THE CHARACTERISTICS OF ULTRAFILTRATES OF PLASMA

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I

On the Establishment of Equilibrium Conditions during the Process of Ultrafiltration

It has been tacitly assumed by many investigators that the fluid formed by filtration through partially semipermeable membranes will be at diffusion equilibrium with the original fluid. In case one of the indiffusible constituents of the original fluid has an ion in common with a diffusible constituent, the so called Donnan membrane equilibrium would be set up if time adequate for the attainment of equilibrium be allowed. There is an important question as to whether the equilibrium would be set up within the time that the ultrafiltrate is passing through the pores of the filtering membrane. Under experimental conditions when a collodion membrane is used as the ultrafilter, the distance across the membrane is relatively great, that is, of the order of magnitude of tenths of a millimeter, and the time during which the filtered fluid remains on the membrane is so short, that is, a matter of minutes, that it might be supposed that equilibrium conditions would never be set up if they depended upon diffusion equilibrium being reached across the whole membrane. Actually, however, we must conceive of the diffusion equilibrium as being set up across the hypothetical plane bounding the region within which the indiffusible constituents can move freely, and separating that region from the region they cannot enter, presumably because of diminutive pore size. That is to say, looking at the membrane as a collection of pores between solid blocks the membrane equilibrium must be set up across the plane of entrance to the pores. Then, unless the fluid moves so rapidly through the pores that diffusion equilibrium cannot occur, we

should expect to find the membrane equilibrium conditions set up in the fluid of the pores, the limiting factor being the rate of flow of fluid compared with the rate at which diffusion equilibrium is established at a given distance from the point of constant composition.

In order to calculate the distance through which diffusion equilibrium will be substantially complete in a given time, one can use a special application of Fick's diffusion law, which is

$$\frac{dC}{dt} = k\left(\frac{d^2C}{dx^2}\right),$$

when C is the concentration at any distance x and time t, and k is the diffusion constant.

Andrews and Johnston (1924) have derived the integral for the case of diffusion in a single dimension into a slab, which has the form:

$$\frac{C}{C_1} = 1 - \frac{8}{\pi^2}\left[e^{-\frac{\pi^2}{4}\cdot\frac{kt}{a^2}} + \frac{1}{9}e^{-\frac{9\pi^2}{4}\cdot\frac{kt}{a^2}} + \frac{1}{25}e^{-\frac{25\pi^2}{4}\cdot\frac{kt}{a^2}} + \ldots.\right]$$

This series may be simplified by neglecting all terms except the first which are insignificant when diffusion equilibrium is more than 36 per cent complete. The equation then becomes

$$\frac{C}{C_1} = 1 - \frac{8}{\pi^2}\left(e^{-\frac{\pi^2}{4}\cdot\frac{kt}{a^2}}\right)$$

In this expression C is the average concentration difference from the original plasma concentration over the distance x; C_1 is the equilibrium concentration difference due to the Donnan effect; k is the diffusion constant of the solute under consideration; and the other symbols have their usual meanings. It is assumed that diffusion is in one plane and that the concentration at the inside wall of the membrane is constant and equal to that in the body of the interior fluid from which ultrafiltration is occurring.

The diffusion constant of sodium chloride in water, according to Landolt-Börnstein, is 0.94 cm.^{2} per day. Using this value for k one can calculate the ratio $\frac{C}{C_1}$ at various values of t and a. In Fig. 1 are plotted the values of a for the times at which equilibration is 95 per cent complete. This proportion may be taken because the difference

between plasma concentration and final ultrafiltrate concentration is of the order of magnitude of 20 mg. per cent chloride, and 95 per cent of this difference represents the limit of experimental accuracy in determination. It is to be seen that at 1 second such an approach to equilibrium would be established at a distance of 0.03 mm. from the plane of separation. In the same figure is shown the maximum rate of flow through the pores of the membrane, calculated from the data of

