Kontrolltieren änderte sich nicht. Der Test 1 Woche nach Operation ergab, dass alle operierten Tiere hypoaktiv waren. Dieser Befund wird auf die noch unvollständige Verheilung der Operationswunde zurückgeführt. Die Ergebnisse des Open-Field-Tests sind unabhängig von den Testtagen.

Nach der hier vorliegenden Untersuchung ändert sich bei adulten Wistar-Ratten nach Pinealektomie nur das Erkundungsinteresse ursprünglich hypoaktiver Tiere. Somit wird hier nur eine der 3 Komponenten, die die Emotivität ausmachen, durch die Pinealektomie beeinflusst. Das Erkundungsinteresse (motorische Aktivität) wird erhöht. Ähnliche Befunde erhoben Reiss[8] und Sampson und Bigelow[9] an in der Jugend pinealektomierten Ratten, wobei diese Ratten nach Pinealektomie alle ein höheres Erkundungsinteresse zeigten als scheinoperierte und Kontrolltiere. Hierbei ist zu beachten, dass die Testsituation (Grösse und Konstruktion des Open-Field, Beleuchtung, Umfeld) Einfluss auf das Testergebnis haben. Ausserdem reagiert jede Ratten-Species anders auf die Testsituation. Interessant ist in diesem Zusammenhang, dass Verhaltensforscher als Ursache für späteres emotives oder nicht-emotives Verhalten fehlende oder stark ausgeprägte stimulierende Einflüsse in den ersten 3 Lebenswochen der Ratte sehen[10,11]. Die Emotivität der Ratte ist im Open-Field nicht mit absoluter Sicherheit zu bestimmen, da auch Gewöhnungseffekte eine grosse Rolle spielen[7,12]. Biochemisch hängt die Emotivität der Ratte vom Gehalt des Gehirns an Indol- und Catecholaminen ab[13–15]. Vom Indolamin Serotonin, das in den Pinealzellen eine sehr hohe Konzentration besitzt[16,17], ist bekannt, dass es über das Zentralnervensystem die motorische Aktivität der Ratte beeinflusst und einen sedierenden Effekt ausübt[3,18]. Das erhöhte Erkundungsinteresse pinealektomierter hypoaktiver Ratten lässt sich durch den verminderten Serotoningehalt des Gehirns und den Wegfall der sedierenden Wirkung erklären. Das nach Pinealektomie unveränderte Erkundungsinteresse hyperaktiver Ratten könnte an einem vor der Operation niedrigeren Serotoningehalt in den Epiphysen dieser Tiere liegen. Diese Vermutung wird gestützt durch die Beobachtung von Reiss[8], der bei motorisch sehr aktiven Ratten eine niedrigere Pinealzelldichte fand, was an einen niedrigeren Serotoningehalt in der Epiphyse denken lässt. Da sich die Häufigkeit von Urinieren und Defäkation nach Pinealektomie nicht ändert, darf vermutet werden, dass diese Komponenten der Emotivität, zumindest bei der Wistar-Ratte, nicht von der Epiphyse gesteuert werden. Um näheren Aufschluss über die Tätigkeit der Catecholamine im Komplex «Emotivität» bei der psychogenetisch nicht selektierten Ratte zu erhalten, wäre es interessant, die Konzentration im Gehirn vor und nach Pinealektomie zu bestimmen. Nach dem jetzigen Stand der Kenntnis scheint die Epiphyse nur eine Teilkomponente der Emotivität, nämlich die Lokomotionsaktivität zu beeinflussen.

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The physical state of alkali ions in a Halobacterium: Some NMR results

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Summary. Nuclear magnetic relaxation measurements of the ions Na^+, Rb^+ and Cs^+ in a Halobacterium sp. are described. The results support a theoretical model which involves the binding of intracellular alkali ions.

Members of the genus Halobacterium grow in media of high salinity, typically 3.5 M NaCl. They have a very high intracellular ion content of about 4 M K^+ and 1.5 M Na^+. Even in a virtually non-metabolizing state the bacteria possess a remarkable ability to retain potassium, indicative of some kind of specific binding of K^+ ions[4–6]. It has been postulated that the K^+ specificity may partly result from preferential hydration of K^+ over Na^+ in water of restricted mobility[7,8]. Nuclear magnetic resonance (NMR) can give information on the dynamics of the ions. We here present pulsed NMR relaxation measurements[9] of the ions Na^+, Rb^+ and Cs^+. Rb^+ and Cs^+ are used as probes for the K^+ ions because they can be observed much more easily with NMR than K^+. The measurements indicate that the fluctuating molecular environment of the ions comprises motions which occur at a rate slower than 10^8 sec^{-1}. This holds in particular for the intracellular ions, which substantiates some kind of binding of these ions.

