that time was only between 0.26–0.28 mEq/kg. No lithium was detected in the serum or brain in rats given distilled water or NaCl.

Discussion. The present findings indicate that the LiCl treatment used by JOHNSON in rats causes physical discomfort, extensive damage in the peritoneal cavity, and serum lithium concentrations in the toxic range soon after the injection[5]. Thus, it is unlikely that JOHNSON's findings were due to effects of LiCl on behavioral mechanisms alone, since the LiCl injection he used probably caused several adverse effects in the rats.

The cause of the intestinal damage produced by the 6 *M* LiCl injection is not exactly known. Hypertonicity alone cannot account for it since the hypertonic NaCl injection produced far less damage than the LiCl treatment. Administration of LiCl by i.p. injection also cannot account for the damage since i.p. injection of isotonic LiCl does not cause intestinal hemorrhage (unpublished observation). Further experiments to determine the cause of the intestinal damage produced by the highly concentrated LiCl solution were not carried out because the treatment appeared to be inhumane so that such studies would have been unethical.

Experiment 2. Sixteen 100-day-old rats were randomly divided into 2 equal groups and given a stomach load (10 ml/kg) of either 0.15 *M* NaCl (control group) or 0.15 *M* LiCl (lithium group) twice a day for 10 days. The test employed by JOHNSON[2] to determine the effect of environmental stimuli on rearing was used. It was carried out on a blind basis. A rear was recorded on a hand-operated counter each time the rat raised its head at least 9 cm above the floor of the vertical transparent tube, 46 cm tall and 23 cm internal diameter. After the test, blood and brain samples were taken for determination of lithium concentration by flame photometry.

Results. The lithium concentrations in the serum and brain in the lithium group were 0.79 $\pm$ 0.03 mEq/l and 0.81 $\pm$ 0.02 mEq/kg, respectively. No lithium was detected in the serum and brain in the control group.

Rearing frequencies were significantly lower in the lithium group than in the control group only during the first 5 min of the test (Table). Rearing frequencies decreased significantly during the test in the lithium group and in the control group ($p < 0.05$). Replacement of the withe card by the black one failed to affect rearing significantly in the lithium group or in the control group.

Discussion. The mode of LiCl administration used in Experiment 2 was chosen because it does not produce adverse effects in the stomach or intestine in rats[6], it produces serum lithium levels in the range recommended for the use of lithium salts in the treatment of affective disorders[7], and it enables brain lithium levels to reach a steady-state[8].

The present findings agree with previous reports of reduced rearing in rats given LiCl[2,8–10], but provide no support for JOHNSON's hypothesis that an alteration in the rat's response to environmental stimuli is responsible for the effects of LiCl on rearing; the change in environmental stimuli produced by replacing the white card by the black one led to no difference between rearing in the control and the lithium group. JOHNSON[2] failed to compare the effect of a change in environmental stimuli on rearing in control and lithium-treated animals. In addition, he administered LiCl as a hypertonic i.p. injection that has several nonspecific adverse effects (see Experiment 1). Consequently, there is so far no direct evidence to support JONHSON's hypothesis and, therefore, it cannot be considered to be correct.

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Similarities between Sodium Channels in Excitable Membranes and in Epithelia

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Summary. The inhibitory effects of the pyrazine derivative, amiloride, on sodium transport in an amphibian epithelium has been studied as a function of pH. It is concluded that the charged (guanidinium) group interacts with a negatively charged acid grouping in the membrane. Similarities between sodium channels in excitable membranes and epithelia are highlighted.

At the present time there is considerable interest in macromolecules of cell membranes which control or mediate special cell functions. This note reports on some similarities between the characteristics of two different membrane components which hitherto had not been suspected. While both components mediate the same function – transmembrane translocation of sodium ions – the mechanism by which this is achieved in the two situations is probably very different.