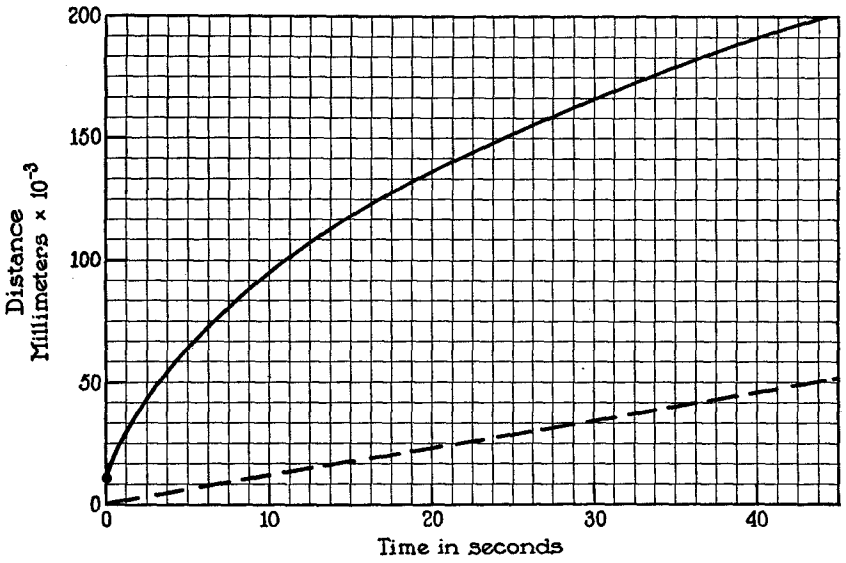

FIG. 1. The distance over which diffusion equilibrium is substantially complete in relation to time. The solid line shows the course of the diffusion equilibrium distance with time. The lower broken line shows the distance over which fluid is passing in the pores of the membrane according to the calculations in the text.

Hitchcock (1926) for the dimensions and the number of pores per unit area of membrane, and our own figures for the rate of flow across the membrane. According to Hitchcock the minimum pore radius in such membranes is 0.3×10^{-6} cm. and the minimum frequency of distribution is 7×10^{10} per cm.^{2} According to our observations the maximum rate of filtration was 2.27×10^{-5} cm. per second, assuming that the whole surface was permeable. Taking Hitchcock's figures, the minimum pore area is 0.198 cm.^{2} per cm.^{2} of membrane surface.

Using this figure it appears that the maximum velocity of flow through the pores of the membrane is 1.14×10^{-4} cm. per second.

This figure represents the maximum velocity, according to the data at hand, which the fluid flowing through the pores of the collodion membrane in our ultrafiltration experiments would have. It is apparent from the graph that at this rate of flow equilibrium will certainly be substantially complete while the fluid is moving through the pores. It is also obvious that with membranes having a thickness of 0.2 mm. diffusion equilibrium would be complete in the time available even if that equilibrium had to occur across the whole membrane. Thus it is apparent that one should expect to find the membrane equilibrium set up even during the dynamic process of ultrafiltration, by virtue of the fact that the mechanical flow of fluid is relatively very slow compared with the rapidity with which diffusion equilibrium is set up over short distances. It seems obvious from our measurements and calculations that at least until the rate of flow through the pores reaches a speed of 5×10^{-2} mm. per second, which is 50 times the speed we calculate to occur, diffusion equilibrium should be established.

II

The Exact Measurement of the Concentration of Electrolytes in Ultrafiltrates

There are hundreds of papers upon the subject of the composition of ultrafiltrates of plasma, but in none of them is there any clear evidence as to whether or not the distribution of ions is influenced by the membrane equilibrium. For the most part investigators have been concerned with the question as to whether one or another constituent of plasma is freely diffusible. The criterion has usually been the existence of identical concentrations of the constituent in the plasma and the ultrafiltrate. Obviously this cannot be the correct criterion if the proteins influence the ionic activity or if the Donnan effect occurs. In most instances, furthermore, investigators have neglected to calculate their results on the basis of the water content of the fluid rather than the volume. Inasmuch as in the case of plasma an error of about 8 per cent results from such neglect, it is not surprising that the conclusions from such observations should be inadequate. Neu-

hausen and Pincus (1923) found that collodion membrane ultrafiltrates of plasma showed nearly the same concentration of sodium chloride as the plasma from which they were derived. In their experiments there was a random difference which they did not attempt to interpret or analyze. Several other investigators have made similar observations. Particularly in view of the fact that physiologists are assuming that certain ultrafiltrates or dialysates of plasma such as ascitic fluid and other edema fluids should show differences in the concentration of ions predicted by the Donnan membrane equilibrium, it seems desirable to make an exact study of the ultrafiltration process itself with sufficiently careful analytical methods, in order to be able to establish the existence or non-existence of such a membrane equilibrium.

In such a study it becomes necessary in the first place to determine with adequate methods what differences if any exist between the concentration of various ions in the plasma and ultrafiltrates from it, and to see whether changes in the base bound by protein, which would be anticipated to affect the membrane equilibrium distribution ratio, do actually alter it. For this study we have chosen the sodium, potassium, and chloride ions for measurement. Some of the finer points in analytical technique, which may be disregarded when less exacting results are wanted, have had to be closely attended to in this investigation.

