PRESSURE–VOLUME RELATIONSHIP OF THE FUNDULUS EGG IN SEA WATER AND IN SUCROSE*

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INTRODUCTION

The fact that the eggs of some teleosts, *Fundulus* (4, 5), trout (1, 6, 13), and salmon (12) possess a plasma membrane which is impermeable to water remains an enigmatic problem. Because of the absence of D_2O exchange under conditions of osmotic filtration and diffusion, Ussing (15) suggested that the plasma membrane of the trout egg was composed of a continuous lipide phase containing no pores, and allowing no solution of water molecules. Experiments in which pressure and volume were measured simultaneously and continuously have shown that the impermeable quality of the plasma membrane of *Fundulus* egg occurs only in activated or fertilized eggs, and that it is acquired during the activation process (5). This has been confirmed for the salmon egg by studies of D_2O exchange (12). The problem of interest which prompted the experiments reported herein concerns the changes which occur during activation and which ultimately lead to the impermeable state of the plasma membrane.

Some features of activation in the *Fundulus* egg have been described previously (4), and except for minor details, appear to be the same for other teleost eggs. Briefly, the outer enclosing chorion of the *Fundulus* egg becomes inelastic shortly after contact with sea water, and thereafter maintains a constant volume. Upon successful insemination or activation, numerous small vesicles in the peripheral aspects of the egg proper rapidly disintegrate and appear to release some colloidal material between the chorion and the egg proper. Upon imbibition of water, the colloid swells and causes the formation of a perivitelline space which separates the chorion from the egg proper. Two

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other distinct features accompany the gradual growth of the perivitelline space. Since the chorion is inelastic, the perivitelline space enlarges at the expense of the egg proper which decreases in volume and surface area. Also, a relatively high colloid osmotic pressure, 150 mm. Hg when stabilized, is formed in the perivitelline space, and is transmitted throughout the entire egg enclosed within the chorion.

It has been suggested (4, 5) that the shrinking of the egg proper is related to the increasing perivitelline pressure. The purpose of this paper is to describe some experiments by which the pressure-volume relationship can be evaluated. In order to strengthen the evidence obtained from studying the normal progression of pressure increase, the events were modified or reversed by immersing the eggs in a hypertonic sucrose solution. In sucrose, because of a slow rate of penetration through the chorion, the pressure and volume changes were sufficiently slow to allow both to be measured. In addition, the degree of the changes produced also served as a severe test of the pressure-volume relationship.

Material and Method

As in previous experiments, pressure and volume changes were followed simultaneously on the same unfertilized egg activated by puncturing with a micropipette. The details of the method and apparatus have been described elsewhere (4). In essence, intracellular pressure was measured with a mercury manometer connected to a micropipette which was inserted deep into the interior of an egg. Volume was determined by measuring the diameter of the spherical egg. In most experiments, internal pressure was allowed to develop to its full extent after activation in sea water. When pressure was stabilized, the external sea water was removed, and a known volume of a test solution of sucrose was added. The volume of the test solution was large enough compared to the amount of sea water adhering to the chorion and its jelly strands, so that no significant dilution occurred after mixing. The concentrations of sucrose ranged from 0.01 M to 1.9 M, and were usually made up in sea water so as to avoid the complications of salt dilution. Occasionally, test solutions prepared in distilled water were used. Eggs of *Fundulus heteroclitus* were used in most experiments, although eggs of *F. majalis*, used occasionally, gave similar results.

RESULTS

A. Effect of Sucrose on the Internal Hydrostatic Pressure

As in earlier experiments (4), an internal hydrostatic pressure gradually developed when the egg was activated by the puncture with which the measuring micropipette was introduced. In some of the graphs showing the effects of sucrose, only the stabilized portion of the time course of internal pressure is shown since the earlier portion has been discussed previously. The effect of sucrose is osmotic, and is, in general, quite similar to that exerted by ions, but differing in amplitude and duration. The differences, as will be shown

later, can be attributed to the relative readiness with which these substances penetrate the chorion. The latter is a porous structure, and contrary to Loeb's conclusions (8, 9), is freely permeable to both water and ions (5). Since the ionic compositions of sea water and perivitelline contents are essentially the same, the effects of test solutions of sucrose, which were made up in sea water, are due entirely to the sucrose present. Depending on the concentration of sucrose, the effects are varied. At a concentration of 0.01 M, corresponding to an osmotic pressure of 150 mm. Hg, sucrose exerts the least effect. In Fig.

