zyme synthesis. The present experiment does not provide information from which the mechanism of increase can be determined. Increased enzyme synthesis might account for the increased MDH activity observed with training but would not seem to be a likely mechanism in the increased activity associated with acute exercise. Whatever the mechanism, it is noteworthy that, in the rat, an increased activity of plasma MDH is elicited by two quite different forms of exercise, namely swimming and treadmill running and as stated previously(3), the failure of acute exercise to increase plasma MDH activity in the trained animal offers possibility of using plasma MDH activity as a criterion of training.

Any form of exercise, particularly if unusual to the rat, could constitute a form of stress and this, rather than the physical activity *per se*, might conceivably cause the observed alterations of enzyme activities. It would seem worthwhile to investigate the possible role of stress by carrying out experiments inducing stress in the absence of physical activity, using adrenergic blocking agents, and using various hormones. Such experiments are currently planned.

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Sodium and Potassium Excretion in Rats Treated Chronically with Morphine.* (31553)

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Several investigators have shown that rats developed tolerance to the antidiuretic action of narcotic analgetics with ensuing polyuria as treatment was continued (1,2,3,4). Since the acute antidiuretic action of morphine in the rat is mediated in part by release of antidiuretic hormone(5), one suggestion is that this tolerance may arise by virtue of the rats becoming less responsive to endogenous antidiuretic hormone(1,2,3). Chronically morphine treated rats were less sensitive to the antidiuretic action of vasopressin(1,2). Also Shimai *et al*(1) found decreased concentrations of ADH in the blood and decreased Gomori staining granules in the hypothalamo-hypophyseal system. Newsome *et al*(3) working with levorphanol found that the blood from tolerant rats which were polydipsic and polyuric bioassayed for large concentration of ADH-like material. If indeed this material were ADH, the hypothesis of tolerance through a change in ADH mediated mechanism would be further supported.

Since sodium and potassium ion depletion is known to diminish responsiveness to vasopressin(6), the present study investigated the excretion of these ions in rats developing tolerance to the antidiuretic effect of morphine.

Material and methods. Sprague-Dawley male rats weighing between 250 and 280 g were treated subcutaneously at 8 a.m., 4 p.m. and 12 p.m. with morphine sulfate, 8 mg/kg. This t.i.d. dose was increased by 8 mg/kg every day, except on the fifth day when the increase was 16 mg/kg. By the 15th day, the

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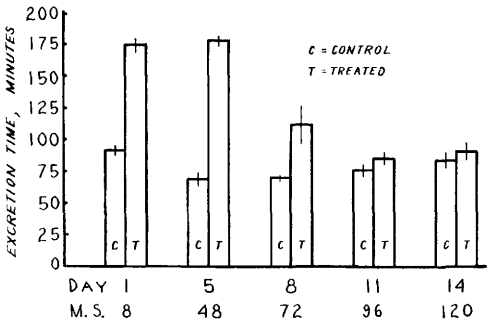

FIG. 1. Effect of morphine sulfate on excretion time of urine in water loaded rats. Abscissa gives treatment schedule with morphine sulfate, M.S., t.i.d. Each determination represents the mean of at least 16 rats in each group. Vertical lines indicate standard errors.

rats were receiving 128 mg/kg, t.i.d. Control rats were given t.i.d. injections of distilled water.

The antidiuretic action of morphine was determined by gavaging the rats with a water load of 5% of the body weight and measuring the excretion time according to the method of Burn *et al*(7). These measurements were made every 3 or 4 days as indicated in the *Results*. In another experiment, the rats in groups of 2 were kept in metabolism cages and urine was collected in Erlenmeyer flasks. Food was withheld but free access to water was provided during the 16-hour collection period from 4 p.m. to 8 a.m. After measuring the urine volume with 50 ml graduated cylinder, samples of urine were put in the freezer for later determination of potassium and sodium. On the 15th day, the rats were decapitated one hour after administration of the last dose of morphine. Samples of blood were taken and plasma osmolalities measured with a Fiske Osmometer. Pieces of muscle from the hind leg were extirpated, weighed and stored in the freezer. Water content of the muscles was measured by drying the tissue at 90°C in a platinum crucible until constant weight was obtained. The difference between this weight and the initial wet weight was taken as the water content. The crucibles were then put in a muffle furnace at 550°C overnight and after appropriate dilution, sodium and potassium contents were measured with a Baird Atomic flame photometer.

