Results and discussion. The Table shows that metabolic properties of myocardial tissue were not modified at the lanthanum concentrations tested. Therefore, in all successive experiments using spontaneously beating atria, the highest concentration of lanthanum (50 μM) has been used. In these experimental conditions, lanthanum causes a significant decrease in peak tension: the effect is already evident after 5 min, reaches the maximum (about 30% diminution) after 30 min, and remains practically constant thereafter (Figure 1). Since lanthanum does not modify metabolic properties of cardiac muscle, it can be excluded that the development of the hypodynamic state was due to a lack of energy.

Effect of different concentrations of lanthanum on the oxygen consumption in guinea-pig heart muscle

Treatment		Concentration (μM)	O_2 consumption[a] (μl/min/g tissue)	P
–	(12)	–	2.29 ± 0.06	–
Lanthanum	(12)	5	2.39 ± 0.04	n.s.
Lanthanum	(12)	50	2.28 ± 0.05	n.s.

[a] Mean ± SE.
Number of determinations is given in brackets.

The investigation of calcium distribution in isolated atria after each incubation period has shown that no differences exist between control and lanthanum-treated preparations (Figure 2). The mean total Ca content of control atria was 6.69 ± 0.30 γ/100 mg fresh tissue and 6.59 ± 0.18 in atria exposed to lanthanum. These results demonstrate that lanthanum does not produce any modification of total tissue calcium content; therefore, it can be concluded that changes in Ca content are not responsible for the decline in peak tension. Figure 2 also depicts the ^{45}Ca exchange curves. As shown in this graph, the size of total exchangeable fraction was not significantly different in controls and lanthanum-treated atria (69.9 ± 4.1 and 66.6 ± 1.9 respectively). On the contrary, a considerable difference was found in the rate of exchange between control and treated atria. In fact, in the former group equilibrium was attained after 30 to 45 min, whereas in lanthanum-treated atria calcium exchanges proceeded considerably slower, reaching equilibrium in about 60 min.

For each of the curves obtained it was possible, according to CARRIER et al.[5], to distinguish 2 components: an early phase of fast exchanging processes, and a second phase of slowly exchanging processes. The fast phase of the calcium exchanging curve is considered to represent calcium located in membraneous structures facing the extracellular space[5], whereas the slowly exchanging phase probably represents intracellular membrane-bound calcium[8]. Lanthanum significantly decreases the rate of the former phase, whereas the second one seems not to be affected. Therefore, it can be concluded that lanthanum inhibits transmembrane calcium movements and seems not to affect the intracellular calcium compartment.

This investigation also confirms that the fast exchanging compartment is correlated to the contractile force developed by heart muscle.

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The Effect of Oxine-5-Sulphonic Acid on the Hepatic Drug Metabolism in the Rat

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Summary. Oxine-5-sulphonic acid inhibits the metabolism of aminopyrine in the rat liver in vitro. The characteristics of this inhibition vary according to whether the oxidative *N*-demethylation of the substrate is determined by the formation of the metabolite 4-aminoantipyrine or by the production of formaldehyde.

Recent investigations have indicated that certain quinoline derivates are potent inhibitors of the drug-metabolizing enzyme systems of liver microsomes. Ethoxyquin was shown to be a competitive inhibitor of the rat hepatic microsomal biphenyl-4-hydroxylase and of ethylmorphine-*N*-demethylase in vitro[2]; in addition, the glucuronidation of *p*-nitrophenol and 4-methylumbelliferone was inhibited by oxine (8-hydroxyquinoline) in enzyme preparations also obtained from rat liver microsomes[3].

The present paper concerns the influence of the oxine-5-sulphonic acid (OSA) on the hepatic microsomal drug metabolism in vitro.

Methods. Male Wistar rats, weighing between 180 and 220 g, were used in all the experiments. The animals were exsanguinated, then the livers were perfused with cold 1.15% KCl and removed. The tissue was homogenized in 3 volume of ice-cold 1.15% KCl, using a Potter-Elvehjem homogenizer coupled with a motor-driven pestle. The homogenates were centrifuged at 10,800 *g* for 20 min to remove cell debris, nuclei and mitochondria. After discarding the floating fat layer, the 10,800 *g* supernatant fraction was further centrifuged for 90 min at 105,000 *g* using a Beckman L5-50 ultracentrifuge. The resulting supernatant fraction was collected and the microsomal pellet resuspended in 1.15% KCl. Protein concentration was determined according to the LOWRY[4] procedure using bovine serum albumin as the standard. The microsomes were diluted to a concentration of 3 mg of protein/ml and the microsomal suspension was then recombined

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Table I. Influence of various concentrations of 8-hydroxyquinoline-5-sulphonic acid (OSA) on the hepatic metabolism of aminopyrine in vitro