Material and methods. A Halobacterium sp. from the Dead Sea was studied. The growth conditions and the method of ionic analysis are described elsewhere[10]. The bacteria were concentrated by centrifugation into pellets of about 1 g. The extracellular space accounts for about 60% of the total pellet volume. A small amount of RbCl or CsCl was added to the growth medium of some samples; at least half of the intracellular K^+ was thereby replaced by Rb^+ or Cs^+. The transverse nuclear magnetic relaxation rate R_2[9] was measured at room temperature with a home-made pulsed NMR spectrometer system[11] at a NMR frequency $\omega_0/2\pi$ of 14 MHz. For ^{23}Na and ^{133}Cs the customary Carr-Purcell-Meiboom-Gill pulse sequence[9,12] was used, whereas R_2 of ^{87}Rb was determined from the rapidly decaying signal after a single pulse. The observed

NMR relaxation decay was analyzed by means of a least-squares fit by computer to a single- or double-exponential function. The observed signal intensity was determined from a comparison with the NMR signal intensity of salt solutions with a known ion content. Further details on the measuring procedures are given elsewhere[11,13].

Results and discussion. The theoretical background for the NMR relaxation of ions in cells has been given for nuclei with spin $^3/_2$, such as ^{23}Na and ^{87}Rb[13–16]. The relaxation proceeds via the nuclear quadrupolar interaction. The relaxation rate is determined by fluctuations in the molecular environment of the ions, e.g. as a result of temporary chelation of ions by macromolecules. A single pool of ions will show 2 components in its transverse NMR relaxation if slow fluctuations, at a rate $\tau_c^{-1} \lesssim \omega_0$, occur in the ionic environment. $\omega_0 \simeq 10^8$ s^{-1} in our experiments. 40% of the signal then relaxes with a slower relaxation rate R_{2s}; the remaining 60% relaxes faster with a rate R_{2f} and may be difficult to detect experimentally. Such slow fluctuations can result either from the diffusion of ions in anisotropic domains of a dimension of 100 A or larger[13,16], or from the binding of an ion in a specific site for a time $\tau_c \lesssim \omega_0^{-1}$[15,16]. ^{133}Cs has a nuclear spin of $^7/_2$, and the relaxation theory is more complex[17,18]. In this case the slowest relaxing fraction comprises 19% of the total signal intensity. The remaining signal comprises three components which relax much faster.

The results of our measurements are summarized in the table. Within experimental error all the sodium ions contribute to the NMR-visible signal. 2 fractions are clearly resolved in the NMR relaxation, the relative intensities of which correspond nicely to the expected 4:6 ratio. The sodium signal stems mainly from extracellular ions: at least 80% of the sodium in the sample is located in the intercellular space, where the Na^+ concentration is much higher than in the cells[5]. R_{2s} and R_{2f} neither are very different from each other, nor are they very much enhanced relative to the relaxation rate in a salt solution. This indicates that the average environment of an extracellular sodium ion does not differ drastically from that in a salt solution, but that some slower fluctuations are present. Interactions of the sodium ions with the cell surface could be responsible for the observed relaxation behavior.

Rb^+ and Cs^+ replace K^+ and are consequently expected to be located within the cells. This is supported by the fact that no NMR-signal of Rb^+ or Cs^+ was detected in the growth medium. For cesium only a fraction of the expected signal intensity is resolved, which corresponds roughly with the 19% intensity expected for the slowest relaxing fraction. A bad signal-to-noise ratio hampered an estimate of the observed intensity of the rubidium signal. Since we observe only 1 relaxation rate for rubidium where 2 are expected theoretically, we identify the observed signal with the slower relaxing fraction. The observed relaxation rates of Rb^+ and Cs^+ are much faster

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Transverse nuclear magnetic relaxation rates R_2 of alkali ions in a Halobacterium sp.

Ionic nucleus studied	^{23}Na	^{87}Rb	^{133}Cs
Number of samples studied	7*	2	2**
Number of resolved signal fractions	2	1	2
Total NMR-visible signal intensity	80 ± 20%	40% ***	15 ± 5%
Relaxation rate(s) R_{2s} of the slower relaxing signal fraction, and the intensity relative to the total NMR-visible signal intensity	81 ± 19 sec^{-1} (40 ± 10%)	8700 ± 1000 sec^{-1}	15 ± 2 sec^{-1} (45 ± 15%) and 35 ± 5 sec^{-1} (55 ± 15%)
Relaxation rate R_{2f} of the faster relaxing signal fraction	240 ± 23 sec^{-1} (60 ± 10%)	> 20,000 sec^{-1}***	> 20,000 sec^{-1}***
Relaxation rate R_2 in a salt solution	18.4 ± 0.4 sec^{-1}	400 ± 30 sec^{-1}	0.21 ± 0.02 sec^{-1}

All measurements were performed at room temperature; NMR frequency 14 MHz. The relaxation rates R_2 are the inverse of the NMR relaxation times T_2. *The sodium relaxation results were the same, irrespective whether K^+, Rb^+ or Cs^+ was accumulated in the cells, or whether the NaCl concentration in the growth medium was 1.7 or 3.5 M. **A third cesium-containing sample was grown in a medium containing 1.7 M NaCl instead of 3.5 M. Despite the presence of a large amount of Cs^+ in this sample, a cesium NMR signal could not be detected. ***These values have not been measured directly; see text.

than in a salt solution, and the enhancement of the relaxation rates is much larger than that for the extracellular sodium ions. The NMR-invisible fraction of the cesium and the rubidium signal must have a relaxation rate R_{2f} larger than 20,000 sec^{-1}, which is the detection limit of our spectrometer. The relaxation decay of $^{39}K^+$ ions in another Halobacterium sp. has been observed[19]. In that case a considerable enhancement of the relaxation rate seems to exist as well. The large enhancement of R_{2s} and a large R_{2f} both indicate that slow fluctuations in the molecular environment of the intracellular ions, at rates slower than about 10^8 sec^{-1}, contribute substantially to the transverse NMR relaxation.