(*a*) *Methods*

Collodion sacs for ultrafiltration were prepared from a solution of 10 gm. of dry pyroxylin in 100 cc. of equal parts of alcohol and ether, to which 5 per cent of glycerin was added. The collodion solution was poured into chemically clean 25 x 200 mm. Pyrex test-tubes. The excess was drained by inverting during slow rotation. After 5 minutes rotation the casting was repeated and the test-tube was set up inverted on a piece of dry filter paper for a period of $\frac{1}{2}$ hour, after which the membrane was removed from the test-tube and immersed in distilled water saturated with chloroform, where it was kept at refrigerator temperature until use. A large number of membranes were made at one time and used for a number of experiments. They were tested as to their impermeability for protein, and practically all membranes made by this method were found to be impermeable. The sacs were blotted dry with clean filter paper before use. They were then rinsed three or four times with plasma and tied securely to perforated rubber stoppers. They were filled through the holes of the stopper and were then placed in a glass jacket

fitted with two side arms for flushing with air of proper composition as noted below. Pressure was applied over the fluid in the sac and maintained constant by the use of a large air pressure bottle, using a mercury manometer to measure pressure. The air used in the pressure bottle and over the fluid in the sac, as well as in the jacket, was obtained from a large reservoir maintained at the CO_2 tension to which the plasma had been equilibrated and possessing the same vapor tension as the plasma, by having been passed through 0.9 per cent sodium chloride. In this way possible errors due to evaporation and changing CO_2 tension were eliminated. The fluid for analysis was measured by weight inasmuch as it is impossible to calibrate a pipette to deliver accurate volumes of fluids of varying surface tension and viscosity, such as are possessed by plasma and ultrafiltrate. This is a very important point, and failure to take it into account leads to serious error. It is not necessary to weigh each sample of a given fluid if the same pipette is used for the successive samples, since a single weighing will obviously determine what the pipette will deliver of a given fluid. In practise it is therefore only necessary to weigh one quantity of every specimen, and this was done with the specimen used for determining total solids and water.

The chlorides were determined by the digestion method of Van Slyke (1923) during the major part of this investigation. In our first twenty experiments we attempted to use the Whitehorn method but found that, although with certain samples of plasma results obtained agreed with those obtained by the theoretically more accurate digestion method, there was usually a difference showing a larger quantity of chloride in plasma by the Whitehorn (1921) method. This difference we believe to be due to the fact that in making the dilution accompanied by protein precipitation the volume of the protein precipitate is neglected. It seems that this introduces a systematic error of at least 3 per cent, which would enter in only in the case of the protein-containing fluid and would therefore completely invalidate the method for comparison of plasma with its ultrafiltrate. We have found the digestion method to be reliable under all circumstances under which we have tested it. The sodium was determined by the uranyl zinc acetate method of Kolthoff and Barber (1928). For the determination of sodium the plasma samples (1 cc.) were ashed by repeated evaporation to dryness with 1 cc. concentrated nitric acid to which 3 or 4 drops of superoxol had been added. Usually two or three evaporations were necessary. The analysis of sodium has been one of the major difficulties in the approach to the problem under investigation, and we have therefore carried out a rather careful study of the analytical accuracy of this method. The results which we have to present depend for their validity upon the accuracy with which the ions in question can be determined, and since the method is relatively untried, we carried out 50 consecutive analyses of the same sample of plasma to estimate the probable error of the method. The results are given in Table I. In the sample of plasma containing 338.9 mg. per cent sodium the maximum deviation was 1.70 per cent. The mean deviation was 0.515 per cent and the probable error of the mean 0.059 per cent. It is to be seen that a very high degree of accuracy is possible. Potassium was determined by the colorimetric technique

of Breh and Gaebler (1930), using the sodium-silver-cobaltinitrite method. In analysis of known solutions we have found this method to be fairly accurate, although not so accurate as the sodium method. Potassium has been found to behave somewhat differently from sodium in the ultrafiltration process, and as high a degree of accuracy is not necessary in order to make sure of this difference.

