The Mössbauer spectrum of $^{57}$Fe in rassvumite was taken by means of a 1024 multichannel analyzer and an electromechanical drive system operated at constant acceleration and a symmetric triangular velocity waveform. During the measurement the absorber and source ($^{57}$Co/Rh) were held at room temperature.

The spectrum (Fig. 1) exhibits two resolved lines which were fitted by least-squares methods to one doublet and assigned to iron in tetrahedral sites (Table 2). The rather small half-width $I' = 0.25 \text{ mm/s}$ of these lines confirms this assignment to one crystallographic site. The quadrupole splitting $\Delta E_Q = 0.53 \text{ mm/s}$ indicates a distinct deviation of the Fe-S tetrahedron from cubic symmetry. The existence of only one doublet instead of two, which had to be assigned to Fe$^{2+}$ or Fe$^{3+}$, respectively, can be explained by electron delocalization including the Fe$^{2+}$ and Fe$^{3+}$ ions in neighbouring, crystallographically equivalent sites leading to an intermediate hyperfine interaction in the Mössbauer spectrum. This interpretation is confirmed by comparison of the isomer shift $\delta = 0.29 \text{ mm/s}$ in rassvumite with the isomer shifts $\delta = 0.60 \text{ mm/s}$ of Fe$^{2+}$ in FeCr$_2$S$_4$ and $\delta = 0.16 \text{ mm/s}$ of Fe$^{3+}$ in KFe$_2$S$_4$ [6], where Fe$^{2+}$ and Fe$^{3+}$, respectively, are also found in tetrahedral coordination. The isomer shift in rassvumite is intermediate in value indicating an electron density at the iron nuclei between pure “ionic” Fe$^{2+}$ or Fe$^{3+}$, respectively, which may be referred to valence fluctuations faster than $\approx 10^{-8} \text{ s}$, e.g. the mean lifetime of the excited iron nucleus. These interactions between neighboured Fe ions are facilitated by the small average Fe–Fe distances of 271 pm [3].

Using the empirical formula of Hoggins and Steinink [7] and the average Fe–S distance of 226.4 pm the isomer shift of Fe in rassvumite was calculated by Clark and Brown [3] to be $\delta = 0.30 \text{ mm/s}$, which is in fairly good agreement with the experimental value $\delta = 0.29 \text{ mm/s}$ of this work. The temperatures of the rassvumite synthesis correspond to its formation under “ pegmatic” conditions.

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Table 2. Mössbauer parameters* of $^{57}$Fe in rassvumite, KFe$_2$S$_4$, taken at room temperature

<table>
<thead>
<tr>
<th>$\Delta E_Q$ [mm/s]</th>
<th>$\delta$ [mm/s]</th>
<th>$\Gamma$ [mm/s]</th>
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<tbody>
<tr>
<td>0.53</td>
<td>0.29</td>
<td>0.25</td>
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* $\Delta E_Q = 1/2 e^2 \frac{qQ(1 + 1/3\lambda^2)^{1/2}}{\mu}$ = quadrupole splitting ($\pm 0.01 \text{ mm/s}$), $\delta$ = isomer shift relative to Fe at 295 K ($\pm 0.01 \text{ mm/s}$), $\Gamma = $ full width at half peak height ($\pm 0.01 \text{ mm/s}$)

Fig. 1. Mössbauer Spectrum of $^{57}$Fe in rassvumite, KFe$_2$S$_4$, taken at room temperature. The solid line is a least-squares fit of one doublet to the data

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Proton-Induced Ion Channels Through Lipid Bilayer Membranes

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We reported recently that acetylcholinesterase (ACHEase) can induce ion channels in lipid bilayers in the presence of acetylcholine (ACh) [1]. It was suggested that channel induction might involve a change in the state of the membrane resulting from protonation of lipid head groups [2] due to local pH changes produced by substrate hydrolysis [3]. In the following, we present direct evidence that ion channels [4] can be induced in phospholipid membranes by acidification of the bulk solution on one side of the bilayer, by addition of HCl, acetic acid, or by hydrolytic production of protons using purified AChase.

Bilayer membranes containing the appropriate phospholipids were prepared [5, 6] between two compartments containing unbuffered 1 M KCl (pH ca. 6.5). Acid was added only to one compartment so that, at positive applied voltage, protons in the acidified compartment would be attracted to the bilayer.

Figure 1 shows an experiment with a membrane made with 1-α-phosphatidylcholine Type II-S (Sigma), a crude mixture of soybean lipids. The membrane, of resting conductivity 2 pA/V (2 pS), initially displayed no significant appearance of channels (Fig. 1a at 0.3 μM H$^+$). On addition of small aliquots of 0.1 N HCl, it started to display signs of opening and closing of resolved ion channels (Fig. 1b, 200 μM H$^+$). Channels of manifold conductivities (between 6 and 20 pS in Fig. 1b) occurred, but opening predominated so that, after 1 h, a conductance equivalent to several thousand pS was observed (not shown). The pH was then lowered drastically, to a value approaching 1, by adding 2 N HCl. Membrane conductivity decreased immedi-
Induction was presumably due to protons, since similar activation was achieved with acetic acid. In further experiments, purified AChase [8] was incorporated into the bilayer as described [1]. In such membranes, ion channels could be induced both by its specific substrate ACh (see [1]) and by phenyl acetate, which is also rapidly hydrolysed by the enzyme [9]. A trace showing 200 pS channels induced with phenyl acetate is shown in Fig. 2b. Clearly, channel induction was not due to a direct effect of the substrate on the enzyme, since in control experiments hydrolysis was complete within seconds after its addition.

