SPECIAL ARTICLES

RADIOACTIVE ION EXCHANGES IN LIVING PROTOPLASM

PENETRATION curves for the entrance of radioactive ions into living cells of *Nitella coronata* have been described by Brooks,[1] who has also suggested that the initial increase in ion concentration in protoplasm during the first phase, which is at a maximum in about 15 minutes, is due to an exchange of a radioactive ion for some inactive ion. In the present study, the loss of radioactive ions from the protoplasm of *Nitella* by means of exchange was investigated. Ion exchange is here used to connote the replacement of an ion, possibly bound to an electrolyte by another ion. Ion exchange studies on barley roots have been described by Jenny and Overstreet[2] and on *Elodea* by Mazia.[3]

Nitella were taken from large outdoor ponds, separated into single cells and allowed to remain in pond water at 10° C. for a week. Only turgid cells with a length of 3.5 to 4.5 cm were used, in order to secure material which was as uniform as possible. Cells were immersed in 0.01 M solutions of K*Cl or Na*Cl dissolved in pond water at pH 8.2. After 15 minutes the cells were removed from the radioactive solutions, washed in triple distilled water, and the number of radioactive impacts measured with a Geiger-Müller counter. Cells were then placed in 0.01 M solutions of either LiCl, NaCl, KCl, RbCl or CsCl. Cells placed in either quartz-distilled CO_2-free water or in an ion-free 0.02 M sucrose solutions were used as controls. The temperature was $15 \pm 0.1°$ C. After a 15-minute immersion, measurements of radioactive counts on each cell were made after 1, 2, 3, 5 and 10 minutes. Whole cells were used, the method of measuring consisting simply of placing each cell on a glass slide and putting this slide a known distance from the Geiger-Müller counting tube. The cell could then be returned to its solution and subsequent loss of radioactivity followed. There is no error because of the presence of sap, since there is no loss of Na* or K* to the sap as shown by separate analyses of the sap, protoplasm and wall in 15 minutes. The curves for the loss of radioactive ions from *Nitella* were exponential and followed equation (1)

$$C_p = C_o e^{-kt}, \qquad (1)$$

where C_p is the concentration of radioactive ion in the protoplasm, C_o the initial concentration of ions in the protoplasm, k a constant giving the slope of the logarithmic curve and t the time of immersion of the cell in the solution of inactive ion. The values of k which were found are given in Table I, and they represent the rate of loss of ions from the cell.

TABLE I

Ion Exchanged	Value of k for					
	Li	Na	K	Rb	Cs	HOH
Na*	0.33	0.26	0.23	0.20	0.19	0.05
K*	0.12	0.16	0.83	2.00	...	0.05

[1] S. C. Brooks, *Proc. Soc. Exp. Biol. Med.*, 38: 856–858, 1938.

[2] H. Jenny and R. Overstreet, *Proc. Nat. Acad. Sci.*, 24: 384–392, 1938.

[3] D. Mazia, *J. Cell. Comp. Physiol.*, 11: 193–203, 1938.

Some preliminary experiments with Rb*Cl and the alkali cation series indicate that in the exchange of Rb* for Li, Na, K, Rb or Cs, the results are similar to those in which K* was used, but the rate of loss is much greater.

Further preliminary experiments on the ion exchange properties of the vacuolar membrane of *Nitella* have been carried out by studying the changes in concentration of either K* or Rb* in both sap and protoplasm after the cell had been immersed in radioactive solutions for 24 hours, to allow for accumulation in the vacuole, and then placed in solutions of inactive alkali cations. The rate of loss of ions from the protoplasm was slower than when the cells had been immersed only 15 minutes. In the sap there was an initial rise after one minute and then a falling off in concentration. In the case of anions, where radioactive phosphorus (as $HPO_4^=$) was used and its exchange for the series F, Cl, Br, I, tested, there is a more rapid loss of P* in F^- than in I^- solutions.

In summary one may say that in the case of K*, the rate of loss followed the Hofmeister series, Rb^+ was the fastest and Li^+ the slowest in replacing K*. For Na* the Hofmeister series was reversed, Li^+ was the most rapid and Cs^+ the slowest in replacing Na*. In distilled water the rate of loss of ions was very low. It is suggested that there are at least two factors in operation, namely (1) a permeability effect which in the case of Na* reverses the lyotropic series because ions may not directly exchange, but, rather, be removed because of a decrease in the number of exchange positions on the membrane, and (2) the lyotropic series which governs the loss of ions from the cell in the case of K*.

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THE DENATURATION OF PROTEINS BY DETERGENTS AND BILE SALTS

ALL the synthetic detergents and bile salts I have tried denature ordinary proteins such as hemoglobin and egg albumin at the isoelectric point and keep the denatured isoelectric protein in solution. Some detergents, such as Duponol PC,[1] in sufficiently high concentration, can prevent the precipitation of denatured protein by trichloracetic acid, tungstic acid and acid ferric sulfate.

Screenivasaya and Pirie[2] observed that the syn-

[1] Sodium dodecyl sulfate is the C_{12} compound of the series $CH_3(CH_2)_nCH_2OSO_3Na$. Duponol Special WA Paste (du Pont) consists mainly of the C_{12} compound. Duponol PC is a mixture of the C_{10}–C_{18} compounds.