TABLE I

Analysis of Sodium Method

50 consecutive analyses of 1 cc. samples of one specimen of blood

Sodium	Deviation from mean	Sodium	Deviation from mean	Sodium	Deviation from mean
Mg. per 100 gm. H_2O	*per cent*	*Mg. per 100 gm. H_2O*	*per cent*	*Mg. per 100 gm. H_2O*	*per cent*
340.1	0.35	337.7	0.35	340.3	0.41
340.7	0.52	336.8	0.61	339.8	0.26
336.7	0.64	336.7	0.67	338.2	0.20
338.6	0.09	342.3	1.00	338.2	0.20
339.6	0.20	336.4	0.73	341.8	0.84
335.6	0.96	340.0	0.32	341.2	0.67
341.2	0.67	338.2	0.20	335.7	0.93
335.9	0.87	335.0	1.13	337.4	0.44
341.3	0.70	340.3	0.41	340.5	0.46
339.5	0.17	340.0	0.32	340.3	0.41
340.3	0.41	338.8	0.03	342.0	0.90
338.3	0.17	336.5	0.70	342.2	0.96
336.5	0.74	338.8	0.03	341.9	0.87
340.8	0.55	338.2	0.20	337.0	0.55
341.2	0.67	337.1	0.52	337.7	0.35
338.9	0.00	340.0	0.32	339.5	0.17
332.9	1.70	338.3	0.20		

Mean of all determinations = 338.9 mg. per cent.

Mean deviation = ± 0.515 per cent.

Standard deviation $= \sigma = \frac{\sqrt{\Sigma d^2}}{n} = 0.6139$ per cent.

Probable error of the mean P.E.$_m$ $= \frac{0.6745\sigma}{\sqrt{50}} = 0.0585$ per cent.

Sodium, chloride, and potassium were determined in duplicate in each instance. The hydrogen ion concentration of the plasma was measured with the quinhydrone electrode using a potentiometer and galvanometer of adequate accuracy. The carbon dioxide content of the plasma was determined by the method of Van Slyke and Neill (1924). In equilibrating the plasma with definite tensions of CO_2, the CO_2-containing air was stored in a Douglas bag and its composition was determined by the Haldane method.

Ultrafiltration was carried on at a pressure of 100 mm. of mercury and the rate of filtration was from 2 to 4 cc. per hour through approximately 50 cm^2. area of membrane. After establishing the pressure at the commencement of filtration, the surrounding jacket was removed and the first 0.5 cc. of filtrate was absorbed from the wall of the collodion sac by dry clean filter paper, in order to eliminate the error of dilution due to the water retained in the pores of the membrane.

(*b*) *The Concentration of Sodium and Chloride in Ultrafiltrate and Plasma*

The first question to be decided is as to whether the concentration of the several ions under investigation is identical in an ultrafiltrate and in the plasma from which it came. In Table II are shown the data

TABLE II

	Proteins	Solids	$[\overline{Cl}]$	$[\overset{+}{Na}]$
	per cent	*per cent*	*mm. per kg. H_2O*	*mm. per kg. H_2O*
Plasma	5.9	6.48	128.6	151.1
Residue	7.4	7.96	126.6	157.9
Mean plasma			127.6	154.9
Ultrafiltrate	0	1.07	132.7	141.8

$$r_{\text{chloride}} = \frac{[\overline{Cl}]_p}{[\overline{Cl}]_u} = 0.963 \qquad r_{\text{sodium}} = \frac{[\overset{+}{Na}]_u}{[\overset{+}{Na}]_p} = 0.916$$

of a typical experiment. It is to be noted that the concentration of sodium and chloride in the water of the ultrafiltrate is different from that of the original plasma, while the concentration in the residue left in the collodion sac is likewise different from the original. The ultrafiltrate was being formed from a plasma of changing composition; presumably, therefore, the concentration of the ultrafiltrate was not constant through the whole process, but was gradually changing. It is therefore the composition of an average ultrafiltrate that we measure, and it is consequently improper to compare it with the concentration in the original plasma; rather one must compare it with the average concentration of the fluid over the time during which it was filtered. Taking the mean of the residue and the original plasma, and comparing

with the ultrafiltrate, which is an average, the ratio one obtains is the true concentration ratio. In the case of sodium one finds the concentration is less in the ultrafiltrate than in the plasma. In the case of chloride one finds more in the ultrafiltrate than in the plasma from which it came.

It is important to note that this inverse behavior of sodium and chloride would be predicted if their distribution were determined by the forces entering into a membrane equilibrium when an indiffusible anion is present. This may be taken as suggestive but not conclusive evidence that the membrane effect produces the concentration differences.