Results. In Fig. 1, an acute dose of morphine sulfate, 8 mg/kg, on day 1 produced definite antidiuresis as indicated by the increase in excretion time over control. By the eleventh and fourteenth days of treatment, there was no difference between treated and controls even though the dose had been progressively increased. Thus, tolerance had developed to the antidiuretic effect of morphine even though the dose of morphine had been raised daily up to 120 mg/kg on day 14.

Data in Table I indicate that chronic treatment with morphine increased the excretion of sodium on the 4th, 8th and 11th days of treatment. The increase in urinary potassium seemed to follow the pattern of sodium excretion although consistent differences were not obtained. No differences in urine volumes were found.

In order to see whether the increased excretion of ions in the urine occurred along with depletion in the tissues, plasma and muscles were analyzed. Table II indicates that the concentration of sodium and potassium in these tissues was not changed. Since chronic treatment with morphine had been reported to cause retention of water in muscles (8), water content of muscle was measured on day 15. Water content was not different: 77.5 $\pm$ 0.2% (S.E.) for tolerant rate and 77.3 $\pm$ 0.1% for controls. Furthermore, plasma osmolalities were not different at this time: 295 $\pm$ 3 and 297 $\pm$ 4 mOsm/l for control and treated groups respectively.

Discussion. In initial treatments morphine produced a definite increase in sodium excretion. Tolerance to this effect developed so that on the 14th day of treatment, differences were no longer observed between morphine and control groups. Although the output of potassium was significantly increased on the 4th day, the effects on potassium excretion were not so striking. In contrast to our data, Tomizawa *et al*(2) reported rats chronically treated with morphine excreted less sodium while retaining sodium in tissues. Their rats appeared to be in a diuretic phase since the excretion time to a water load was shortened and the daily urine output was greater than in controls. Thus

TABLE I. Mean Urinary Excretion of Sodium and Potassium.

Day of treatment	Morphine sulfate, mg/kg, t.i.d.		Mean urinary volume, ml/16 hr	Sodium concentration ± S.E.		Potassium concentration ± S.E.	
				mEq/l	μEq/100 g/16 hr	mEq/l	μEq/100 g/16 hr
4	32	Treated	14	77 ± 7*	393 ± 36*	108 ± 5*	544 ± 69*
		Control	15	38 ± 5	189 ± 18	70 ± 3	354 ± 20
8	72	Treated	13	84 ± 5*	395 ± 94*	90 ± 10	415 ± 38
		Control	15	45 ± 7	238 ± 35	65 ± 9	331 ± 41
11	96	Treated	15	91 ± 11*	562 ± 77*	114 ± 24	641 ± 54*
		Control	13	55 ± 10	213 ± 35	87 ± 10	343 ± 28
14	120	Treated	9	89 ± 8	269 ± 26	90 ± 8	281 ± 23
		Control	12	60 ± 8	221 ± 25	89 ± 9	312 ± 22

* $P < .05$, control *vs* treated assessed by "t" test. These values represent the mean of at least 12 rats for each group.

these results may represent what happens at a late phase. It would appear then that initially morphine produces increased sodium excretion which returns to control values and then decreases as treatment is continued. This picture of the occurrence of several phases to the development of tolerance is seen even better in the results of Newsome *et al*(3) with levorphenol. Their excretion time data showed an initial phase of antidiuresis followed by a return to control times and subsequently a phase of diuresis with decreased excretion times. It appears that sodium excretion is increased during the antidiuretic phase, back to control levels during the intermediate phase, and decreased during the diuretic phase.

