Method. 49 adult cats (from 2.0 to 3.5 kg), anesthetized by i.p. Nembutal of 25 mg/kg, were used.

The preliminary experiments demonstrated[7] that practically uniform levels of dopamine in the right and the left caudate in 9 untreated cats, and that the caudate dopamine depletion consequent to destruction of the substantia nigra in 17 cats is affected by the site of destruction rather than the duration after operation; the destruction of the rostromedial part of the substantia nigra provides maximal diminution (Figure 1).

In 23 cats, arranged in pairs, the substantia nigra on one side was destroyed by radio-frequency current which produced lesion of ca. 2 mm in diameter with consequent diminution of dopamine in the homolateral caudate nucleus[6]. The target is either of midportion or the rostromedial portion or both of the midportion and rostral portion of the substantia nigra.

One in each pair was then given CDP-choline daily in an i.m. dose of 200 mg, and the other in each pair, a comparable volume of saline injected by same route for 2 to 5 weeks. After that, the cats were sacrificed for dopamine assay. The brain was cut at frontal 12 mm from the aural orifice; the anterior half was taken out to extract the bilateral caudates, which was frozen immediately in liquid nitrogen whereas the remaining portion of the brain, which was utilized for confirmation of the site of destruction and for histological examination, was perfused with 10% formalin.

Tissue dopamine assays were performed by the method described by ANSELL and BEESON[7]. As the parameter for comparison between the treated and the control group, the rate in percentage of dopamine depletion of caudate on the treated side comparing with the non-treated side i.e., (left caudate dopamine/right caudate dopamine) $\times 100$ was used.

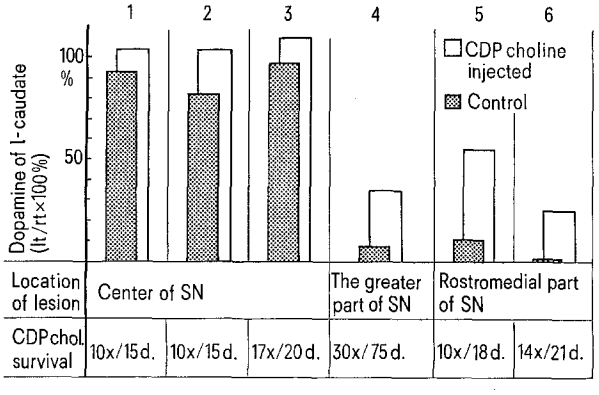

Fig. 2. Interrelation between CDP-choline and dopamine in the caudate. To cats with dopamine depletion in the caudate by destruction of the substantia nigra, CDP-choline was injected i.m. in a dose of 200 mg daily into one in each pair and saline into the other in each pair, respectively. CDP-chol./Survival: duration of CDP-choline administration and the number of days between destruction of the substantia nigra and extraction of the caudate. 3 cats were used in groups 2 and 3, and those with disadvantageous data were adopted. It can be seen from the chart that cats treated with CDP-choline showed higher dopamine levels than the untreated controls.

Results. Figure 2 illustrates the results of the experiments. 1. 3 pairs out of 4 pairs of cats revealed destruction of the midportion of the substantia nigra. Controls in these pairs displayed diminution rates of 82, 93 and 98%; thus only a slight depletion. CDP-choline treated cats, on the other hand, showed rates of 104, 104, and 108% respectively; thus the dopamine levels were consistently higher than those in the untreated controls. It follows that the treated animals showed no diminution of dopamine at all, whereas the dopamine level diminished slightly in untreated cats.

2. There were 2 pairs of drop-outs among the 3 with extensive destruction of the midportion and rostral portion of the substantia nigra, and the experiment was successful in the remaining pair. The rate of diminution shown by the control in this pair was 8%, while the treated cat displayed a rate of 36.7%, indicating a dopamine level approximately 4 times as high as that in the untreated control.

3. There were 2 successful pairs out of 3 pairs with destruction of the medial portion of the rostral end of the substantia nigra, in which the experiment was successful. The control cat in 1 pair (No. 5) showed a dopamine diminution rate of 12%, whilst the treated one in this pair displayed a rate of 57%, about 5 times as high. In the other pair (No. 6), the rate shown by the control was 0% (viz. disappearance of dopamine in the caudate on the side of destruction) and that by the treated cat was 27%; hence an obvious difference.