The voltage dependent sodium channels of excitable membranes behave as if they contain a selectivity filter in series with a voltage sensitive ion gate[1,2]. The selectivity filter is blocked by the toxins, tetrodotoxin and saxitoxin[3] and also by hydrions when the external pH is lowered[4,5]. Analysis of the voltage dependence of the blocking action of hydrions in frog nodes indicated that the selectivity filter behaves as a singly ionized acid grouping, located one quarter of the way across the membrane from the outside, with a pKa of 5.4 at zero potential[6]. The toxins have a high affinity for sodium channels (around 10^9 M^{-1}) and interact with a probable stoichiometry of 1:1. Tetrodotxin (pKa 8.8) is less active at alkaline pH[7] suggesting that the cationic form is more

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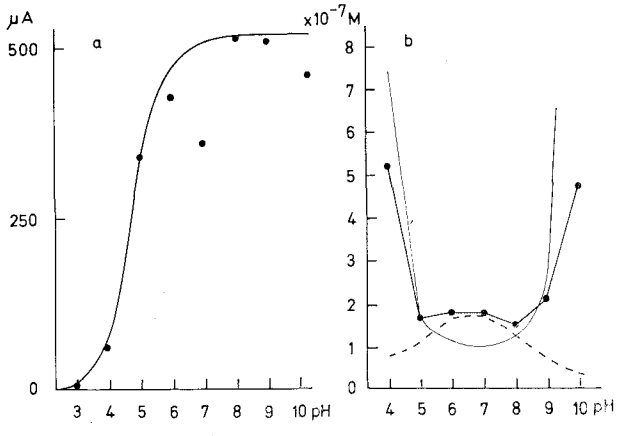
active than the zwitterion in blocking sodium channels. Specific binding of the toxins to sodium channels is impaired at acid pH values[8] indicating that cationic forms and protons compete for occupation of the binding sites. However, unlike the inhibition of sodium permeability by protons, the blocking effects of the toxins do not appear to be potential dependent[9].

Thus the evidence suggests that sodium channels in excitable membranes contain a singly ionized acid grouping, which interacts with the cationic form of the toxin molecules, other parts of the toxin molecule being important for increasing the total binding energy with the channel. The acid grouping of the channel may be required to lower the free energy required to dehydrate sodium during its passage through the selectivity filter[10].

Sodium ions also move through the mucosal membranes of sodium transporting epithelia, such as those of the kidney, colon, salivary duct and a number of amphibian epithelia. Considerable attention has been devoted to the movement of sodium through the mucosal membranes of amphibian epithelia. In contrast to sodium flux in excitable membranes, where the fully activated I/V relations are either linear or obey the Goldman equation[11], the movement of sodium into epithelial cells appears to be by facilitated diffusion[12]. Little attention has been given to the selectivity mechanism of sodium channels in epithelia, although it is known that lithium can be readily handled by the channels[13]. Tetrodotoxin has little or no effect on sodium ion movement in epithelial membranes[14], however, the pyrazine derivative, amiloride (*N*-(*N*-amidino)-3, 5-diamino-6-chloropyrazine carboamide), has a high affinity for the sodium entry sites in epithelia[15, 16].

Materials and methods. Sodium transport in isolated frog abdominal skin (*Rana temporaria*) was measured as short circuit current (SCC). The solution bathing both sides of the skin had the following composition (m*M*) NaCl, 111; KCl, 2; $CaCl_2$, 1 and glucose, 11. The serosal solution was buffered at pH 7.6 with *tris* buffer (5 m*M*). The mucosal solution was unbuffered, the pH being controlled with a pH stat (Radiometer). Amiloride was added only to the mucosal bathing solution. K_m values for amiloride at different pH values of the mucosal solution were taken as the drug concentration giving 50% inhibition of the amiloride sensitive current.