TABLE III

Concentration Ratio for Sodium in Consecutive Ultrafiltrations of Several Samples of Dog's Plasma Equilibrated with Air Containing 6 per cent CO_2

0.952	0.900*	0.918	0.911
0.914	0.913*	0.955	0.951
0.940	0.908†	0.929	0.914
0.902	0.919*	0.978	0.916
0.930†			

Mean = 0.926
σ = ±0.021
P.E.$_m$ = ±0.004

* Average of three complete ultrafiltrations on one sample of plasma.
† Average of two such complete runs on one plasma.

In order to determine whether the difference between the concentration of sodium in the water of the plasma and in the water of the ultrafiltrate is a significant one in relation to the possibilities for analytical error in the method, it is necessary to study a series of observations. In Table III are shown the results with seventeen samples of plasma representing twenty-three complete ultrafiltration experiments. The mean value for the concentration ratio for sodium in ultrafiltrate as compared with the plasma from which it was derived is 0.926. The difference between this and the ratio 1.000, which would exist if there were an equivalence in concentration of the two, is 0.074. This value is more than three times the standard deviation and is nearly twenty

times the probable error of the mean of this series. There is obviously a significant difference. Comparing the probable error of the mean for the concentration ratio with the probable error of the mean of a series of determinations of a single sample of plasma, as shown in Table I, it is to be noted that the probable error of the former is about seven times the probable error of the mean in the latter. This is due to the fact that in the calculation of a "concentration ratio" analytical values in three separate fluids must be dealt with. The random variations due to the inherent inaccuracies of the technique therefore enter in three times, and although they may cancel each other out—and usually do—they will on occasion be in the same direction and therefore cumulative.

In the case of chloride the concentration ratio r has been found to have an average value of 0.969 for plasma equilibrated with CO_2-containing air, as is shown in Table V. There is thus a difference of 0.031 between the ratio observed and that which would occur if the concentration of chloride were identical in plasma and ultrafiltrate. The standard deviation in this series, which represents twenty-three complete ultrafiltrations on thirteen samples of plasma, is 0.009 and the probable error of the mean is 0.0016. One can state with assurance that the distribution of chloride in plasma and ultrafiltrate is unequal and that therefore some factor such as the membrane effect must enter into the process.

(c) The Influence of Changing the Amount of Base Bound by Protein

In the course of these experiments it was observed that there was a correlation between the bicarbonate content of the plasma and the sodium and chloride concentration ratios. We therefore compared the concentration ratios for each sample of plasma at different hydrogen ion concentrations produced by equilibrating portions of the plasma with differing tensions of CO_2 in air. In Table IV are presented the analytical results of one such complete experiment. It is apparent that raising the pH by diminishing the bicarbonate increases the difference between the concentrations in ultrafiltrate and plasma. That is to say, at the higher pH there is more chloride and less sodium ultrafiltered. In this connection it should perhaps be noted again that the pertinent plasma concentration is the average between the original and the residue.

TABLE IV

Influence of Changes in the pH upon the Distribution Ratio in Ultrafiltration

	Original plasma	Residue	Ultrafiltrate	Distribution ratio	Residue	Ultrafiltrate	Distribution ratio	Residue	Ultrafiltrate	Distribution ratio	Average ratio for subexperiments	
(*a*) pH 6.95	422.6	390.0	421.7	0.964	408.6	423.9	0.980	385.5	424.7	0.952	0.965	Chloride
(*b*) pH 7.75	422.0	378.5	432.5	0.926	373.7	429.8	0.926	371.4	430.0	0.923	0.925	mg./100 gm. H_2O
(*a*) pH 6.95	350.0	360.2	332.3	0.936	367.0	332.6	0.928	364.1	330.7	0.926	0.930	Sodium
(*b*) pH 7.75	349.0	365.2	329.2	0.922	366.3	327.2	0.915	366.1	328.2	0.920	0.919	mg./100 gm. H_2O

Bicarbonate content of plasma at pH 6.95 was 15.6 millimols; in the plasma at pH 7.75 it was 10.5 millimols. The correction has been made for CO_2 in solution.

TABLE V

Concentration Ratio for Cl and the Change with Alterations in pH

Experiment No.	r (pH about 7.00)	r (pH about 7.70)	Δr
1	0.958	0.935	−0.023
2	0.973	0.961	−0.012
3	0.972	0.963	−0.009
4	0.964	0.943	−0.021
5	0.962	0.953	−0.009
6	0.960	0.939	−0.021
7	0.967	0.938	−0.029
8	0.971	0.940	−0.031
9	0.992*	0.951*	−0.041
10	0.976*	0.957*	−0.019
11	0.972†	0.930†	−0.042
12	0.962†	0.944†	−0.018
13	0.965*	0.925*	−0.040
Mean	0.969	0.944	−0.024
σ	±0.0087	±0.0116	±0.0118
P.E.$_m$	±0.0016	±0.0022	±0.0022

* Average of three complete experiments on the same plasma.

