Permeability of cellophane membranes to parotid proteins during dialysis[1]

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Summary. Pooled parotid saliva was dialyzed in cellophane membranes against water for periods of up to 1 week and loss of proteins was monitored by acrylamide gel-electrophoresis. A gradual loss of cationic proteins was observed whereas anionic proteins were not appreciably affected. Loss of the cationic proteins could be greatly reduced by performing dialyses against dilute electrolyte solutions rather than water. These effects were attributed primarily to electrostatic charges associated with the dialysis membranes.

The tendency of proteins to pass through cellophane membranes during dialysis depends to some extent on protein molecular sizes and on the solvents used, the escape rates being nearly always fastest with distilled water but relatively slow with dilute salt solutions[3]. Another factor affecting escape rates of proteins is electrostatic charge. Since the intrinsic electrostatic charge on cellophane dialysis membranes is negative in aqueous solutions, positively charged solute particles tend to pass through the membranes more readily than negatively charged particles[4]. Human parotid and submandibular saliva contain both acidic and basic proteins, and it has been demonstrated by electrophoresis that some of the basic proteins are lost during dialysis of parotid or submandibular saliva samples against water for 30–48-h periods[5,6]. The present study was undertaken to determine the nature and extent of protein loss upon dialysis of parotid saliva specimens for varied lengths of time against water or dilute electrolyte solutions.

Materials and methods. Parotid saliva was collected from young male naval personnel[7] under stimulation with sour lemon drops (Regal Crown Sour Lemon Candies, Tootsie Roll Industries, Chicago, Ill.), and the individual collections were pooled. Sections of 8/32″ seamless Visking membranes (No. 8, Union Carbide Corp., Chicago, Ill.) were soaked for 10–30 min in deionized water and one end of each section was knotted. 10 ml volumes of the saliva were dialyzed in the membranes at 4 °C for periods ranging from 15 min to 168 h. All dialyses involved a total of at least 80 outer fluid volumes of water or dilute electrolyte per saliva sample volume. The electrolyte solutions included 0.2 and 0.3% NaCl, and 0.1 M tris-EDTA-borate buffer, pH 9.0. Dialyses were performed without agitation except, as noted for particular samples, when a rotating dialyzer (Pope Scientific, Menomonee Falls, Wisc.) was used.

Following dialysis the membranes were emptied, rinsed twice with deionized water, and the saliva solids were recovered by lyophilization and weighed. The proteins that passed through or precipitated onto the membranes during dialysis of several saliva samples against water were also recovered. The dialyzable protein fractions were simply lyophilized whereas the precipitated fractions were resolubilized in 0.2% NaCl, dialyzed against water for 20–45 min, and lyophilized. All saliva samples that were dialyzed against the electrolyte solutions were dialyzed briefly against water in this same manner prior to lyophilization to reduce levels of electrolytes that might otherwise interfere with the electrophoretic separations.

The lyophilized protein preparations were subjected to horizontal acrylamide gel-electrophoresis for 5 h in 0.1 M tris-EDTA-borate buffer, pH 9.0. Protein components were delineated with Wool Fast Blue BL stain. Other details of the electrophoretic procedure have been described in previous reports[8,9].

Results and discussion. The table shows the rate of total-solid loss for 3 lots of pooled parotid saliva that were dialyzed against water. Approximately 40% of the total solids were lost after 30 min and 60% after 3 h of dialysis. Additional tests of saliva samples under these conditions showed that the loss during the first 30 min primarily involved electrolytes and by 3 h the bulk of the uric acid had also passed through the membranes[10]. The rate of loss after 3 h gradually decreased until approximately 25% of the original material remained within the 72–168-h range. Electrophoretic patterns showed no appreciable differences

