nuclear quadrupole relaxation times. By the conventional $\pi - \pi/2$ pulse sequence the spin-lattice relaxation time, T_1, of ^{23}Na was determined for a sample with the composition (by weight) 64 % lecithin, 16 % sodium cholate, and 20 % water. Since the magnetization decays exponentially with the pulse spacing, one T_1 suffices to describe the experiments. It was found that $T_1 = 1.33 \pm 0.05$ ms, which is an order of magnitude less than the value observed in an aqueous sodium chloride solution ($T_1 =$ 60 ms). Thus a strong interaction between the membrane surfaces and the sodium ions is evident also from this measurement.

Of course, the binding of sodium ions to membrane surfaces is a well-known phenomenon. The details of this interaction are, however, not known and the reason for this is above all a lack of suitable experimental methods. The method proposed in this communication presents several advantages, *e.g.* the negligible perturbation of the system studied and the sensitivity to small changes in interaction strengths. From this preliminary study it may be concluded that it should be possible to use small cations as NMR probes for the examination of interactions close to the membrane model surface. We are currently attempting to investigate certain aspects of ion binding to membrane models by this method, *e.g.* the competition between sodium and other alkali ions and the effect of cholesterol on ion binding.

Experimental. The lecithin was extracted from egg yolk and purified according to the procedure described by Singleton *et al.*[9]

The lamellar mesophase was prepared by adding water to a mixture of sodium cholate and lecithin kept under nitrogen atmosphere. One sample was also prepared from commercially available lecithin (from BDH) and was found to give essentially the same NMR spectrum as the sample obtained from lecithin prepared in our laboratory. The continuous-wave ^{23}Na NMR spectrum was recorded with a Varian V-4200 NMR spectrometer as described elsewhere.[8] The T_1 measurements were performed at 25°C and 23.81 MHz with a Bruker B-KR 322 s pulsed NMR spectrometer.

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Isoelectric Fractionation, Analysis, and Characterization of Ampholytes in Natural pH Gradients. II. Buffering Capacity and Conductance of Isoionic Ampholytes. A Correction

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In the article [1] of the above title published in 1962, there is, unfortunately, a physico-chemical inconsistency which the author has felt irritating ever since; yet its practical importance has not been considered great enough to warrant a correction in the scientific press. The method of isoelectric focusing, however, is now in a state of rapidly growing importance, and

for that reason an error in the basic theory can no longer be tolerated. The inadvertence comes from a failure to distinguish between intrinsic and hybrid dissociation constants.

An imaginary experiment with a hypothetical ampholyte, containing one acidic and one basic group, the pK_a-values of which were both assumed to be 7, was discussed in an earlier article [2] (1956). These dissociation constants were of course meant as intrinsic ones although this was not clearly stated. The conclusion that such a substance, dissolved in water, would have 1/4 in the anionic, 1/4 in the cationic, 1/4 in the zwitterionic, and 1/4 in the undissociated form was quite correct, in spite of what was written in the foot-note on p. 458 in the 1962 article. Consequently, the maximum degree of ionization of an isoionic ampholyte is ½, provided that the zwitterion is not regarded as an ion.

Intrinsic dissociation constants, however, cannot be measured experimentally; they can only be calculated on the basis of certain plausible assumptions. The 1962 article is neither concerned with intrinsic dissociation constants, nor with imaginary experiments. It only deals with constants experimentally measurable by acidimetric titration. These constants differ from the intrinsic ones, and titration of the hypothetical substance defined above would give K_a values of 2×10^{-7} and $\frac{1}{2} \times 10^{-7}$ (*cf.* MacInnes,[3] p. 397). Such constants lying close together are generally called hybrid constants since both protolytic groups are active in both dissociation steps. The inadvertence in the 1962 article consists in the failure to point out that hybrid constants were concerned and in missing the fact that hybrid pK values can never come closer together than log 4. Consequently, $pI - pK_1$ has a lower limit of log 2. The equation for the degree of ionization:

$$\alpha = \frac{2}{2 + 10^{pI - pK_1}}$$

is correct, but the conclusion that its upper limit is 2/3 is false. With $pI - pK_1 =$ log 2, the upper limit for α turns out to be ½ in complete agreement with the 1956 article.

As a consequence of the false conclusion, Fig. 1 in the 1962 article also becomes misleading in that the curve starts at $pI - pK_1 = 0$, which is impossible. The revised curve presented here, with $pK_2 - pK_1 = 2(pI - pK_1)$ on the abscissa, starts at $pK_2 - pK_1 =$ log 4. The ordinate is now given in units of the maximum buffering capacity, (ln 10)/4, of a monovalent weak protolyte. The curve shows that this buffering capacity is still retained at a pK difference of about 1.6 pH units. For $\Delta pK = 3.5$ pH units, the buffering capacity in the isoionic state is still as good as at the edges of a buffering range comprising 3 pH units around the pK of a monovalent weak protolyte.

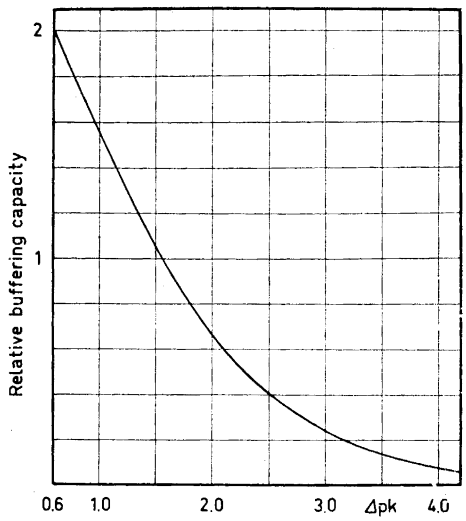

Fig. 1. Relative buffering capacity, in units of the maximum capacity of a monovalent weak protolyte, of an ampholyte in the isoionic state as a function of the pK difference between the dissociation steps on either side of the isoionic point.

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