† Average of two such experiments.

TABLE VI

Change in Concentration Ratio for Sodium with Alteration of Hydrogen Ion Concentration of Plasma

Series 1		Series 2	
Δ pH	Δr	Δ pH	Δr
+0.67	−0.014*	†	−0.021
+0.61	−0.021*	†	−0.061
+0.80	−0.023‡	†	−0.068
+0.95	−0.004‡	†	−0.068
+0.85	−0.011*	†	−0.038
Mean	−0.015		−0.051
σ	±0.006		±0.018
P.E.$_m$	±0.002		±0.005

* Figure represents the average of three complete runs on one sample of plasma.

† pH was not measured but CO_2 content determinations indicated Δ pH approximately + 0.90.

‡ Figure represents average of two complete runs.

In order to be sure that random variations are not so large as to destroy the significance of the differences we have consistently observed to be as indicated above, it is necessary to make a statistical analysis of a comparable series. Table V shows the data of a number of similar experiments so analyzed. In the last column is shown the change in concentration ratio with the change in pH from 7.00 to 7.70. The average Δr is -0.024, and the probable error of the mean is only 0.002. All values of Δr have the same sign, and there seems to be no reasonable room for doubt that the pH determines the value of r. Increasing the alkalinity of the plasma, that is, increasing the base bound by protein, causes more chloride to appear in the ultrafiltrate, and *vice versa*.

In a similar way the data for all comparable observations on sodium are presented and analyzed in Table VI. Here again one invariably finds that r diminishes with increases in pH. The regularity of the difference is not so great as with chloride, probably because of the greater inaccuracies of the sodium method, but in spite of that fact there is obviously a significant change in the concentration ratio for sodium with a change in hydrogen ion concentration.

(*d*) *The Behavior of Potassium in Ultrafiltration from Plasma*

The concentration ratio for potassium $r = \frac{[K_u]}{[K_p]}$ has been found to be 0.901 when the plasma is about pH 7.00. When the pH is increased to around 7.70 the concentration ratio has been found to fall to 0.816, as indicated in Table VII. Thus in the case of potassium as well as the other ions the hydrogen ion concentration determines the concentration ratio.

In Table VIII are presented for comparison the average concentration ratios for sodium, chloride, and potassium in all comparable experiments. The great difference in concentration ratios for these three ions is unmistakable. If the activity coefficient of each of the ions were the same, and all of the distribution differences were due to the membrane equilibrium phenomenon, the concentration ratios should be identical for all ions. Obviously, then, either the activity coefficients are different for the several ions or the membrane equilibrium effect of indiffusible ions is not the sole factor in bringing about

the distribution differences. The simplest explanation of these findings is probably to be found by making the plausible assumption that the activity coefficients are different. If one were to assume that the activity coefficient of the chloride ion in plasma is the standard for comparison for the other ions, then the sodium ion activity is less than the chloride, while the potassium ion activity is still lower than the sodium.

TABLE VII

Concentration Ratios for Potassium and the Change with Alteration in pH

Experiment No.	r (pH about 7.00)	r (pH about 7.70)	Δr
14	0.905*	0.845*	−0.060
15	0.897†	0.809†	−0.088
16	0.900†	0.804†	−0.096

* Average of three complete experiments on one sample of plasma.

† Average of two experiments.

TABLE VIII

Comparison of Average Concentration Ratios for Cl, Na, and K in all Experiments

	r (pH about 7.00)	r (pH about 7.70)
Cl	0.969	0.942
Na	0.928	0.913
K	0.901	0.816

(*e*) *The Calculation of the Magnitude of the Membrane Equilibrium Effect*

It is not certain that the concentration changes produced in the ultrafiltrate by altering the amount of base bound by protein in the plasma are connected with the membrane equilibrium phenomenon. It is altogether possible that other factors might operate to bring about the same effect, and it is therefore of interest to see how closely one can account quantitatively for the observed phenomena by calculations from known data, using the assumption that the distribution of sodium and chloride on the two sides of the ultrafilter membrane is actually determined by the Donnan membrane equilibrium.