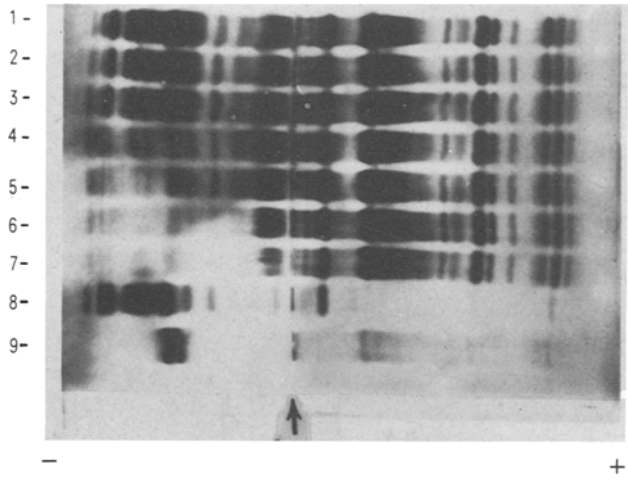

Fig. 1. Electrophoretic patterns after dialysis of parotid saliva samples against water for various periods. 1: control; 2–7: sequence of dialyses for 4, 8, 16, 24, 48 and 120 h; 8: fraction passing through membrane during 168 h, except for material from first 2 h of dialysis, primarily inorganic salts, which was discarded; 9: fraction precipitating in membrane during 168 h, recovered by dialysis vs 0.2% NaCl for 48 h, dialysis vs water 20 min, lyophilization. Arrow indicates origin.

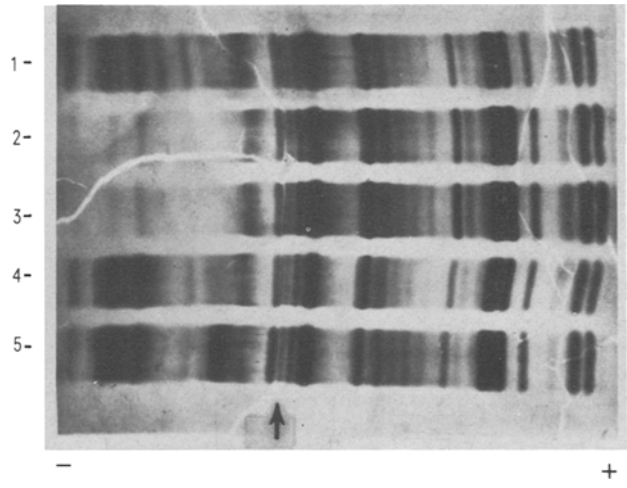

Fig. 2. Electrophoretic patterns after dialysis of parotid saliva samples against water and dilute electrolyte solutions. 1: control, using water with rotating dialyzer 2.5 h; 2 and 3: 168 h, using water, with rotating dialyzer first 2.5 h; 4: 168 h, using 0.3% NaCl 167 0.25 h and water for final 45 min; 5: 168 h, using the tris-EDTA-borate buffer 167 0.25 h and water final 45 min. Arrow indicates origin.

Percentage recovery of nondialyzable solids after dialysis of 10 ml parotid saliva specimens against water for various periods

Period of dialysis (h)	Pool No. 1	Pool No. 2	Pool No. 3	Mean
None	100.0 (0.1043 g)	100.0 (0.1176 g)	100.0 (0.1171 g)	100.0
0.25	73.4	74.7	68.7	72.3
0.50	61.4	62.0	56.4	59.9
0.75	57.1	53.6	54.1	54.9
1	50.0	46.5	–	48.3
2	43.5	42.8	42.7	43.0
3	40.3	40.3	40.0	40.2
4	39.5	40.5	38.6	39.5
5	39.2	38.8	37.5	38.5
6	36.8	38.9	36.4	37.4
8	–	–	35.1	35.1
12	31.1	35.7	32.4	33.1
16	29.5	32.5	28.9	30.3
24	26.8	31.7	30.3	29.6
48	22.7	29.2	27.2	26.4
72	21.4	25.4	28.4	25.1
96	19.5	25.1	28.2	24.3
120	–	24.5	24.4	24.5
168	–	23.3	28.0	25.7