4. Histological examination: Degeneration of nerve cells of the pars compacta of substantia nigra in parallel with the diminution of caudate dopamine concentration was in evidence. The cats treated with CDP-choline showed milder degenreation and loss of nerve cells in the substantia nigra than the untreated cats did.

Conclusion. CDP-choline is proved to exert a significant ($0.001 < P < 0.01$) protective effect against diminution of dopamine in the caudate derived from ipsilateral destruction of the substantia nigra. The mechanism might be postulated that phospholipid metabolism of injured nerve cells in the substantia nigra and nigrostriatal tract is improved by CDP-choline and, as a result, the production of dopamine is augmented and the transporting activity is also ameliorated.

Zusammenfassung. Es ist anzunehmen, dass CDP-Cholin (Cytidin-Diphosphat-Cholin) auf Verminderung von Dopamin im Nucleus caudatus einen signifikanten ($0.001 < p < 0.01$) Abwehreffekt ausübt, was durch homolaterale Destruktion der Substantia nigra verursacht wird.

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Selective Calcium-Alkali Metal Exchange in a Synaptic Membrane Protein[1]

It is well-recognized that bioelectric phenomena are associated with differential fluxes of alkali metal ions across permselective membranes; and although a great deal is known about the kinetics of the ionic fluxes, little is known of the chemical architecture of the membrane, particularly concerning its permselectivity. The problem has been investigated using a variety of materials ranging from artificial ion exchange systems to intact muscle and nerve[2,3]. Although such studies have yielded valuable information on the selectivity sequences of

inorganic ions and the physio-chemical factors governing them, they have provided little information concerning the chemical nature of the biomacromolecules involved in selectivity. One approach to this problem has been to utilize monomolecular films of lipids and proteins derived from natural excitatory membranes; and, by measuring such properties as surface potential, pressure, and adsorption, to study interactions with inorganic ions, ATP, and various psychotropic drugs[4]. A particularly useful preparation has been a 'hydrophobic' protein isolated from synaptic membranes of rat and beef brain[4,5].

In the present study a monomolecular film of the synaptic protein at an air-water interface has been used to investigate selectivity of various alkali metal ions. The protein used was prepared from synaptic membranes isolated from beef cerebellum by procedures described elsewhere[4,5]. Purification of the protein involved solubilization in 0.2% sodium dodecyl (SDS) in 0.1 *M tris*, pH 7.5 and repeated fractionation on a Sepharose 6B column. Removal of the SDS ions was accomplished by a combination of dialysis and gel filtration (Sephadex G-25). A similar preparation was obtained by using 0.5% sodium deoxycholate (DOC) instead of SDS, since deoxycholate could be more readily removed than SDS from the protein. The final protein, SP, had a molecular weight of 42,000 in SDS or DOC and over 500,000 in the absence of detergents, Surface adsorption of radiotracer Ca^{2+} and ATP to films prepared from SP was performed as described elsewhere[5,6]. Briefly it consisted of measuring

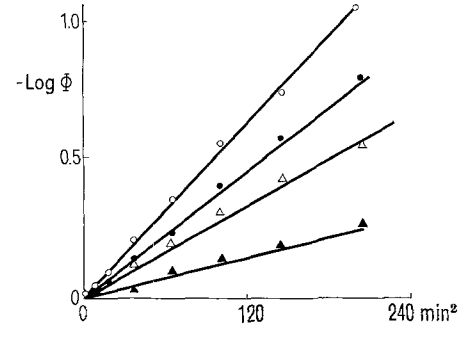

Fig. 1. Time course of displacement of surface protein Ca^{2+} by various cations. At 0 time 30 µl of 3 *M* electrolyte was injected into subsolution and Ca^{2+} desorption measured by decrease in surface radioactivity. Φ = fraction of adsorbed Ca^{2+}; (▲), LiCl; (△), NaCl; (●), CsCl; (○), KCl. Each point is the average of 5 determinations and the s.d. was generally less than 10% of the mean. Note: the plot for Rb was almost identical to that for Na and is not shown.