In order to calculate the magnitude of the Donnan distribution ratio one must know accurately the amount of base bound by protein, which most investigators have not attempted. It is possible, however, to calculate more simply and accurately the difference in the ratio r which would be expected when the pH of the plasma is altered by a change in the CO_2 tension with which it is equilibrated. From this difference, as the derivation below indicates, can be calculated the change in the base bound by protein which presumably caused it. The change in the base bound by protein was experimentally measured in these experiments by determination of the differences in the $\bar{H}CO_3$ content. This is, of course, also a measure of the change in the total concentration of diffusible anions in the plasma, since the bicarbonate is the only diffusible anion that appreciably alters with a change in CO_2 tension. Thus by comparing the calculated with the observed values for the change in concentration of diffusible anion, or conversely in base bound by protein, one can test whether the membrane equilibrium theory satisfactorily accounts for the observed distribution ratios.

Let r_a = distribution ratio in the more acid condition.
r_b = distribution ratio in the more basic condition.
$[\bar{Cl}_{pa}]$ = average concentration of chloride in millimols in the plasma during ultrafiltration at the higher CO_2 tension.
$[\bar{Cl}_{ua}]$ = Concentration of chloride in the ultrafiltrate at the higher CO_2 tension.
$[\bar{A}_{pa}]$ = Concentration of all diffusible anions in the plasma at the higher CO_2 tension.
$[\bar{A}_{ua}]$ = Concentration of all diffusible anions in the ultrafiltrate at the higher CO_2 tension.
Subscript *pb* indicates concentration in the plasma during ultrafiltration at the lower CO_2 tension and higher alkalinity.
Subscript *ub* indicates concentration in the ultrafiltrate at the higher alkalinity.

From the classical Donnan equation:

$$r_a = \frac{[\bar{Cl}_{pa}]}{[\bar{Cl}_{ua}]} = \frac{[\bar{A}_{pa}]}{[\bar{A}_{ua}]} \quad (1),$$

and

$$r_b = \frac{[\bar{Cl}_{pb}]}{[\bar{Cl}_{ub}]} = \frac{[\bar{A}_{pb}]}{[\bar{A}_{ub}]} \quad (2).$$

From the law of electrostatic neutrality and the fact that $\overset{+}{\mathrm{N}}\mathrm{a}$ is the quantitatively important cation one can state that:

$$[\overset{+}{\mathrm{N}}\mathrm{a}_{ua}] = [\bar{A}_{ua}] \tag{3},$$

and

$$[\overset{+}{\mathrm{N}}\mathrm{a}_{ub}] = [\bar{A}_{ub}] \tag{4}.$$

By substitution in (1) and (2)

$$[\bar{A}_{pa}] = r_a \times [\overset{+}{\mathrm{N}}\mathrm{a}_{ua}] \tag{5},$$

and

$$[\bar{A}_{pb}] = r_b \times [\overset{+}{\mathrm{N}}\mathrm{a}_{ub}] \tag{6}.$$

For convenience one can define:

$$\Delta[\bar{A}_p] = [\bar{A}_{pa}] - [\bar{A}_{pb}] \tag{7}.$$

Since with a change in CO_2 tension the only diffusible anion whose concentration changes is the bicarbonate ion,

$$\Delta[\bar{A}_p] = \Delta[\bar{\mathrm{H}}\mathrm{CO}_{3p}] \tag{8},$$

where $\Delta[\overline{\mathrm{H}}\mathrm{CO}_3]$ is the difference in millimols between the bicarbonate content of the plasma in the more acid and the more basic conditions.

If one substitutes the experimentally determined values given in Table IV in equations (5) and (6) one finds:

$$[\bar{A}_{pa}] = 0.965 \times \frac{331.2}{2.3} = 138.9$$

and

$$[\bar{A}_{pb}] = 0.925 \times \frac{328.2}{2.3} = 131.9.$$

Therefore from equation (7) $\Delta[\bar{A}_p] = 7.0$. The measured value of $\Delta[\bar{\mathrm{H}}\mathrm{CO}_3]$, which should equal $\Delta[\bar{A}_p]$, is 5.1. Computation for thirteen such complete ultrafiltrations shows that the calculated average value for $\Delta[\bar{A}_p] = 5.4$ mm., while the experimentally determined quantity $\Delta[\bar{\mathrm{H}}\mathrm{CO}_3] = 4.8$ mm. The agreement between the calculated and the

observed values is sufficiently close to permit the conclusion that the membrane equilibrium theory will account quantitatively for the facts observed.

