retardé. Ces modifications, qui reflètent des altérations dans la maturation des systèmes inhibiteurs et excitants du système nerveux central au cours de la croissance persistent jusqu'à la 5e semaine post-natale. Ces résultats indiquent que la nicotine est capable de provoquer des modifications dans le développement du cerveau à l'état fœtal.

D. B. HUDSON, E. MEISAMI[23] and PAOLA S. TIMIRAS

Department of Physiology-Anatomy, University of California. Berkeley (California 94720, USA),
18 August 1972.

[23] Present address: Department of Biology, Faculty of Science, University of Tehran, Tehran, Iran.

Effect of Alkali Cations on the Interaction Between Detergents and Erythrocyte Membranes

The degree of complement (C)-mediated lysis of mammalian erythrocytes (E) with high K^+ and low Na^+ content, including human E, depends on the alkali metal cation present in the reaction mixture[1-3]. Thus, the highest degree of lysis was obtained in 145 m*M* K^+, and the lowest, in Na^+. Arrangement of the alkali metal cations according to their ability to enhance C lysis resulted in the selectivity series K > Rb > Li > Cs > Na or K > Li > Rb > Cs > Na[1,3]. The facilitating effect of K^+, Rb^+ and Cs^+ occurs primarily on the final stage of the reaction of C with E[2]. Therefore, it became important to ascertain whether the effect of alkali cations was specific for C lysis or whether it also occurred in hemolysis of non-immune nature. We explored this problem using the detergents Triton X-100 (Tr) and Na desoxycholate (DOC). We also investigated whether the alkali cations modify the degree of solubilization of purified E membranes (M) caused by Tr and DOC.

Material and methods. Blood from normal humans was collected in ACD solution and used within 2 days. The E were washed 3 times at 4°C with veronal buffer containing 145 m*M* NaCl, 0.5 m*M* $MgCl_2$ and 0.15 m*M* $CaCl_2$, pH 7.3[4] (Na buffer). The E were suspended in cold Na buffer and standardized photocolorimetrically to 6.25×10^8 E per ml. For hemolysis studies 0.4 ml were transferred to tubes and centrifuged. To the sedimented E was added 1 ml ice-cold Na buffer or buffers identical to the Na buffer except that 145 m*M* K, Rb, Li, or Cs was substituted for 145 m*M* Na. Then an aqueous solution of the detergent was added (25 µl of 0.2% [v/v] Tr or 50 µl of 0.9% [w/v] DOC) to yield 0.076 m*M* Tr or 1.03 m*M* DOC. The E were suspended and incubated at 37°C for 1 h, with mixing. The reaction was terminated by addition of 2 ml of an ice-cold veronal buffer identical to the buffer used initially, centrifugation of the unlysed cells, and measurement of the degree of lysis[4]. E suspensions subjected to this procedure but incubated without detergent served as blanks.

M solubilization studies were performed with M obtained from human E that were washed at 4°C wiht isotonic phosphate buffer, pH 7.4, and lysed with 5 m*M* phosphate buffer, pH 7.4[5]. The M were washed twice with this buffer and twice with Na buffer. Then they were suspended in Na buffer and the concentration was adjusted to an O.D. of 0.5 at 550 nm, which corresponded to 4.4×10^9 *M* particles per ml, as determined with a Coulter counter. 2 ml were transferred to tubes which were centrifuged at 30,000 g for 20 min. To the sedimented *M* was added 1 ml ice-cold Na buffer or buffers containing the other alkali metal ions. Then an aqueous solution of the detergent was added (50 µl of 10% [v/v] Tr or 50 µl of 10% [w/v] DOC) to yield 7.4 m*M* Tr or 11.5 m*M* DOC. The procedure then continued as described above for hemolysis of E. The reduction in turbidity at 550 nm was used to measure the degree of M solubilization.