in band intensities for samples dialyzed for 8 h or less, but over longer periods of dialysis the cathodal bands gradually decreased in intensity. The anodal bands showed essentially no changes with longer dialysis periods. These observations are illustrated in figure 1 which presents patterns of representative samples from pool No. 3 of the table. Pattern 1 is a control sample that had been concentrated by ultrafiltration (UM-1 Diaflo Membrane, Amicon Corp., Lexington, Mass.) without dialysis. Patterns 2 through 7, of samples dialyzed for 4–120 h, show a loss of cathodal proteins apparently progressing from the more rapidly-migrating components (at the left in figure 1) towards the origin as the period of dialysis was increased. By contrast, the anodal protein patterns of these samples showed essentially no differences. Pattern 8, of proteins permeating the membrane during a 168-h dialysis, is seen to represent predominantly the more rapidly-migrating cathodal proteins, while pattern 9, of proteins that had precipitated onto the membrane during this period, shows faint anodal bands along with 2 prominent cathodal bands. Some denaturation of the basic proteins apparently occurred during the 168-h dialysis against water. This could account for the failure to recover certain slowly-migrating components (patterns 8 and 9) that had been evident initially (patterns 1–5) but were diminished during prolonged dialysis (patterns 6 and 7).

Figure 2 shows the contrasting electrophoretic patterns obtained by 168-h dialysis of parotid saliva samples against water and dilute electrolyte solutions. The dialyses against water (patterns 2 and 3) resulted in the loss of most of the cathodal proteins, whereas such losses were greatly diminished by using 0.3 NaCl or the electrophoretic buffer as dialytic solvents, as shown respectively in patterns 4 and 5. On the other hand, the corresponding patterns for the anodal proteins were all quite similar. Pattern 1 represents a control sample.

It was concluded from these observations that the electrostatic surface charges on the cellophane membranes significantly affected the permeability of the membranes by the parotid proteins. This was especially evident for dialyses conducted in water. The escape rates appeared to be considerably influenced by the net positive charges on the proteins, as was illustrated by the progressive diminution of cathodal bands of figure 1 (patterns 3 through 7), with essentially no changes evident among the corresponding anodal bands. However, the protein escape rates were markedly decreased when the dialyses were performed in dilute electrolyte solutions, as shown by the contrasting cathodal patterns of figure 2.

Molecular size was undoubtedly a factor that could have affected the protein escape rates. Craig et al.[3] found that proteins up to 45,000 daltons could pass through Visking cellophane membranes during dialysis for 80 h against dilute acetic acid. Escape rates of the proteins were closely correlated with their mol.wts, except that the escape rates for some strongly basic proteins and peptides were somewhat higher than would otherwise have been expected from their molecular sizes. Parotid saliva is known to contain several basic proteins of 12 000 daltons or less[11,12]. Although the mol.wt range of the basic components shown in figure 1, pattern 8, has not yet been fully established, the contrasting cathodal patterns of figure 2 show how the escape of these components can be retarded by conducting the dialysis against dilute electrolyte solutions rather than water.

The findings of this study underscore the importance of minimizing periods of dialysis of basic salivary proteins or peptides in water when using common dialysis membranes such as Visking cellophane tubings. When long-period dialyses are deemed necessary it appears to be more practical to conduct them primarily against dilute electrolyte solutions, limiting the final dialyses against water to relatively brief periods.

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2 Deceased.

3 L.C. Craig, T.P. King and A. Stracher, J. Am. chem. Soc. *79*, 3729 (1957).
4 C.J.O.R. Morris and P. Morris, Separation methods in biochemistry, p. 777. Interscience Publishers, New York 1964.
5 S. Holen, N. Haber and I. Tuck-Weiss, J. dent. Res. *40*, 470 (1961).
6 C. Dawes, Arch. oral Biol. *8*, 653 (1963).
7 P.B. Carroll, J. dent. Res. *46*, 1044 (1967).
8 T.S. Meyer and B.L. Lamberts, Biochim. biophys. Acta *107*, 144 (1965).
9 T.S. Meyer and B.L. Lamberts, Arch. oral Biol. *13*, 839 (1968).
10 T.S. Meyer and B.L. Lamberts, unpublished observations.
11 I.B. Holbrook and P.C. Molan, Biochem. J. *149*, 489 (1975).
12 B.J. Baum, J.L. Bird, D.B. Millar and R.W. Longton, Arch. Biochem. Biophys. *177*, 427 (1976).