Results obtained with a piece of frog (*R. temporaria*) abdominal skin (7.5 cm^2). Amiloride was added to the mucosal bathing solution. The sodium current (μA) was taken as that current which was sensitive to amiloride (10^{-4} *M*), and is shown versus pH in (a). The line is the titration curve for monobasic acid with a pKa of 4.8. K_m values for amiloride at different pH values are shown in (b) as solid symbols. The thin continuous line indicates the predicted behaviour (see text) of K_m with pH, scaling the values so that theoretical and experimental values coincide at pH 5. The pKa of amiloride was taken as 8.7. The interrupted line shows values of the true K_m for amiloride in the presence of 111 m*M* sodium, calculated from measured values of K_m together with pH, K_{amil} and K_R (see text).

Results and discussion. With the mucosal solution controlled at pH values between 5–10 amiloride, in sufficient concentration, caused complete inhibition of SCC. However, at pH 3–4 not all of the current was sensitive to amiloride. The residual, amiloride resistant, current may have been due to a mucosal to serosal proton flux. The Figure shows the result of a single experiment. The sodium current (amiloride sensitive SCC) varied with pH in a way which suggested that a grouping with a pKa of around 5 was responsible for controlling sodium entry. Deviations from the theoretical titration curve were not systematic, as was found when the results from several experiments were pooled. Amiloride, like the toxins referred to earlier, has a guanidinium group. The pKa of amiloride is only 8.7, yet guanidine itself has a pKa of 13.6. The pKa is lowered by the presence of the pyrazine ring and particularly the carbonyl group, as for example in acetylguanidine (pKa 8.26). As with tetrodotoxin the affinity of amiloride is reduced at alkaline pH, due presumably to the decline in the fraction of the guanidinium form. At acid pH values protons compete with amiloride for the binding sites as shown by the reduced affinity at pH 4. Thus both tetrodotoxin and amiloride appear to require a guanidinium group for blocking activity, the only difference being that this charge is effectively neutralized by zwitterion formation in tetrodotoxin and loss of a proton in amiloride.

If it is assumed that only the charged form of amiloride competes with protons for a singly ionized acid grouping in the mucosal membranes with a pKa of 4.8, and that combination of this grouping with either a proton or amiloride prevents the sodium translocation process, then the relationship between the true and measured K_m for amiloride is given by

$$K_{measured} = K_{real}\left[1 + \frac{K_{amil}}{[H^+]} + \frac{[H^+]}{K_R} + \frac{K_{amil}}{K_R}\right]$$

where K_{amil} and K_R are the dissociation constants of amiloride and the membrane receptor respectively. This simple kinetic approach gives predictions which are reasonably consistent with the experimental results. The Figure is typical of several experiments, and experiments with two other inhibitors of sodium transport (triamterene and *N*-(*N*-benzylamidino)-3, 5-diamino-6-chloropyrazine carboxamide) with widely differing pK's and affinities also gave results consistent with this approach.

Thus the similarity of the interactions between sodium channels in excitable membranes and tetrodotoxin, and epithelial sodium channels and amiloride is striking. In both instances the inhibitors have guanidinium groups,

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which appear to interact with an acid grouping with a pKa of around 5. Also, for both types of blocker the effect of pH modifies this interaction in a predictable way when the pK's of the drugs and receptors are taken into account. It is not known if the amiloride binding site which controls sodium access in epithelia is a selectivity filter controlling entry to a carrier mechanism or whether it forms an integral part of a carrier.

The blocking effects of tetrodotoxin (and saxitoxin) have been demonstrated in a wide variety of excitable tissues in both vertebrates and invertebrates. Similarly, amiloride sensitive sodium channels are found not only in mammals, but in other vertebrates and lower forms such as insects and annelids[17]. The wide distribution of both types of channel and the similarities outlined in this report suggests they may have a common ancestry.

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Conditioned Suppression: Dissociation of Learning in Baclofen Treated Rats

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Summary. In rats, baclofen induced a memory deficit related to a dissociation of learning. Baclofen given prior to training or prior to testing had no effect on the amnesia elicited by diazepam.