Results and discussion. The results (Table) indicate that the alkali metal cations markedly influence the degree of lysis of human E caused by Tr and DOC, in a manner that is characteristic for each detergent. Thus, the activity series obtained with Tr was K > Rb = Cs > > Na ⩾ Li, and that with DOC was Li > Rb = Cs > K > Na. In contrast, the degree of E M solubilization produced by Tr and DOC was independent of the alkali metal ion present in the reaction system. Identical results

[1] M. M. E. DE BRACCO and A. P. DALMASSO, Immunology *17*, 559 (1969).
[2] A. P. DALMASSO, R. LELCHUK and E. D. DE ISOLA, Fedn Proc. *30*, 472 (1971).
[3] J. P. LEDDY, P. A. THIEM, P. F. LEBLOND, R. I. WEED and P. K. LAUF, J. Immun. *108*, 475 (1972).
[4] E. A. KABAT and M. M. MAYER, *Experimental Immunochemistry*, 2nd edn. (Charles C. Thomas, Springfield, Illinois, USA 1961), p. 149.
[5] J. T. DODGE, C. MITCHELL and D. J. HANAHAN, Archs Biochem. Biophys. *100*, 119 (1963).

Effect of alkali metal cations on degree of hemolysis of human erythrocytes and on solubilization of human erythrocyte membranes by Triton X-100 and by Na desoxycholate

Alkali metal ion present during reaction (145 m*M*)	% Erythrocytes hemolyzed (Mean ± S.E., 8 experiments[a])		% Membranes solubilized (Mean and range, 4 experiments[a])	
	Triton	DOC	Triton	DOC
Na	19.6 ± 5.4	22.7 ± 4.1	32.1 (17.2–38.8)	76.1 (60.8–63.0)
K	87.5 ± 1.4	30.9 ± 6.5	36.2 (33.5–38.8)	77.2 (64.8–84.4)
Rb	76.1 ± 5.9	80.7 ± 6.1	32.5 (25.3–38.8)	79.1 (68.6–84.4)
Cs	76.3 ± 6.3	80.4 ± 9.0	34.3 (29.5–41.7)	81.5 (68.6–88.7)
Li	17.7 ± 2.6	93.7 ± 0.7	25.0 (20.2–27.6)	81.5 (68.6–88.7)

[a] Each experiment was carried out with type 0, Rh^+ erythrocytes obtained from different donors.

were obtained in a second experiment using the conditions described above, except that the buffers were free of Mg and Ca ions. Finally, in studies performed as above but with buffers in which the alkali metal cations were replaced by 100 mM of the chloride salts of the alkali earth cations, the following effect was found on the degree of hemolysis (Mean $\pm$ S.E., 8 experiments). Tr: Ca, 84.7 $\pm$ 2.8; Sr, 58.6 $\pm$ 6.0; Ba, 58.1 $\pm$ 8.8; and Mg, 18.0 $\pm$ 4.5. DOC: Ca, 90.6 $\pm$ 4.1; Sr, 51.6 $\pm$ 10.0; Ba, 38.4 $\pm$ 11.0; and Mg, 7.2 $\pm$ 0.5. In contrast, the degree of M solubilization caused by the detergents was unaffected by the various alkali earth cations.

The observation that the alkali cations modify the degree of detergent-induced lysis of intact E is of interest in regard to the multiple effects of such ions on M phenomena. It is unclear why there is a selectivity effect of the alkali cations on hemolysis but not on M solubilization caused by Tr and DOC. M solubilization reflects a profound disruption of M structure and thus may be independent of the relatively subtle changes imparted on the M surface by the alkali cations. These modifications are, however, sufficient to alter the degree of hemolytic processes caused by relatively minor, often localized, alterations produced by hemolysins on M structure. The finding of similar degrees of E M solubilization by the detergents in the various cationic media indicates that the modifications caused by the alkali cations on the degree of lysis of intact E result from their interaction with the M itself rather than from a fluid phase effect of the cations on the solubility or micellar arrangement of the detergent. The activity series of alkali metal ions upon C lysis[1,3] is unrelated to those found in lysis caused by Tr and DOC. However, the series obtained with alkali metal cations on the final stage of C lysis[2] was similar to that obtained in Tr induced lysis.

Resumen. Los distintos cationes alcalinos monovalentes (145 mM) y divalentes (100 mM) modifican marcadamente el grado de hemólisis de eritrocitos humanos provocado por Triton X-100 y por desoxicolato de Na (DOC). La serie de actividad con cationes monovalentes en hemólisis por Triton es K > Rb = Cs > Na $\geqslant$ Li, y por DOC es Li > Rb = Cs > K > Na. Por el contrario, el grado de solubilización de membranas eritrocitarias producido por Triton o DOC es independiente de los distintos cationes alcalinos.

