newborn rats, but immunosympathectomy blocks that response (Table 1), innervation seems significant in transmitting this effect of the additional lighting. Zweig *et al.* (*7*) reported that when lighting was prolonged 4 hours into the normally dark period serotonin levels equalled the daytime high. Our data show that such additional lighting produces an intermediate level that is significantly higher than the nocturnal low ($P < .01$) but significantly lower than the daytime peak ($P < .02$). This observation supports the contention that there may be both a nerve-dependent and a nerve-independent serotonin rhythm at this age in the rat (*13*).

In contrast to the adult, light stimulus in the immature rat can reach the pineal by a route involving the retina (*7*). The receptor for this route is located somewhere in the head, but a more specific localization is unknown (*7*). Since denervated glands do not respond to additional illumination, the receptor for the involved neural pathway is probably not within the pineal body itself, but within the central nervous system—perhaps the hypothalamus.

This report of a serotonin rhythm after immunosympathectomy has been confirmed in pineals from 20-day-old rats sympathectomized by superior cervical ganglionectomy at the age of 8 days (*13*).

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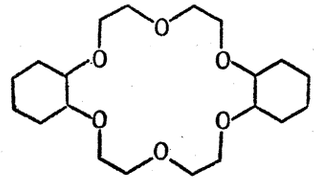
Binding of Alkali Metal Ions by Cyclic Polyethers: Significance in Ion Transport Processes

Abstract. *Values for the formation constant (log K), the change in enthalpy ($\Delta H°$), and the change in entropy ($\Delta S°$) have been determined for the interaction of lithium, sodium, potassium, rubidium, and cesium ions with the two isomers of the cyclic polyether, 2,5,8,15,18,21-hexaoxatricyclo[20.4.0.0^{9,14}] hexacosane. The stability order of these metal ions with either isomer is identical to the permeability order for these same metal ions with the structurally related antibiotics, valinomycin and monactin.*

Considerable research effort, particularly among biochemists and physiologists, has been directed toward understanding the phenomenon of ion transport through cellular membranes. One hypothesis suggests that carrier molecules are important in the active transport of ions. Experimental justification for this hypothesis is found in the fact that many substances markedly affect active ion transport in systems consisting of mitochondrial, chloroplast, or reconstituted biological membranes. Of special interest among substances affecting ion transport are cyclic molecules of low molecular weight containing residues joined by amide, ester, or ether linkages, or any combination of these linkages; for example, antibiotics of the valinomycin and actin classes and certain of the recently synthesized cyclic polyethers, such as 2,5,8,15,18,21-hexaoxatricyclo[20.4.0.0^{9,14}] hexacosane (*1*)



I

We propose that compound I may serve as a model compound for thermodynamic investigations of active transport processes inasmuch as this compound (i) increases the permeability to alkali metal ions (*2*) of reconstituted biological membranes in the sequence

$$K^+ > Rb^+ > Cs^+ > Na^+ > Li^+ \qquad (1)$$

and (ii) is structurally similar to certain of the antibiotic molecules, such as valinomycin (*3*) and monactin (*2*), which also exhibit this property with the same metal ion sequence. Consequently, calorimetric titration and potentiometric studies of the interaction of alkali metal ions with the *cis* and *trans* isomers of compound I, IA and IB, have been initiated. Results show that sequence 1 also holds for the thermodynamic stabilities of the complexes formed by these metal ions with isomers IA and IB.

The sample of compound I (*4*) containing the isomer mixture was purified by passage through an acid-washed aluminum oxide column with heptane used as eluent. The isomers were then separated by passing the purified sample through a Woelm aluminum oxide column (activity grade 1) and varying the eluent among hexane-ether mixtures. The column was stripped with methanol, and the isomers were recrystallized from ether and hexane. Isomers IA and IB have melting points of 61° to 62°C and 69° to 70°C, respectively; they can also be distinguished by their infrared spectra, although it is not presently known which isomer is *cis* and which is *trans*.

Solutions of alkali metal salts (~0.2 and ~0.4*F*) were titrated into solutions of either isomer IA or IB (~0.01*F*) in a precision thermometric titration calorimeter (*5*). Values for the formation constant *K*, the change in enthalpy $\Delta H°$, and the change in entropy $\Delta S°$ were calculated from the calorimetric titration data (*6*). The calculations were aided by an IBM-360 computer.

Table 1. Thermodynamic quantities for the reaction $M^+ + I = MI^+$. Uncertainties are the standard deviations from the average of three to seven runs in each case.

Metal ion M^+	Isomer of compound I	log *K*	$\Delta H°$ (kcal/mole)	$\Delta S°$ (cal/deg mole)
K^+	A	2.01 ± 0.02	− 3.89 ± 0.06	− 3.8 ± 0.2
K^+	B	1.60 ± .03	− 5.18 ± .08	− 10.1 ± .3
Rb^+	A	1.47 ± .02	− 3.48 ± .06	− 5.0 ± .2
Cs^+	A	1.07 ± .06	− 2.00 ± .10	− 1.8 ± .3

A digital *p*H-meter (Orion model 801) equipped with cationic (Beckman 39137) and saturated calomel electrodes was also used to measure the extent of binding of Na^+, K^+, Rb^+, and Cs^+ with isomer IA.

