which the formaldehyde production was evaluated. Studies of GRAM et al.[12] suggested that the time-course of production of 4 AP from aminopyrine in the rat liver seems to be different from that of the production of formaldehyde. The ratio, formaldehyde: 4 AP, produced from a given amount of aminopyrine, markedly varies with animal species, the duration of incubation and the pretreatment of the test animals with inducers. Our experiments indicated that this ratio may also be influenced by the presence of inhibitors, since the increase of the OSA concentration in the enzyme preparations is associated with a parallel reduction of the amounts of formaldehyde produced relative to 4 AP formed (Table II). Thus, in the overall reaction of aminopyrine to formaldehyde, the removal of one methyl group from the tertiary amine is affected by the OSA inhibition more than the demethylation of the metabolite 4-methylaminoantipyrine.

The detailed mechanism, by which OSA produces inhibition of the aminopyrine metabolism, is unknown. Since OSA has the ability to form complexes and addition compounds with various metals[13], it may perhaps react with cofactors or other components of the microsomal membranes. However, the possibility cannot be excluded that OSA may function as an alternative substrate of the microsomal enzyme systems.

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Reexamination of Vertical Activity in Rats Treated with Lithium Chloride

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Summary. Injection of hypertonic LiCl i.p. has several nonspecific adverse effects in rats. No evidence was obtained for JOHNSON's hypothesis that the effect of LiCl on rearing is mediated by environmental stimuli.

JOHNSON[2] observed a decrease in vertical activity (rearing) in rats given an i.p. injection of 6 mEq LiCl/kg 15 min before the activity test compared to rats given an i.p. injection of distilled water. I planned to use the same method of LiCl administration employed by JOHNSON in order to gain information about the mechanism of action of lithium on behavior, but when I injected the hypertonic LiCl solution in rats I found that the treatment was extremely noxious. Thus, my first experiment was carried out to determine whether the LiCl treatment used by JOHNSON had pronounced adverse effects, so that his findings could not be considered to have been due to effects of LiCl on behavioral mechanisms alone.

JOHNSON was interested in the role of environmental stimuli in the effects of LiCl on behavior. He found that rearing was lower in LiCl-treated rats than in controls tested in a relatively narrow vertical transparent tube, and that a change in environmental stimuli produced by replacing a large white card in the rat's visual field by a black one affected rearing in control animals. But JOHNSON's conclusion that environmental stimuli affected control rats and LiCl-treated rats differently was fallacious (non sequitur)[3] because he failed to determine directly the effect of a change in environmental stimuli on rearing in LiCl-treated rats. Thus, my second experiment was carried out to determine whether a change in environmental stimuli affects rearing differently in control rats and LiCl-treated rats.

The effects of environmental stimulation on vertical activity (rearing) in rats given stomach loads of 0.15 *M* NaCl (control) or 0.15 *M* LiCl (lithium group) twice a day for 10 days

Group	White stimulus card			Black stimulus card
	5-min period			5-min period
	1	2	3	1
Control	27.1 ± 9.2	14.0 ± 4.2	8.1 ± 7.6	4.9 ± 3.9
Lithium	15.9 ± 7.4[a]	12.5 ± 3.6	4.4 ± 4.3	2.6 ± 2.2

Values are means ± SD for 8 rats per group. [a] Significantly different from control group at $p < 0.02$.

Materials and methods. Experiment 1. Nine 100-day-old male albino Wistar rats were randomly divided into 3 equal groups and given an i.p. injection of either 6 mEq LiCl/kg (0.1 ml/100 g body weight of 6 *M* LiCl), distilled water (0.1 ml/100 g body weight) or saturated NaCl (0.1 ml/100 g body weight of ca. 5.8 *M* NaCl). The rats were killed 15 min later in ether anesthesia, the peritoneal cavity was opened and color photographs of it were taken[4]. The concentration of lithium in the brain and in serum was determined by flame photometry.

Results. Signs of physical discomfort appeared in the rats given LiCl; they squealed briefly immediately after the injection and then vigorously licked the site of injection for about 1 min. Thereafter, they became prostrate and remained in this position until being placed in the ether jar. Inspection of the peritoneal cavity showed that 40–60% of the small intestine was distended, hemorrhagic and inflammed.

The rats treated with NaCl squealed briefly, licked the site of injection and then became prostrate. However, in contrast to the LiCl treatment, only about 10% of the small intestine was distended in rats given NaCl and hemorrhage in the peritoneal cavity was never observed.

The rats given distilled water neither squealed nor licked the site of injection. They did not become prostrate and showed no signs of damage in the peritoneal cavity.

In rats treated with LiCl, the concentration of Li^+ in the serum ranged between 7.2–11.2 mEq/l 20 min after the injection, while the concentration of Li^+ in the brain at

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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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