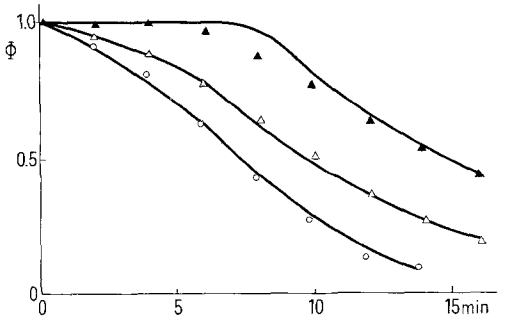

Fig. 2. Φ vs time of displacement. Legend same as for Figure 1. Solid lines represent actual data and symbols refer to points calculated from data in Figure 1 using equation I.

(Geiger-Müller tube and scaler) the increased radioactivity at the surface as the ^{45}Ca adsorbed to a protein film (0.5 m^2/mg at the limiting area) spread over the aqueous subsolution (10^{-3} *M tris*, pH 7.5) containing the radiotracer. In the desorption studies used to measure Ca^{2+} displacement, 10–30 µl of an electrolyte solution was injected into the subsolution after equilibrium adsorption of ^{45}Ca had been attained.

The overall kinetics of the displacement of Ca^{2+} from the surface conform reasonably well (within 90–95% of equilibrium displacement) to the rate law:

$$-\log \phi = k' t^2 \qquad \text{(I)}$$

where k' is the observed rate and ϕ is the fraction of initial Ca^{2+} adsorbed at time t. Equation I was derived from a model based on diffusion and chemical displacement (equation II) by 2 M^+ of adsorbed Ca^{2+}.

$$P - Ca + 2M^+ = P - (M)_2 + Ca^{2+} \qquad \text{(II)}$$

If two M^+ are involved in the rate-limiting step, the rate of disappearance of Ca from the surface will be given by equation III where

$$-d(Ca)_p / dt = k_1 (Ca)_p (M^+)^2_s \qquad \text{(III)}$$

$(Ca^{2+})_p$ is the concentration of adsorbed Ca^{2+}, $(M^+)_s$ is the concentration of M^+ near the surface close enough to react, and k_1 is the rate constant for the displacement. Assuming that $(M^+)_s$ is diffusion controlled and therefore proportional to $t^{1/2}$ equation III becomes

$$-d(Ca)_p / dt = k_1 k_2^2 (Ca)_p t \qquad \text{(IV)}$$

where k_2 refers to the rate contant for diffusion. Integration of equation IV yields equation I and

$$k' = k_1 k_2^2 / 2(2.30).$$

Alternative rate laws based only on diffusion of one or two M^+, displacement by only one M^+, or neglect of diffusion did not fit the data as well.

Results on the rates of exchange of protein-bound Ca^{2+} by the monovalent cations plotted according to equation I are represented in Figures 1 and 2. The observed rates for displacement k' and the equilibrium adsorptivity follow the sequence Li < Na, Rb < Cs < K.

In attempting to account for the selectivity ratio of 2 mobile counter ions for a charged interface, a number of parameters must be considered such as valence, ionic radius and polarizability, hydration number, electrostatic charge and configuration of the fixed polyelectrolyte, and diffusion coefficient of the monovalent cations. The rate of exchange of the monovalent cation with the bound Ca^{2+} increases with increasing ionic radius or decreasing energy of hydration, the two parameters being interdependent[7].

[1] This research was supported by NIH grants No. MH 20142 and No. NS 06827 and with the technical assistance of Judy Straw.

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If it is assumed that the binding force for the cation by the protein is electrostatic and that the cations in the negative sites are hydrated, it would follow that the least hydrated (i.e. largest ionic radius) would be bound most strongly according to Coulomb's law. However, Rb^+ and Cs^+ not only displace less Ca^{2+} than K^+ does at equilibrium but also approach equilibrium more slowly. One explanation might be that the steric factors of the Ca sites on the protein may hinder the exchange with Cs and Rb because of their larger ionic radius. Among the alkali metals, Cs^+ and Rb^+ alone are of sufficient size to form electrostatic complexes with various ligands which can be extracted from aqueous solution by certain substituted hindered phenols[8].

The kinetic analysis implies that two monovalent cations are involved in the rate-limiting step. It was suggested in a previous study involving lipid monolayers[9] that a rate-determining step in ion exchange at the interface may be the removal of water dipoles from the counter ion. Presumably, the rate-limiting step at the protein surface involves the exchange of water between one adsorbed Ca^{2+} and 2 monovalent cations. The free energy of hydration for Ca^{2+} (–378 Kcal/mole) is much more negative, being twice that of any of the monovalent cations.