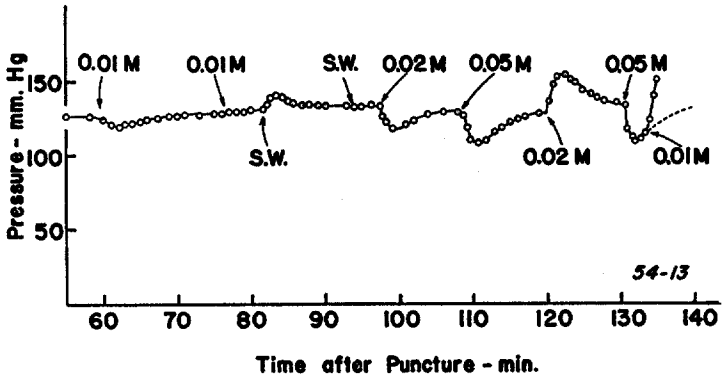

FIG. 1. Effect of hypertonic solutions of sucrose in sea water on the internal hydrostatic pressure. The first immersion in 0.01 M produced a slight decrease because this concentration was slightly hyperosmotic. The pressure returned when 0.01 M penetrated the chorion to establish a new equilibrium which was confirmed by the lack of effect of the second immersion in 0.01 M. The two subsequent immersions in sea water showed a similar process. 0.02 M sucrose when applied to an egg in sea water was hyperosmotic, but became hypo-osmotic if the egg had been first immersed in 0.05 M. At the end of the graph, perivitelline pressure probably would follow a slower return course as shown by the dotted line were the egg allowed to remain in 0.05 M sucrose in a manner similar to the results at 110 to 120 minutes. But changing to 0.01 M sucrose facilitated the pressure return.

1, the small transient decrease of pressure produced by this concentration can be explained by the fact that the perivitelline pressure was not quite 150 mm. Hg, and therefore, 0.01 M sucrose was slightly hyperosmotic. As when immersed in hypertonic salt solutions, water would leave the perivitelline space, accounting for the decline of pressure which would return when sucrose has penetrated the chorion to establish a new equilibrium.

Contrary to this simple manifestation, a 0.1 M sucrose solution in distilled water added to the sea water environment to yield a final concentration of 60 per cent sea water and 0.04 M sucrose produced a complicated sequence of events (Fig. 2). Immediately after the addition, there was a rapid rise in

pressure which was followed by a fairly rapid decline. This decline differed from that observed in hypotonic salt solutions (5) in being more rapid and in undershooting the original pressure to produce a dip before the pressure finally returned. This sequence of events can be explained as being the net result of two oppositely directed processes, one produced by the dilution of sea water, and the other produced by sucrose solution. The dilution of sea water (equivalent to 0.53 M of a uni-univalent salt) to 60 per cent (equivalent