A. P. Dalmasso[6] and Rosalia Lelchuk[7]

Institute of Medical Research, Faculty of Medicine, University of Buenos Aires, Donato Alvarez 3000, Buenos Aires (Argentina), 19 September 1972.

[6] Career Investigator, National Research Council, Argentina. Present address: Veterans Administration Hospital, Univ. of Minnesota Medical School, Minneapolis, Minn. 55417, USA.

[7] Research Fellow, Institute of Pharmacology, Secretary of Public Health, Argentina.

Vascular Effects of Periarterial Mesenteric Nerve Stimulation After Adrenergic Neurone Blockade

During an investigation of the vascular effects of 6-hydroxydopamine in cats, it was observed that stimulation of the periarterial mesenteric nerves after adrenergic blockade increased blood flow in the superior mesenteric artery. This observation led to the studies reported below.

Methods. 13 cats of either sex weighing 3–4.5 kg were anaesthetized with i.p. pentobarbital sodium 40 mg/kg Polyethylene catheters were inserted into an external jugular vein and a common carotid artery for drug administration and systemic arterial pressure measurement respectively. The abdomen was opened in the midline and the adrenal glands were excluded from the circulation by ligatures. The nerve plexus surrounding the main trunk of the superior mesenteric artery was dissected from the vessel and divided. The distal end was laid over bi-polar platinum electrodes and rectangular pulses of 1 msec duration at frequencies of 5–15/s and voltages of 5–15v were delivered for periods of 1–5 min. Blood flow in the superior mesenteric artery was measured with a Biotronex electromagnetic flowmeter using cuff-type probes. Vascular connections between the superior and inferior mesenteric arteries were divided. Intestinal motility was recorded in some animals by means of a balloon inserted into the small intestine and connected to a Statham pressure transducer. Mesenteric vascular resistance (mm/Hg/ml/min) was calculated by dividing arterial pressure by mesenteric arterial flow. Portal venous pressure changes were neglected since this variable changed by less than 2 mm Hg in preliminary experiments in 2 cats. Adrenergic neurone blockade was produced acutely in 9 animals by infusions of bretylium tosylate 1–3 mg/min via a polyethylene catheter tied into the pancreatico-duodenal branch of the mesenteric artery.

Adrenergic neurone blockade was produced in the other 4 cats by giving i.v. 6-hydroxydopamine 50 mg/kg and 75 mg/kg respectively 14 and 7 days prior to the blood flow studies.

Results. The control values (means $\pm$ SE, n = 13) for mean arterial pressure and superior mesenteric arterial flow were 127 $\pm$ 4 mm Hg and 12.4 $\pm$ 1.6 ml/min/kg cat.

Effects of mesenteric nerve stimulation after blockade with 6-hydroxydopamine (4 cats). Stimuli below 8v produced no changes. Stronger stimuli up to 15v, 15/s increased mesenteric blood flow and produced either no change or a slight fall (5-10 mm Hg) in systemic arterial pressure (Figure 1). Intestinal tone and motility were not significantly affected. The mesenteric flow increase was small in magnitude and often slow in onset and development (Figure 1D). Furthermore it usually outlasted the period of stimulation by 2–15 min (mean 5.2 min). The calculated maximum reductions in the vascular resistance of the mesenteric bed in each of the four animals were 41%, 25%, 16% and 12%. These reductions were not significantly altered by pre-treatment with intravenous atropine 0.5 mg/kg.

Effects of mesenteric nerve stimulation after blockade with bretylium (9 cats). Mesenteric nerve stimulation before bretylium produced the expected reduction in mesenteric arterial flow (Figure 1A). Infusions of bretylium 1–3 mg/min into the mesenteric artery increased systemic arterial pressure and mesenteric blood flow in parallel. Both variables returned to pre-infusion values within 20 min of stopping the infusion. After total bretylium doses of 18–40 mg mesenteric nerve stimulation, at 8v or above increased mesenteric blood flow in 6 of the 9 cats. The response resembled that seen in the four cats given