A POSSIBLE RAPID OSMOREGULATORY MECHANISM

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The results of determination of activity of sodium ions in dog plasma, obtained by a potentiometric method using selective glass electrodes, are described. Of the total number of sodium ions 13% are bound with plasma proteins. On the basis of the ability of plasma proteins to bind sodium ions and to liberate them at a time of change in the ionic strength of the surrounding medium, the existence of a rapid mechanism of regulation of the osmotic pressure in the blood plasma is postulated.

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The theory of the neurohormonal system of osmotic regulation, responsible for the final equalization of osmotic pressure, in no way rules out the possibility that more rapidly acting mechanisms exist, based on the physicochemical properties of the blood and producing buffer effects on the introduction of sodium and water into the blood. Plasma proteins may act as buffers, but the problem of fixation of ions by the plasma proteins and the role of this process in osmotic regulation has not been adequately studied.

Opinions differ on the degree of fixation of ions by the blood proteins [3-6, 9]. Most workers report very slight fixation or its complete absence [7, 8]. However, this conclusion is improbable, bearing in mind the strongly helical structure of the plasma proteins with a high negative charge on their molecules.

In this investigation an attempt was made to assess the role of protein-bound sodium and also to examine the possibility that plasma proteins may participate in the mechanism of rapid regulation of the blood osmotic pressure.

EXPERIMENTAL METHOD

Activity of Na^+ ions was determined by means of electrodes made from sodium-selective glass Na – Al – 20-10. The comparing electrode was a calomel electrode connected to the test object by an electrolyte bridge.

The electrodes were calibrated in NaCl solutions with concentrations of $1 \cdot 10^{-1}$, $1 \cdot 10^{-2}$, and $1 \cdot 10^{-3}$ M. Calibration curves were plotted on the basis of coefficients of activity for NaCl solutions [1]. The coefficient of Na^+ activity in blood plasma (γ_{Na}) was calculated from the formula $\gamma_{Na} \frac{A_{Na}}{c_{Na}}$, where A_{Na} represents activity of Na^+ ions in the plasma and c_{Na} the total Na^+ concentration in the plasma.

The potential difference between the cation-selective and reference electrodes was determined with a Vi type TR-1501 electrometer with imput impedance of 10^{14} Ω. Dog plasma was chosen for the experiments. In the corresponding series of experiments it was heated to 60° for 10 min. Glucose and urea were added to the plasma in crystalline form to avoid dilution and in amounts calculated to increase the osmolarity of the blood by 3.5%.

The total sodium concentration in the plasma was measured with a Zeiss flame photometer.

EXPERIMENTAL RESULTS AND DISCUSSION

The Na^+ activity in the plasma in most experiments ranged from 100 to 115 meq/liter and the corresponding Na^+ concentrations varied from 150 to 180 meq/liter. The coefficients of Na^+ activity (γNa) for pure solutions and for plasma were 0.78 and 0.64 respectively. The difference between Na^+ activity in

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TABLE 1. Changes in Na^+ Activity (in meq/liter) in Plasma as a Result of Denaturation and Osmotic Changes

Na^+ concentration in plasma	A_{Na^+} in NaCl solution	A_{Na^+} in native plasma	A_{Na^+} in denatured plasma	ΔA_{Na^+}	Na^+ concentration in plasma	A_{Na^+} in NaCl solution	A_{Na^+} in plasma	A_{Na^+} in plasma + urea	ΔA_{Na^+}	Na^+ concentration in plasma	A_{Na^+} in NaCl solution	A_{Na^+} in plasma + urea	A_{Na^+} in plasma + glucose	ΔA_{Na^+}
177	132	109	120	11	180	134	115	135	20	182	135	106	120	14
182	135	112	124	12	150	116	86	97	11	164	125	109	119	10
154	119	78	115	37	156	120	100	115	15	166	126	115	124	9
156	120	100	118	18	174	130	119	131	12	164	125	106	115	9
176	131	100	137	37	166	26	101	115	14	160	123	115	124	9
164	125	106	124	18										