It is tempting to speculate that ADH is involved in these effects of morphine on sodium and possibly potassium excretion. High doses of vasopressin are known to increase urinary sodium and potassium excretion(9). Based on work of de Bodo(10) and Giarman *et al*(11), one would expect high blood levels of ADH during the antidiuretic phase. What happens to the ADH system on chronic treatment with narcotics? Newsome *et al*(3) found extremely high concentrations of ADH-like activity in the blood of rats during the diuretic phase of chronic levorphanol treatment. These results would be suggestive of a decreased responsiveness to endogenous ADH at the late phase. On the other hand, Shimai *et al*(1) reported initial high concentration followed by extremely low ones as treatment was continued with morphine. In addition, these rats were less responsive to the exogenously administered ADH preparation during the diuretic phase. To further complicate the problem, Marchand and Fujimoto(12) found that morphine affected the rate of disappearance of exogenously administered vasopressin. It is evident that the complexity of the problem requires guarded interpretation of the results at this time.

TABLE II. Mean Sodium and Potassium Concentration in the Muscle and Plasma.

		Sodium	Potassium
		(mEq/kg wet wt ± S.E.)	
Muscle	Control (10)	19.0 ± .9	106.8 ± 4.4
	Tolerant (10)	21.2 ± .4	107.1 ± 2.3
		(mEq/l ± S.E.)	
Plasma	Control (7)	135.0 ± 1.2	3.2 ± .04
	Tolerant (8)	139.8 ± 2.5	3.0 ± .07

( ) = No. of rats.

Summary. Tolerance has been shown to develop to the antidiuretic effect of morphine upon chronic administration. Urinary excretion of sodium and potassium increased initially but returned to control values as treatment was continued for 14 days. No changes in muscle or plasma concentrations of these ions were seen.

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Galactose Metabolism in the Sea Lion.* (31554)

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Galactosemia is characterized by the lack of galactose-1-P uridyl transferase (UDP glucose: α-D-galactose-1-phosphate uridyl transferase 2.7.7.12), an important enzyme of galactose metabolism. When infants afflicted with this inborn error of metabolism are fed galactose, they develop cataracts, cirrhosis, malnutrition, and mental retardation. The milk of the sea lion is devoid of carbohydrate and hence, galactose(1). Sunshine and Kretchmer have shown that neither of the disaccharides lactose or sucrose are absorbed by the sea lion intestine, but that glucose is absorbed and apparently metabolized(2). It seemed possible that this animal had lost its capacity to metabolize galactose and might serve as an admirable model for study of the pathogenesis of galactosemia in man.

Materials and methods. Two California sea lions (*Zalophus californianus*) were investigated. One weighed 12 kg and was approximately 3 months of age; the other weighed 32 kg and was approximately 16 months old. Galactose-1-phosphate uridyl transferase was assayed by our modification of the UDPG consumption assay(3). Galactokinase (ATP: D-galactose-1-phosphotransferase 2.7.1.6) activity was estimated by measuring the phosphorylation of galactose-1-C14 by a minor modification of the system described by Ng *et al*(4). Blood galactose levels were determined by the method of Roth *et al*(5).

Results. The galactokinase and galactose-1-phosphate uridyl transferase activities of the red cells of the infant and sub-adult sea lions are presented in Table I and are compared with values as obtained on normal human subjects. Liver tissue from the infant sea lion was also examined for galactokinase and galactose-1-phosphate uridyl transferase activity. High levels of activity of both enzymes were found, but the procedures used were not adequate for quantitation of enzyme activity in this tissue.

The sub-adult sea lion (32 kg) was given 0.91 g of galactose/kg body weight and serial estimations of blood galactose levels were made on blood obtained by flipper puncture. The results of these studies are shown in Fig.

TABLE I. Galactokinase and P-Gal Uridyl Transferase Activity in Human and Sea Lion Blood Samples.

Specimen		Galactokinase activity, μMole Gal-1-P formed/hr/g Hb	P-gal uridyl transferase activity, μMole UDPG consumed/hr/g Hb
Sea lion	Infant	1.46	11.11
	Sub-adult	1.53	8.62
Human	Infant	2.40*	24.0 (approx.)
	Adult	.91*	24.0

* Average value from Ng, Donnell and Bergren (4).

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