Some biochemical, electrophysiological or pharmacological effects induced by the benzodiazepines seem to be related to their actions on central GABA-(γ-aminobutyric acid)-ergic mechanisms[1]. The involvement of a GABA link in some behavioral effects of benzodiazepines was examined in a previous work[2], and it was found that baclofen – a compound structurally related to GABA – enhanced the food intake of rats placed in a non-familiar situation, as benzodiazepines did. The purpose of this work was to give some information about the role of a GABA-ergic mechanism in the amnesic effect of benzodiazepines[3]. With that aim, an amnesic effect, and a modification of diazepam-elicited amnesia eventually induced by baclofen, were investigated in rats.

Material and methods. The experiments were carried out on male Wistar A.F. rats (180–200 g). The animals were housed 8 per cage with free access to food and water, unless otherwise noted, and maintained in 12/12 h light-dark cycle. The test situation was a (36 × 36 × 30 cm) translucent box. A drinking bottle was located in a corner of the box. A metal drinking-tube terminated the bottle at a height of 3 cm above an electrifiable part of the floor (22 × 10 cm). The rats were deprived of water (but not of food) during the 16 h preceding their introduction into the test apparatus.

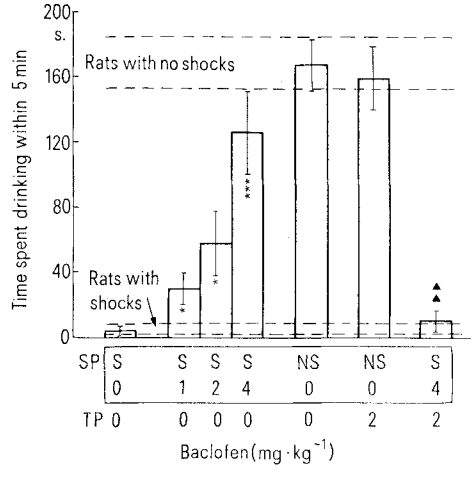

Fig. 1. 'Test phase'. Effects of baclofen given before either the 'shocks phase' (SP) or the 'test phase' (TP) on the drinking inhibition of rats. S, rats with shocks; NS, Rats with no shocks. *, **, ***, indicate that S treated rats differ as compared to S control rats at 0.05, 0.02 and 0.01 level respectively. ▲▲, indicate that S rats, given baclofen before both SP and TP differ at 0.02 level from animals given baclofen only before SP. Vertical bars represent SEM.

'Shocks phase'. Each rat was placed in the test situation and shocked (2 mA) as soon as it started to drink. After this electric shock, the animals remained 1 min in the apparatus, and each time they drank, they were shocked again. Previous experiments have shown that rats generally start drinking within 2 min and did not tolerate more than 2 shocks. The rats which do not drink within 2 min and which receive more than 2 shocks, were eliminated of the study. Rats with no shocks were given a 3 min placement in the apparatus without any shock.

'Test phase'. 4 days after the 'shocks phase', each rat was placed for 5 min in the test situation without any shock. During this period the time spent drinking was recorded to the nearest second with a manually operated chronometer.

Drugs were administered (1 ml/100 g) 30 min before the 'shocks phase' and/or the 'test phase'. Baclofen (β-parachlorophenyl-γ-aminobutyric acid) s.c. and diazepam i.p. were injected, as suspension with acacia gum. The statistical comparison between groups (10 to 12 rats per group) was done using the Student's t-test or Darmois' t-test.

Results. Control rats with shocks exhibit, as compared to control rats with no shocks, a marked drinking behavior inhibition (Figure 1). In rats treated with baclofen 30 min before the 'shocks phase', this drinking inhibition was reduced or even suppressed. Baclofen (2 mg.kg^{-1}) given 30 min before the 'test phase' abolished the effect of the administration of this drug before the 'shocks phase'. At this dose, baclofen had no effect on the drinking time either of rats with no shocks, or of rats with shocks (15 ± 7 sec); Figure 2 shows that baclofen (1 mg.kg^{1}) did not statistically modify diazepam-induced reduction of the drinking inhibition. In rats with shocks, baclofen

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