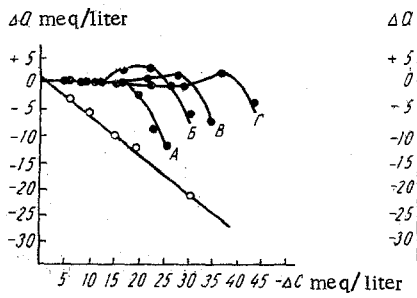

Fig. 1

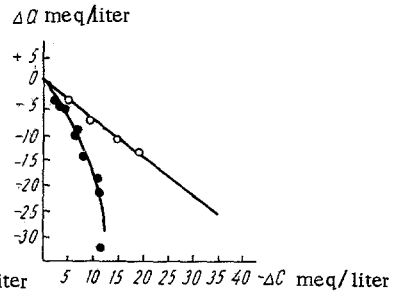

Fig. 2

Fig. 1. Changes in activity of Na^+ ions in plasma during dilution (black circles). Explanation in text.

Fig. 2. Changes in Na^+ ion activity in denatured plasma on dilution. Explanation in text.

aqueous NaCl solutions and in plasma for equality of concentrations thus averaged 20 meq/liter. However, this value varied considerably among different individuals and it evidently depended on the functional state of the animal.

Thermal denaturation of the plasma proteins caused a steep rise in Na^+ activity. In denatured plasma it came close to the Na^+ activity in corresponding aqueous solutions of NaCl (Table 1).

Dilution of the plasma with distilled water caused a marked increase in its buffer properties relative to Na^+. In Fig. 1, where changes in Na^+ concentration during dilution are plotted along the abscissa and changes in activity along the ordinate, the results of dilution of plasma samples containing different concentrations of inactive Na^+ are shown.

For comparison, values of changes in Na^+ ion activity during dilution of aqueous solutions of NaCl are shown. Curve A corresponds to dilution of plasma samples with 18 meq/liter protein-bound NaCl, curve B to dilution of samples with 25 meq/liter, curve C with 27, and curve D with 39 meq/liter. The buffer capacity of the system can be seen to correspond strictly to the concentration of protein-bound Na^+.

Buffer properties of this type may result from the gradual, and not the simultaneous uncoiling of the helical structure of the protein following slight weakening of the degree of screening of negative charges on the molecule. Gradual uncoiling of the albumin molecule with a decrease in ionic strength of the surrounding medium may be due to the frequent disulfide bridges (17) maintaining the native protein structure during uncoiling of some parts of its molecule. This view of the dynamics of albumin denaturation agrees completely with the chemical diagram of human plasma albumin drawn by Anfinsen [2] and illustrated in

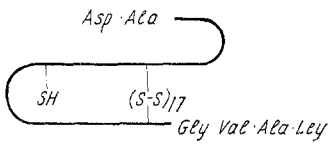

Fig. 3. Chemical diagrams of human plasma albumin.

Fig. 3. If, during denaturation by dilution, S – S bonds begin to break at the "mouth" of the molecule and the loop becomes smaller in the course of dilution, the time must arrive when the loop disappears completely with rupture of the last disulfide bridges. This must be accompanied by liberation of a larger quantity of Na^+ in comparison with the preceding stages.

Changes in Na^+ activity during dilution of denatured plasma are illustrated in Fig. 2 (the axes have the same meanings as in Fig. 1). It can be seen that in this case buffer properties are absent.

It was next decided to examine changes in Na^+ activity in blood plasma after the addition of osmotically active substances in concentration increasing osmolarity by 3-5%.

The results of experiments in which urea and glucose were added to plasma in concentrations of 9 moles/liter are given in Table 1. The change in Na^+ activity was approximately 14 meq/liter for urea and 10 meq/liter for glucose when added to the plasma.

The increase in Na^+ activity following addition of urea is connected with its denaturing action on proteins (it ruptures hydrogen bonds).

The change in Na^+ activity on addition of osmotically active substances such as glucose is evidently connected with their direct or indirect action on the protein buffer system, causing redistribution of ions between the protein molecules and the medium.

Besides the neurohumoral system of osmotic regulation there is thus evidently a mechanism with more rapid action, based on the physicochemical properties of the plasma proteins.

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