

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 phenols⁸.

The kinetic analysis implies that two monovalent cations are involved in the rate-limiting step. It was suggested in a previous study involving lipid monolayers⁹ that a rate-determining step in ion exchange at the interface may be the removal of water dipoles from the counterion. 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:

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

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 , temperature⁶. 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 cerveau bovin. Le pouvoir de divers cations univalents, alcalins 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 équilibrée ont suivi la séquence $\text{Li} < \text{Na}$, $\text{Rb} < \text{Cs} < \text{K}$. 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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Rochester (New York 14642, USA), 23 July 1973.

⁸ F. A. COTTON and G. WILKINSON, *Advanced Inorganic Chemistry* (Interscience, New York 1967), p. 421.

⁹ A. YAMAUCHI, A. MATSUBARA, H. KIMIZUKA and L. G. ABOOD, *Biochim. biophys. Acta* 150, 181 (1968).

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 place¹. 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 pituitaries², 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 rats³. 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 ovaries⁴.

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,

¹ P. N. MARTINOVITCH, O. K. IVANIŠEVIĆ, *Experientia* 26, 95 (1970).

² H. D. MOSIER, JR., and R. A. JANSONS, *Proc. Soc. exp. Biol. Med.* 128, 23 (1968).

³ D. G. DRIPS and F. A. FORD, *J.A.M.A.* 91, 1358 (1928).

⁴ A. MATSUMOTO, *Annot. zool. Japon.* 45, 80 (1972).

Reproducibility of female rats following irradiation of the head region at the age of 8 days with different doses of X-rays

Dose of X-rays (R)	At first pregnancy		When 10-17 months old					
	Animals tested (No.)	Fertile animals (%)	Mated animals (No.)	Pregnant animals (No.)	Ratio pregnant/mated	Littering (\bar{X})	Litter size (\bar{X})	Resorptions (\bar{X})
600	15	87	13	7	0.54	1.7	6.9	1.3
700	19	95	18	8	0.44	1.9	7.7	0.7
800	22	91	20	9	0.45	1.4	5.4	1.0
Total irradiated	56	91	51	24	0.47	1.7	6.7	1.0
Controls	32	94	30	28	0.93	1.7	6.9	0.8

in comparison to 94% of nonirradiated controls. The picture becomes strikingly different when the reproducibility of experimental and control animals is followed during the period of 10 to 17 months of their life. Whereas 28 rats from a group of 30 normal animals produced progeny, approximately half of the irradiated animals never became pregnant during this period. However, the other half of the irradiated rats, regardless of the dose applied, reproduced at about the same rate as did the controls. Also, they did not differ significantly from the controls in respect to the litter size and to the number of resorptions.

We have shown earlier that a single whole-body exposure of 8-day-old female rats to 100 R of X-rays produced sterility in 86% of the animals⁵. It is also known that the irradiation of 1-, 2- or 3-week-old mice with a dose as low as 30 R decreases fertility⁶. In this experiment, almost all the irradiated animals proved to be fertile at the age of 4 months or so, and the differences in reproducibility, represented by an equal ratio of normally reproducible and nonreproducible animals, appeared later on. This suggests that the phenomenon of scattering, if any, might have been of minor importance.

None of the phenomena so far mentioned showed dose-dependence. Pronounced dose-dependence ($r = 0.971$), however, was observed when mortality of the animals was followed. Compared with the 94% survival for the controls, the chances for animals that were irradiated with 600, 700 or 800 R to live longer than 17 months were 77, 65 and 36%, respectively. In animals which died prematurely, regardless of their capacity to reproduce, symptoms of cachexia were plainly visible.

The results presented suggest that the irradiation of the head region of 8-day-old female rats with doses of 600, 700 or 800 R of X-rays does not incapacitate the rat

to reproduce during early months of its reproductive life, but significantly reduces chances for such animal to produce progeny when older than 10 months. The results obtained further suggest that the irradiated animals, within the interval of life considered in this work, if reproducible at all, behave like nonirradiated animals in regard to the frequency of littering and to the litter size. In the population of irradiation animals, visible signs of a rapid process of aging were frequent and were accompanied by a high, statistically dose-dependent, mortality rate⁷.

Résumé. L'irradiation de la tête des rats femelles âgés de 8 jours à des doses de 600, 700 ou 800 R n'a pas d'influence sur la capacité de reproduction au cours des premiers mois du stade adulte, mais réduit celle-ci quand l'âge de 10 mois est dépassé. Parmi les animaux irradiés, on a souvent constaté des signes visibles de vieillissement accéléré et d'un taux élevé de mortalité dépendent statistiquement de la dose.

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⁵ D. SLADIĆ-SIMIĆ, N. ZIVKOVIĆ, D. PAVIĆ and P. N. MARTINOVITCH, in: *Symposium on Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation* (Academic Press, London and New York 1962), p. 327.

⁶ R. RUGH and M. WOHLFROMM, *Atompraxis* 10, 511 (1964).

⁷ This work was supported by a grant from the Serbian Academy of Sciences and Arts.

Regulation in Nodeless Chick Blasoderms

In the chick, GRABOWSKI¹ and GALLERA² have reported that a complete regulation may occur after node excision. Of course such a result implies that a new node is formed, and the main question is to know-how this reconstitution takes place. GRABOWSKI¹ and BUTROS³ have assumed that the node reorganizes itself at the expense of presumptive notochordal cells still included in the upper layer all around the node at the stage of operation. Accordingly, GRABOWSKI¹, as well as GALLERA², have established that blasoderms did not succeed in regulating completely, if

the node was removed at the head process stage, a stage at which all notochordal cells are already invaginated. BELLAIRS⁴ has suggested that the streak area of the full grown streak located behind the node may play this role

¹ C. T. GRABOWSKI, *J. exp. Zool.* 133, 301 (1956).

² J. GALLERA, *C. r. Ass. Anat.* 49, 632 (1964).

³ J. BUTROS, *J. Embryol. exp. Morph.* 17, 119 (1967).

⁴ R. BELLAIRS, in: *Developmental Processes in Higher Vertebrates* (Logos Press, London (1971)).