The results from calorimetric and potentiometric measurements are in agreement and indicate that alkali metal ions have the following order of decreasing affinity for isomers IA and IB

$$K^+ > Rb^+ > Cs^+ \gg Na^+, Li^+ \qquad (2)$$

Thus, the permeability order for compound I has a firm thermodynamic basis, and similar thermodynamic correlations might be expected for structurally related antibiotics.

Neither Na^+ nor Li^+ has a measurable affinity for isomer IA or IB. The potassium ion, Rb^+, and Cs^+ showed stronger interaction with isomer IA than with IB; however, only in the cases listed in Table 1 was the change in heat sufficiently large to permit calculation of the attendant thermodynamic values.

Compound I represents an interesting new class of complexing agents which selectively interact with cations of the alkali metal group. The similarities in behavior of compound I and the above-mentioned antibiotics toward alkali metal cations are not unexpected if one considers the similar uncharged character and macrocyclic structures having cavities ringed with centrally oriented oxygen atoms.

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Surgery in the Rat during Electrical Analgesia Induced by Focal Brain Stimulation

Abstract. *Chronic monopolar electrodes were implanted in the region of the midbrain central gray in eight rats. In three rats, continuous 60 cycle-per-second sine-wave stimulation resulted in an electrical analgesia defined by the elimination of responses to aversive stimulation while general motor responsiveness was retained. Exploratory laparotomy was carried out in these animals during continuous brain stimulation without the use of chemical anesthetics. Following surgery, brain stimulation was terminated, and responses to aversive stimuli returned. Electrodes effective in inducing electrical analgesia at the lowest currents were located at the dorsolateral perimeter of the midbrain central gray. It was concluded that focal brain stimulation in this region can induce analgesia in the absence of diffusely applied "whole brain" stimulation.*

A number of investigators have reported electrical analgesia or electrical anesthesia during diffuse application of electrical current between external scalp electrodes. The usual procedure is to apply electrodes to the opposite sides of the skull and to gradually increase the electrical current until the subject no longer responds to noxious stimulation. Comprehensive reviews of this work have appeared in the recent literature (*1*).

The purpose of the present investigation was to determine if *focal* application of electrical currents alone could induce analgesia or anesthesia without application of currents across the entire brain. Previous work in our laboratory (*2*), together with anatomical studies (*3*) and lesion experiments (*4*), suggested that stimulation of periaqueductal midbrain structures might reduce responsiveness to noxious stimulation.

Chronic monopolar stainless steel electrodes and subcutaneous grounds were stereotaxically implanted in each of eight male Wistar rats. The surgical procedure was essentially that reported by Miller (*5*). Electrodes were directed to areas in and around midbrain central gray at deGroot anterior-posterior coordinates 0.0 (*6*). To test for aversive responsiveness, a method reported by Kreele and Smith (*7*) was used. This involves the application of mechanical pressure to the paws and tail with an "analgesiometer" fashioned from a hemostat. The aversive response of the animal is scored simply as present or absent. All animals responded vigorously to hemostat-applied pressure both before and after implantation of electrodes.

Ten days after electrode implantation, each animal was tested in the following way. Continuous, constant-current, 60 cycle/sec sine-wave stimulation, starting at 5 μa root mean square (r.m.s.), was delivered through the indwelling electrode. At 15-second intervals, the current was increased in 5-μa steps until either aversive responses to hemostat-applied pressure were eliminated or 35 μa was reached. Of the eight electrodes tested, stimulation through three yielded electrical analgesia when the current reached 15, 20, and 30 μa. The three animals showing this effect were then scheduled for laparotomy.

On the day of surgery, the brain stimulation procedure was essentially the same. Since the animals were able to walk about at the levels of current used, they were secured in a supine position for surgery. Abdominal skin, muscle, and peritoneum were sectioned and retracted sufficiently to permit visualization of the abdominal cavity. This was followed by closure of the incisions with wound clips. None of the surgical procedures evoked aversive reactions. It

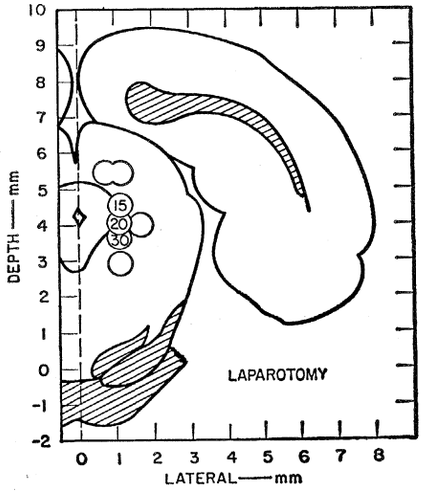

Fig. 1. Composite diagram indicating location of electrode tips through which stimulation produced analgesia.