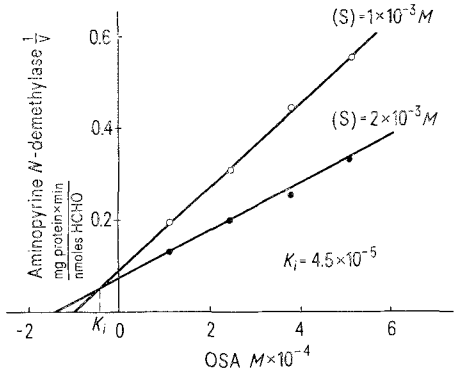
OSA concentration (mM)	Aminopyrine metabolism[a]	Inhibition (%)
No OSA	0.293 ± 0.007[b]	–
0.026	0.262 ± 0.011[c]	10.58
0.13	0.191 ± 0.009[d]	34.81
0.26	0.159 ± 0.004[d]	45.73
0.44	0.148 ± 0.008[d]	49.49
0.65	0.139 ± 0.012[d]	52.56

[a] Activity expressed as nmoles 4 AP formed/mg microsomal protein/min. Procedures for the determination of 4 AP formation and composition of the enzyme mixtures are detailed in Methods. Initial concentration of the substrate: 2×10^{-3} M; incubation time: 1 h at 37 °C. [b] Values are the means (± SE) of 5 experiments. The statistical significance of the changes by OSA as compared to the controls has been calculated by Student's t-test and has been denoted as follows: [c] $p < 0.05$. [d] $p < 0.001$.

Table II. The influence of various OSA concentrations on the formaldehyde and 4 AP produced per unit of aminopyrine added to the microsomal preparations from rat liver.

OSA in the enzyme mixture (mM)	HCHO/aminopyrine[a]	4 AP/aminopyrine[a]	HCHO/4 AP[b]
No OSA	35.29	1.75	20.07
0.13	20.97	1.14	18.30
0.26	14.01	0.95	14.69
0.44	9.22	0.88	10.39
0.65	6.96	0.83	8.34

[a] Ratios are expressed as nmoles of formaldehyde or 4 AP produced per mg of microsomal protein per micromole of aminopyrine added to the incubation mixtures. Initial concentration of aminopyrine: 2×10^{-3} M. [b] nmoles of formaldehyde formed per nmoles of 4 AP formed.



Dixon plots showing competitive inhibition of aminopyrine-N-demethylase activity by 8-hydroxyquinoline-5-sulphonic acid (OSA). Aminopyrine N-demethylase activity (nmoles formaldehyde/mg protein/min) was assayed at substrate concentrations of 1×10^{-3} M (○) and 2×10^{-3} M (●) and OSA concentrations of 1.2–5.2×10^{-4} M.

with the soluble fraction diluted to concentration of 6 mg of protein/ml. The aminopyrine metabolism was assayed in typical incubation mixtures containing $MgCl_2$ (7 mM), glucose-6-phosphate (1.5 mM), NADPH (0.5 mM), 1.0 ml of 0.1 phosphate buffer, pH 7.4, 2.0 ml of supernatant (microsomes + the soluble fraction), aminopyrine (1.0 or 2.0 mM) and desired concentrations of OSA in a final volume of 5 ml. Reaction mixtures were incubated at 37 °C under air in a Dubnoff metabolic shaker.

The N-demethylation of aminopyrine was measured by the quantitation of the metabolite 4-aminoantipyrine (4 AP)[5], or by assaying the formaldehyde production[6].

Results. The action of OSA on the NADPH-dependent metabolism of the aminopyrine in the rat liver preparations is shown in Table I; OSA appeared to have an inhibitory effect on the N-demethylation of the aminopyrine to 4 AP in vitro: a statistically significant decrease of the 4 AP production was observed at OSA concentrations higher than 2.6×10^{-5} M, and the enzyme activity was reduced to about 50% of the control values at OSA concentrations of 4.4×10^{-4} M.

The metabolism in vitro of the aminopyrine, in the presence of different concentrations of OSA, was also evaluated by assaying the formaldehyde production in the enzyme mixtures incubated for 30 min. This experiment confirmed that OSA is an inhibitor of the aminopyrine N-demethylase in vitro (Figure). The inhibition was of the competitive type, and the apparent K_i value was 4.5×10^{-5} M.

Discussion. Although various studies were performed regarding the pharmacology of aminopyrine in combination with OSA, both in animals[7–9] and in man[10,11], the mechanism of the interaction of the above compounds is still unknown. Results of the present investigation indicated that OSA acts as a competitive inhibitor of the aminopyrine N-demethylase in the rat liver. It has been shown[5] that the metabolism of aminopyrine to 4 AP occurs in 2 steps: in the first step aminopyrine is converted to 4-methylaminoantipyrine (4 MAP); this metabolite is then demethylated to 4 AP.

$$\text{Aminopyrine} \longrightarrow \text{4 MAP} + \text{HCHO}$$
$$\text{4 MAP} \longrightarrow \text{4 AP} + \text{HCHO}$$

The second reaction occurs very slowly because it is inhibited by the aminopyrine present in the incubation mixture[12]. It is therefore possible that OSA may reduce the production of 4 AP from the aminopyrine, either by direct inhibition of the enzyme activity or indirectly as a consequence of the higher aminopyrine concentrations remaining in the enzyme mixtures during the incubation.

In the experiments in which the aminopyrine metabolism was measured by the formation of the metabolite 4 AP, the inhibitory effect of OSA on the enzyme activity dramatically increased with the concentration of OSA in the mixtures up to 2.5×10^{-4} M. At the highest OSA concentrations, only a slight increase of the inhibitory effect was obtained, in contrast to the experiments in

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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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