Present theories of ion-channel formation, whether in artificial bilayers or in native membranes, favour the notion of a polypeptide “ionophore” providing the pore through which ion movement occurs. There is, however, no direct experimental evidence bearing on this issue in biological membranes. Our results demonstrate a direct involvement of the lipid bilayer [10] in pore formation in vitro, by a mechanism in which protonic control [11] of phospholipid head groups may induce area fluctuations [12] which destabilize the bilayer lattice; missing-chain defects [13] of defined conductivity (“ion channels”) may consequently arise in the two-dimensional point lattice [14] of the lipid hydrocarbon tail groups. It can be predicted that any other mechanism leading to area fluctuations could similarly induce ion channels. Indeed, resolved ion channels have already been observed in homogeneous lipid bilayers close to the phase-transition temperature [15]. Yet no caloric phase-transition temperature can be detected in the case of diphytanoyl-lecithin [16] nor in the heterogeneous soybean lecithin preparation [17] employed in our studies. The degree of protonation of lipid head groups may, therefore, characterize an order transition in the bilayer, accompanied by area fluctuations, even under conditions of phospholipid heterogeneity which prevail in biological membranes. It is worth noting that the “proton receptors” have already been described in vivo [18]. Finally, the present experiments support our earlier hypothesis that ACh-induced ion channels in membranes containing AChase are pro-

aturally, returning to a level not significantly different from that at neutral pH (Fig. 1 c), although opening and closing of individual channels was still observed. On lowering the proton concentration to 60 mM with 1 M KOH, the membrane reverted to a state of greatly increased conductivity (Fig. 1 d). This was not due to irreversible damage inflicted by the KOH, since further addition of HCl again reduced the membrane conductivity dramatically (Fig. 1 e).

As expected, ion currents were voltage-dependent, and their direction was reversed upon reversing the sign of the applied voltage (see traces on an enlarged time-scale, Figs. 1 f, g). Mean channel lifetimes appeared longer when the applied voltage was negative (reducing the expected pH gradient across the membrane).

Similar observations were made with pure synthetic diphytanoyl-lecithin [7] (Avanti), as can be seen in Fig. 2a, and with a mixture containing synthetic dioleoyl-lecithin (90% w/w) and cholesterol [20]. Channels were again observed in a critical pH range, ca. 2–3, in the bulk solution.
duced within the lipid bilayer as a consequence of ACh hydrolysis [1, 19]. In this way, other membrane-bound hydrolases such as ion-specific ATPases might similarly induce ion channels through the phospholipid bilayer by the protons produced during hydrolysis in vivo.

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17. Unpublished observations

Early Receptor Potential of Pineal Organ and Lateral Eye of the Pike

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Using intraretinal microelectrodes Brown and Murakami [2] recorded fast summed potentials after an intense flash. This so-called early receptor potential (ERP) is characterized by its very short latency and has been observed in all vertebrate and invertebrate eyes investigated so far [3]. It is probably generated by the direct action of light on the visual pigment in the outer segment of photoreceptors and produced by charge displacements within the pigment molecule [1]. In mixed retinae, the ERP is said to be generated mainly by cones [5] and is, therefore, of particular interest for research on pineal photoreceptors which are cone-like in structure.

So far successful recordings of rapid photoreponses to brief, high-intensity flashes were only obtained from the epiphysis of Rana catesbeiana [6]. Using a similar technique, we recorded these potentials from the exposed pineal organ and the eye cup of the pike, Esox lucius. Regardless the flash luminance and state of adaptation the shape of pineal ERP resembled that of the lateral eye including a R1 component appearing after a very short latency (peak time 0.1 ms) and a R2 component (peak time 0.5 ms) of opposite polarity. A photoresistant potential component, as described for the eye cup, was occasionally seen in the pineal organ.

Recovery in the dark of the ERP after bleaching was similar in pineal organ and eye cup. After an intense flash the amplitude increased from zero to about 50% of the dark-adapted value within

Fig. 1. ERP recovery in the dark of the R2 amplitude of the pineal organ (c) and the lateral eye cup (●) after a light flash equivalent to a steady exposure of 1.7 x 106 cd·m−2. The measurements denote the amplitude of the R2 component at different dark intervals after the bleaching flash, 100% being the amplitude after 2 h in darkness

Fig. 2. Early receptor potentials of the pineal organ (A) and the lateral eye cup (B) of the pike in response to flashes of different spectral lights carrying equal numbers of quanta. Measurements taken 5 min after a constant conditioning flash. The action spectra (C, D) are the means (C) including the standard deviations (D) of five animals. Measurements performed during light adaptation (C, dots and D) and after selective adaptation to red light (C, circles). Dartnall's nomogram v.p. 617 nm (C) and 650 nm (D) are drawn in full, v.p. 533 nm (D) by dashed line