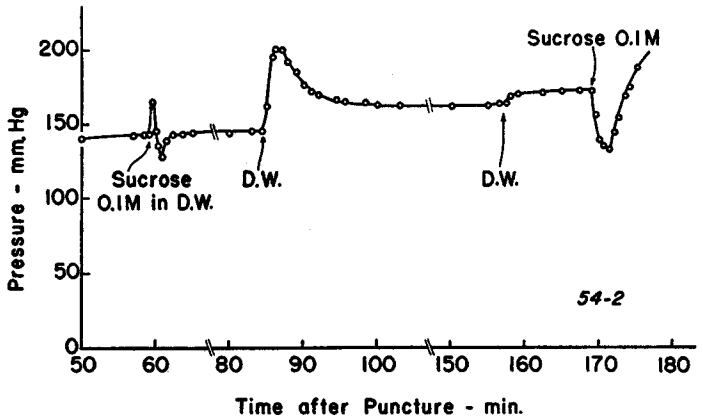

FIG. 2. Effect of 0.1 M sucrose in distilled water on the internal hydrostatic pressure. The first immersion produced a final concentration of 60 per cent sea water and 0.04 M sucrose. As a result, a diphasic response was elicited. That was due to salt dilution (causing pressure rise) and hyperosmoticity of sucrose (causing pressure decrease). The diphasic response was abolished after the salts were washed out by distilled water. The first wash at 82 minutes produced a large increase of pressure which then returned. At 120 minutes (not shown) a second wash produced a smaller rise. At 156 minutes, a third wash produced a very small change. After these washings, addition of 0.1 M sucrose in distilled water only produced the effect of hyperosmotic sucrose solution.

to 0.32 M) provided an osmotic gradient for water to enter the perivitelline space. The presence of 0.04 M sucrose on the outside, however, exerted the opposite effect since the perivitelline colloid was 0.01 M. The first process obviously exceeded the second in magnitude to produce an initial rise of pressure. The second process must not only have prevailed during this time to account for the rapid decline of the pressure, but also it must have outlasted the first to produce the transient dip in pressure. Evidence in support of this explanation is provided by the fact that the same concentration of sucrose when added to the same egg after the latter had been washed several times in distilled water produced only a marked decrease in pressure (Fig. 2). Un-

doubtedly, most of the salts in the perivitelline space had been washed out by the distilled water, so that the initial effect of salt dilution was no longer present.

In higher concentrations of sucrose, from 0.3 M to 1.9 M in distilled water or in sea water, the pressure was reduced to the atmospheric level or a slightly negative value for a varying period of time before the pressure finally began

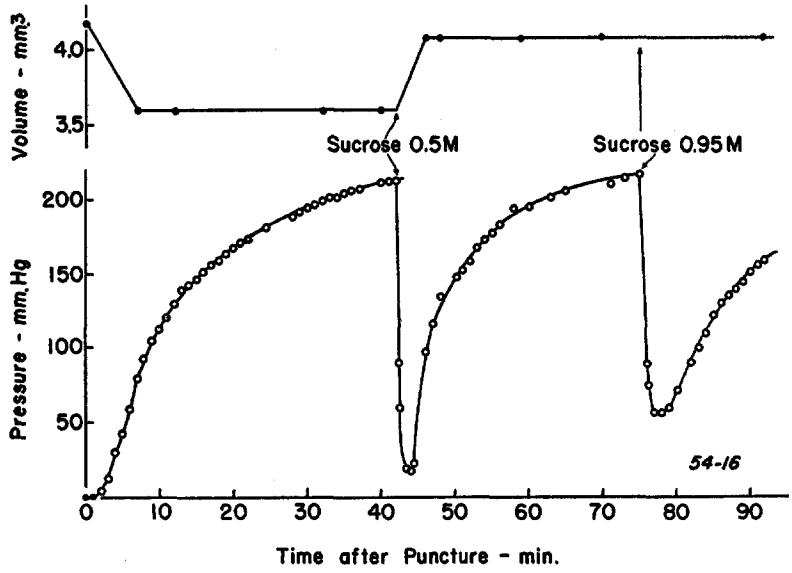

FIG. 3. Effect of highly hypertonic solutions of sucrose in sea water on the perivitelline pressure and the volume of the egg proper. In this egg, the pressure (lower tracing) was higher than average in the fully activated state, at which time the volume (lower tracing) was stabilized at a minimum. Addition of 0.5 M sucrose in sea water induced a precipitous fall of the perivitelline pressure and a simultaneous swelling of the egg proper. Upon the return of pressure, most eggs so treated showed a decrease of volume. In this case, no shrinkage was measured, suggesting that the egg proper had become incompressible at this time. Later immersion in 0.95 M sucrose caused only a pressure change without any further volume change since no previous shrinkage occurred.

to return (Fig. 3). This effect was also observed when mannitol was used instead of sucrose, showing that the effect was due only to the slowness with which the non-electrolytes crossed the chorion, and not to any specific action of sucrose. When the higher concentrations were used, the chorion often collapsed, producing a large indentation which then pressed on the egg proper. With time, the indentation in the chorion gradually refilled. But as long as a visible deformity was present, no pressure was recorded, the latter beginning

to return only after the last trace of the indentation was refilled frequently with some abruptness.