[2] M. Screenivasaya and N. W. Pirie, *Biochem. Jour.*, 32: 1707, 1938.

thetic detergent sodium dodecyl sulfate[1] in 0.5 per cent. solution slowly splits the large tobacco mosaic virus protein into smaller molecules and separates the nucleic acid from the protein, but does not denature the protein as shown by solubility and digestibility tests. Unfortunately the tests for denaturation were not carried out in an altogether satisfactory way. In any case, tobacco mosaic virus is much more resistant to sodium dodecyl sulfate than hemoglobin or egg albumin.

Hemoglobin is denatured by extraordinarily small amounts of Duponol PC. The addition of only 2 mg of Duponol PC to 10 mg of beef methemoglobin dissolved in 10 cc of pH 6.8 phosphate buffer solution suffices to denature the protein. This denaturation of methemoglobin in dilute solution can be followed optically. Native methemoglobin is a brown compound with a band in the red. Denatured methemoglobin in neutral Duponol solution is a red compound with no band in the red.

If 0.5 gm of Duponol PC is added to 10 cc of 2.5 per cent. hemoglobin and the excess Duponol is then removed by dialysis against water, the hemoglobin remains in solution after the dialysis and retains the color and spectrum of denatured methemoglobin. This result shows the great affinity of Duponol PC for denatured methemoglobin. The dialyzed hemoglobin is precipitated by 0.1 saturated ammonium sulfate, which does not precipitate native methemoglobin. It is digested by trypsin, which does not digest native methemoglobin. Duponol PC in sufficiently high concentration can prevent the precipitation by 0.1 saturated ammonium sulfate and can inactivate trypsin. This is why the excess Duponol is removed by dialysis before the solubility and digestibility tests.

It takes about 10 times as much of the bile salt, sodium glycholate, as of Duponol PC to denature hemoglobin.

0.2 N trichloracetic acid does not precipitate hemoglobin in 1 per cent. solution if 1.3 per cent. Duponol PC is present.

Hemoglobin can be estimated colorimetrically as denatured methemoglobin in 1 per cent. Duponol PC solution. This colorimetric procedure has two advantages over the usual acid hematin procedure. The amount of light adsorbed does not change with time and it is the same whether the Duponol is added to oxyhemoglobin or to methemoglobin.

Bacteria do not grow in the hemoglobin solution containing 1 per cent. Duponol, not even at 37° C.

The synthetic detergents and the bile salts all have the same type of hydrophobic-hydrophilic structure. Each synthetic detergent and bile salt consists of a large hydrophobic part with a small hydrophilic part attached to it. In actual chemical structure, the detergents and bile salts vary considerably. The hydrophilic group may be an acid group, such as OSO_3^-, or a basic nitrogen group. The hydrophobic part may consist of a long straight fatty acid chain, or it may contain a naphthalene or other ring structure.

The denaturation of protein and the solution of denatured protein by detergents and bile salts which differ widely in chemical composition must be attributed to the one property all the detergents and bile salts have in common, their general hydrophobic-hydrophilic character.

Bile salts have been used to extract the photosensitive protein pigment of the eye[3] and to extract a chlorophyll compound from the chloroplasts of spinach.[4] Chlorophyll, I have found, can be extracted from spinach by Duponol PC much more effectively than by bile salts. It is now clear, however, that a protein extracted by bile salts or detergents may no longer be in its original native undissociated form.

Physiologically, the bile salts emulsify fats, activate lipase and promote the absorption of various substances. The possibility must now be considered that the physiological reactions of the bile salts, like their reactions with proteins, depend not on their specific structures, but on their general hydrophobic-hydrophilic character, and that other substances with the same general hydrophobic-hydrophilic character can act as physiological substitutes for the bile salts.

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THE CONFIGURATION OF THE GLUTAMIC ACID OF ADENOCARCINOMA PROTEIN

RECENTLY, Kögl and Erxleben[1] have reported that the glutamic acid of malignant tumor proteins is present in partly racemized form. Chibnall, Rees, Tristram, Williams and Boyland,[2] using the Foreman procedure, found only l(+) glutamic acid in Crocker mouse sarcoma and in bronchial carcinoma. Kögl and Erxleben[3] have offered evidence to show that the Foreman procedure is unsuited for the isolation of the unnatural glutamic acid isomer (d(−) glumatic acid). It is reported in a recent issue of *Science News Letter* (36:37, 1939) that the results of Kögl and Erxleben have been confirmed by Dr. E. Schroeder, of the Biochemical Research Foundation of the Franklin Institute.

[3] W. Kühne in L. Hermann, "Handbuch der Physiologie," Leipzig, 1879, 3: 264.
[4] E. L. Smith, SCIENCE, 88: 170, 1938.
[1] Kögl and H. Erxleben, *Zeit. f. physiol. Chem.*, 258: 57, 1939.
[2] A. C. Chibnall, M. W. Rees, G. R. Tristram, E. F. Williams and E. Boyland, *Nature*, 144: 71, 1939.
[3] F. Kögl and H. Erxleben, *Nature*, 144: 111, 1939.