It had been shown for a stearic acid monolayer that the adsorption isotherm for Ca^{2+} at a given concentration of a monovalent cation M can be expressed by the following equation:

$$K = \frac{a_M{}^2\phi}{a_{Ca}(1-\phi)^2} \exp \frac{2w(1-2\phi) - RT}{RT}$$

where K denotes the equilibrium constant, a, activity in mole fraction, ϕ, the fraction of monolayer sites occupied by Ca^{2+}, w, the interchange energy, R, gas constant, and T, temperature[6]. If the rate of Ca^{2+} adsorption to a stearic acid film is measured with varying concentrations of the various alkali monovalent metals initially in the subsolution prior to application of the SP, it was found to be the same regardless of the type of cation. An essentially similar finding was obtained with the protein film in place of stearic acid. An experimental plot of ϕ vs C_{Na} for a stearate monolayer can be shown to be indistinguishable from that obtained by substituting corresponding values obtained with the protein into equation V, assuming $w = -0.23$ kcal/mole and $K = 3.4$ mole/l. It was of interest that the values of w and K for SP and stearic acid were reasonably similar, a fact which points to the involvement of acidic amino acids (glutamic and aspartic) as likely binding sites of SP. In preliminary studies a relatively good agreement between the experimental data with Na^+ and equation V was obtained when the protein film was used, a finding which further supports the validity of equation V. It would, therefore, appear as if the kinetics of Ca^{2+} adsorption to a lipid film were generally applicable to that of a protein.

Résumé. Cette note concerne l'adsorption du Ca^{45} sur une couche interfaciale de protéine hydrophobique extraite de membranes synaptiques de cervelet bovin. Le pouvoir de divers cations univalents, alkalins métalliques de faire échange avec le Ca a été étudié. Conformément à une loi cinétique basée sur la diffusion et le déplacement chimique, les constantes d'échange et l'adsorptivité équilibreé ont suivi la séquence Li < Na, Rb < Cs < K. On en a conclu que l'étape limitant la vitesse de la réaction dépend de l'échange d'eau entre un Ca absorbé et 2 cations univalents et que dans la séquence, la position du Rb et du Cs (le moins hydraté) peut dépendre de facteurs stériques.

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The Effects of *X*-Ray Irradiation of the Head Region of 8-Day-Old Female Rats on Their Reproductive Capacity when 10–17 Months Old

Recently, we have reported that X-irradiation (500, 600, 700 or 800 R), applied in infancy to the head region of female rats, had no influence on the first pregnancy, size of the first litter or on the time at which the opening of the vaginal orifice took place[1]. These results were consistent with the findings of MOSIER and JANSONS, who reported that, following neonatal head-irradiation, the content of gonadotropins in the 121-day-old pituitaries was about the same as that of control pituitaries[2], and with the findings of DRIPS and FORD, who observed that the time of opening of the vaginal membrane was not affected by neonatal irradiation of the hypophysis in female rats[3]. MATSUMOTO confirmed our results that no marked effects of head-irradiation of infant female rats could be detected on the subsequent development of the follicles and *corpora lutea* in the ovaries[4].

In this work we have been interested in the effects of head-irradiation of infant female rats on the survival and the reproductive capacity during the advanced periods of their sexual activity, i.e., from 10 to 17 months of age.

Materials and methods. The head region of a number of 8-day-old female albino rats of a close-bred Wistar strain was exposed to 600, 700 or 800 R of X-rays from a Siemens set under the following conditions: 200 kV, 16 mA, filter – Cu 0.5 mm, FSD – 34 cm, dose rate – 107 R/min. Sexually mature animals were mated with potent males. After the first littering, the irradiated females and their controls were kept in isolation up to 9 months of age. From that moment onward one normal male, replaced by another every month, was kept in the same cage with 4 experimental or control females during the whole period of experimentation. After each delivery the animals were laparotomized and their uteri examined for the number of implantations and resorptions.

Results and discussion. As can be seen from the Table, in which the results of the experiment are summarized, 91% of the total of irradiated animals, when tested at the age of 4 months, proved to be capable of reproducing,

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