We can contrast this with the rate of fluctuation of the hydration sphere of an alkali ion in solution, which is of the order of 10^{11} sec^{-1}[20]. Slow changes in the environment of the intracellular ions are expected if the ions are bound. The NMR results therefore substantiate that the intracellular ions K^+, Rb^+ and Cs^+ are somehow bound in Halobacterium.

The meaning of the 2 resolved fractions in the relaxation of the cesium signal is not clear. One might speculate that they reflect 2 different types of intracellular environment, each characterized by different values of their relaxation rate R_{2s}. This possibility is not entirely hypothetical, as other experiments on intracellular K^+ point to 2 different types of ionic environments for the intracellular ions[8].

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Contact inhibition and the movement of metal, glass and plastic beads within solid tissues

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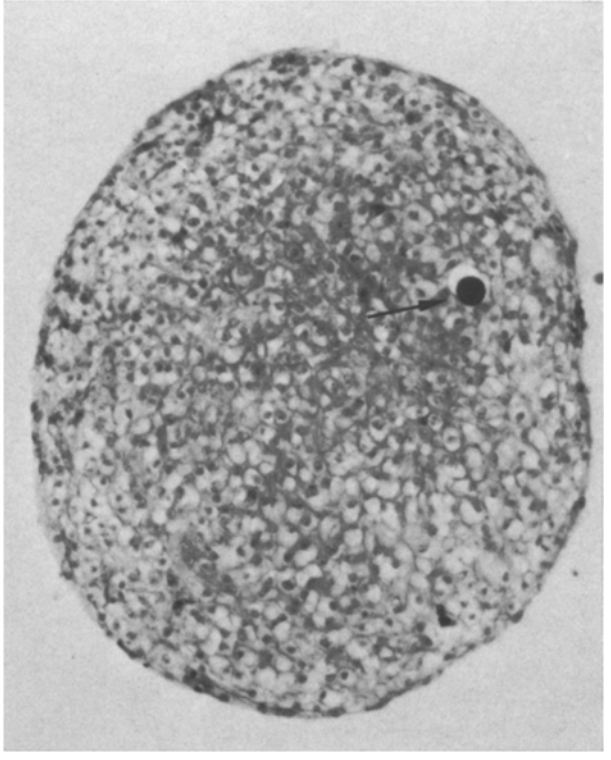
Summary. Cell-size spheres of metal, glass and plastic can move from surface to subsurface positions within solid tissue masses in culture, demonstrating that the observed movement of cells in similar circumstances may not be due to 'active cell locomotion'.

If 'contact inhibition of cell movement' stops cell locomotion in the direction of contact with another cell, then cells in contact on all sides with other cells might be expected to show little or no movement[1,2]. Evidence for just such an expectation has been reported[3,4]. A small piece of embryonic chick tissue was allowed to fuse in culture with another bit of embryonic tissue previously labeled with tritiated thymidine. Autoradiographic inspection of histological sections prepared after several days of culture revealed essentially no movement of labeled cells across the boundary between the labeled and the unlabeled tissues.

Apparently contradictory results have been reported with a slightly different experimental procedure[4,5]. In this experiment single, labeled cells were seeded onto the surfaces of unlabeled tissue masses in culture and the subsequent movement of labeled cells was monitored by autoradiography. After 2–3 days of culture a substantial number of single, labeled cells were found within the interiors of tissue masses. Control experiments demonstrated that the observed cellular displacement was due neither to the presence of holes or fissures in the tissue masses through which single cells passively circulated, nor to the pre-treatment of isolated cells with proteolytic enzymes. The same tissues were used in these seeding experiments as were used in the earlier fusion experiments.

Evidence is reported in this communication which indicates clearly that the displacement of single cells from the surface to the interior of a tissue mass is not alone sufficient evidence to conclude that cells 'actively move'[6] in such cases. I repeated the seeding experiments substituting beads of metal, glass and plastic for labeled cells. The beads (Duke Scientific Corporation, Palo Alto, California) were spherical and of approximately cell size: polystyrene, 9.8 micron average diameter; aluminum, 7–15 microns; glass, 10–15 microns; and stainless steel, 8–18 microns. Smooth, round fragments of 5 day em-



Section through a fragment of heart tissue. Arrow indicates a stainless steel sphere which was seeded onto the surface of the fragment and has moved to the interior during 3 days of culture (×250).

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