(f) The Influence of Ionic Activity upon the Distribution Ratios

It is interesting to consider the possible reason for the differing distribution ratios for the several ions. It seems most likely that these differences are due to differing ionic activities for the several ion species. If the assumptions are made that the activity of the chloride ion is not influenced by the presence of the protein in plasma and that the sodium ion activity in the ultrafiltrate is equivalent to its activity in other solutions of similar ionic strength, it is possible to calculate the activity coefficient of the sodium ion in the plasma from the observed distribution ratios. Stadie and Sunderman (1931) have shown that the chloride ion activity is not lowered in hemoglobin solutions, whereas the activity of sodium in hemoglobin combination is markedly lowered. It is furthermore possible to calculate the difference in ionic activity with a change in the hydrogen ion concentration of the plasma. For the more acid condition one may write

$$r_a = \frac{[\bar{Cl}_{pa}]}{[\bar{Cl}_{ua}]} = \frac{\alpha_{ua}[\overset{+}{Na}_{ua}]}{\alpha_{pa}[\overset{+}{Na}_{pa}]}$$

and

$$\frac{\alpha_{pa}}{\alpha_{ua}} = \frac{1}{r_a} \times \frac{[\overset{+}{Na}_{ua}]}{[\overset{+}{Na}_{pa}]}$$

For the more basic condition

$$\frac{\alpha_{pb}}{\alpha_{ub}} = \frac{1}{r_b} \times \frac{[\overset{+}{Na}_{ub}]}{[\overset{+}{Na}_{pb}]}$$

where α_{ua} represents the activity coefficient for the sodium ion in the ultrafiltrate, α_{pa} the same in the plasma, and other symbols as before. In our observations at pH 7.0 summarized in Table VIII the activity ratio $\frac{\alpha_{pa}}{\alpha_{ua}}$ for sodium is equal to 0.958, which implies that the activity of sodium in the plasma is depressed somewhat more than 4 per cent

by the presence of the protein. Similarly in the case of potassium one calculates that at pH 7.0 the activity ratio is 0.929, while at pH 7.7 it is only 0.866. Thus it is apparent that the values for the distribution ratios of sodium and potassium lower than the value for chloride can be interpreted as due to the depression of the activity coefficient for the cations by the protein in the plasma. It is impossible from the data at hand to be certain as to the exact magnitude of the effect, but it appears to be very probable that in the altered distribution ratio for cations from that for chloride, one is dealing with an effect of the altered activity coefficient of cations by proteins on the alkaline side of their isoelectric points.

Greene and Power (1931) have preferred to treat their data on dialysis of plasma from the point of view of the degree of dissociation of alkali proteinates. Their experimental findings concerning the percentage inactivation of the several cations by the influence of the protein in the plasma agree with the results presented here.

(*g*) *The Biological Significance of These Studies*

It seems apparent from the results here presented that in order to judge as to whether a biological fluid is or is not an ultrafiltrate of blood plasma it is necessary to take into account the fact that ultrafiltrates apparently come into membrane equilibrium concentration with the fluids from which they are formed. Furthermore, the membrane equilibrium ratio is very considerably altered by changes in the hydrogen ion concentration of plasma over physiological ranges. Consequently ultrafiltrations must be made at the hydrogen ion concentration and carbon dioxide tension existing in the organism before results of such ultrafiltrations can be interpreted in connection with this problem.

These results seem to stress further the importance of more careful study of the activity of the several ions in biological systems, and show the inadequacy of comparing analytical concentrations directly as a test for determining whether or not a fluid is a simple ultrafiltrate.

SUMMARY

1. Calculations from the Fick diffusion law are shown to predict that membrane equilibria should be established during the course of ultrafiltration.

2. It is shown that the chloride ion is more concentrated and the sodium ion less concentrated in the ultrafiltrate than in the plasma from which the ultrafiltrate was derived.

3. It has been found that by increasing the base bound by protein through a reduction in the bicarbonate content the difference between the plasma concentration and the ultrafiltrate concentration for the several ions studied increases.

4. Calculations from the Donnan equation as to the magnitude of the change in base bound by protein at differing hydrogen ion concentrations are in substantial agreement with the observed values, thus rendering it probable that the membrane equilibrium effect is responsible for the change in distribution ratios observed.

5. It is pointed out that the observed difference in the distribution ratio of cations from that of the chloride anion is probably to be explained by the influence of protein in lowering the activity coefficient of cations when on the alkaline side of the isoelectric point.

6. It is pointed out that account must be taken of these observations in any consideration of the rôle of ultrafiltration in the production of any secretion or body fluid.

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