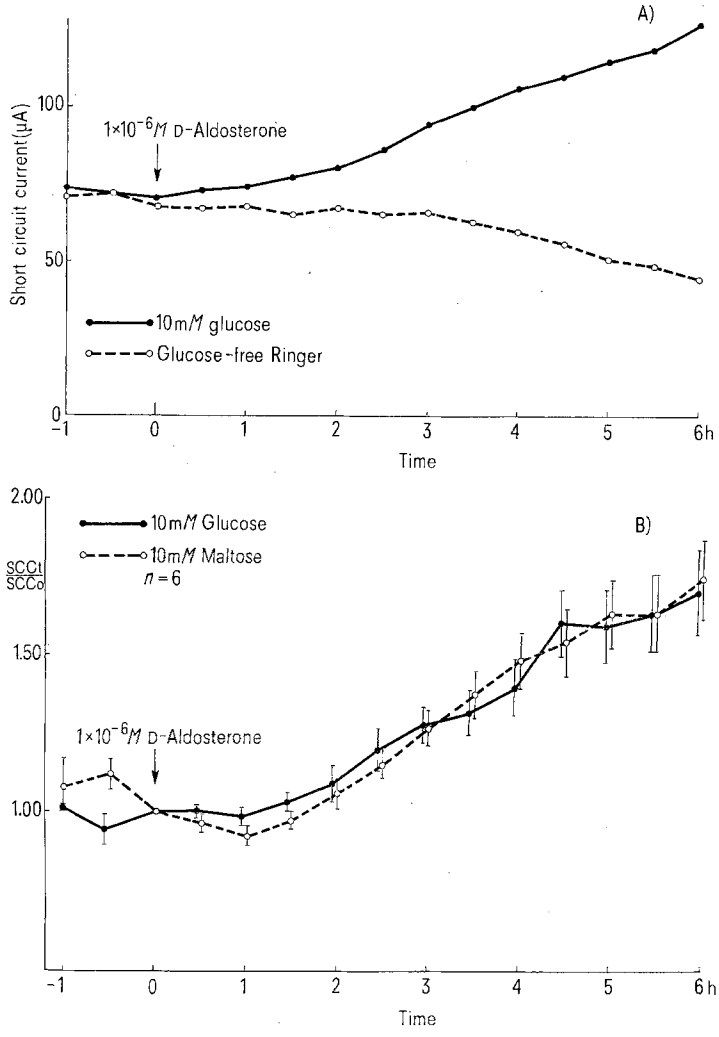

A). The effect of glucose on the aldosterone-activated sodium transport of the frog bladder. B) The effect of D-aldosterone on the SCC in glucose- and maltose-containing Ringer's solution. Vertical line shows 1 S.E.M. The initial value of the SCC in the glucose-containing Ringer's solution was 57 ± 12 μA (mean ± S.D.), and in the maltose-containing Ringer's solution was 48 ± 10 μA (mean ± S.D.).

Results and discussion. The comparison between glucose and maltose as substrate for the aldosterone effect on the SCC of the frog bladder is shown in the Figure B. When glucose was used as a subtrate, the SCC started to increase approximately 90 min after addition of D-aldosterone and the highest value of the SCC was 1.71 times of the value before addition of the drug. In the maltose solution, the SCC also started to increase nearly 90 min after addition of D-aldosterone and the highest value of the SCC was 1.75 times of the value before addition of the drug. At each measuring point of the SCC after addition of D-aldosterone, there was no significant difference of the SCC value between glucose and maltose groups. The conductance did not change during the experiment.

These results indicate that maltose was utilized as a substrate for the aldosterone activating sodium transport of the frog bladder. WESER[1] reported that maltase activity can be found in most organs and that maltose metabolism proceeds via hydrolysis to glucose[6]. Several reports concerning localization of maltase exist: An acid maltase was found in the lysosome-rich light mitochondrial fraction, and this rat liver acid maltase showed all the properties of the lysosomal enzyme[7]. No evidence of localization of maltase activity in membrane fraction of rat liver was found[8]. There has been no report that maltase is localized in membrane fractions, except in the small intestine. These observations suggest that maltose might not be hydrolyzed on the cell-membrane since the enzyme is not located in the cell-membrane except the membrane of small intestine, and that most of the maltose seems to hydrolyze to glucose by maltase after maltose permeates across the membrane. This study suggests that D-aldosterone activates sodium transport of the frog bladder using maltose which permeates across the membrane as a substrate.

Maltose which was purified to 99.5% was supplied from Ohtsuka Pharmaceutical Factory Inc.

Zusammenfassung. Es wird gezeigt, dass Maltose als Energielieferant für die Na-Pumpe in der Harnblase von Amphibien (*Rana catesbiana*) dienen kann.

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