In all concentrations of sucrose, the pressure eventually returned, a fact which could only be explained by the penetration of sucrose through the chorion.

B. Further Evidence of Sucrose Penetration through the Chorion

The following experiment provides conclusive evidence of sucrose penetration through the chorion (Fig. 1). After first washing in 0.01 M sucrose solution and sea water successively to ascertain that the recording system was in order, sea water surrounding an egg with a stabilized pressure of 130 mm. Hg was replaced by a 0.02 M sucrose solution in sea water. A slight dip was produced since the applied solution was hyperosmotic. When the pressure had returned, a 0.05 M sucrose solution in sea water, was placed on the outside. This solution produced a more marked and more prolonged dip. While the egg remained in 0.05 M sucrose solution, the pressure eventually returned again, indicating that a new equilibrium had been reached across the chorion. Now, when a 0.02 M sucrose solution, the same concentration which previously elicited a decrease of pressure, replaced the 0.05 M solution, a transient hump was produced, indicating that 0.02 M was now hypo-osmotic. The stabilized internal pressure, however, has hardly changed from the original level. The occurrence of the hump can only be explained by the presence of 0.05 M sucrose in the perivitelline space to cause a 0.02 M solution to change from hyper- to hypo-osmoticity. Subsequently, when 0.01 M sucrose was substituted for 0.05 M sucrose (Fig. 1), the pressure, which probably would have followed the dotted line in its return, rose more rapidly and, in fact, exceeded the original level. This rapid return and increased pressure must also indicate the presence of 0.05 M sucrose in the perivitelline space, which transitorily was hyperosmotic to 0.01 M sucrose in the bathing fluid.

C. Volume Changes of the Egg Proper Accompanying Pressure Changes

As noted previously (4), after the chorion became inelastic, most of the perivitelline space which appeared was due to a decrease in the volume of the egg proper. This decrease in volume may vary between 20 and 40 per cent; the lower value being shown in the last column of Table I of the present paper. The variation is probably random among different batches of eggs, and does not affect the interpretation of the results of the experiment. In the last column of Table I are shown some values of the volume of the egg proper as a fraction of the whole. For each experiment, three values are shown, the first being that shortly after activation had begun and when some perivitelline space was present. The second value represents the fractional volume after or close to the attainment of a steady state following activation. The last value for each experiment is the fractional volume of the egg proper in

hypertonic sucrose solution obtained when the pressure was at its lowest for the test condition. As indicated in an earlier section, higher concentrations of sucrose often caused a collapse in the chorion which then pressed on the egg proper and induced deformation. For an accurate evaluation of volume from diameter measurement, the egg proper must be spherical, and distortions avoided. Therefore, Table I contains only those values which were not subjected to this complication. From the data, it is clear that in each case of immersion in a hypertonic sucrose solution that results in a significant

TABLE I

Volume of Egg Proper in Sea Water and in Sucrose

Experiment No.	Pressure	Medium	Volume of chorion	Volume of egg	Vol. egg / Vol. chorion
	mm. Hg		*mm.*^{3}	*mm.*^{3}	
54-6	758	Sea water	2.98	2.43	0.814
	902	" "	2.98	2.34	0.782
	755	Sucrose (1.9 M)	2.98	2.80	0.935
54-8	745	Sea water	3.18	2.84	0.894
	854	" "	3.32	2.68	0.808
	756	Sucrose (0.95 M)	3.32	3.02	0.910
54-16	750	Sea water	4.28	4.19	0.978
	957	" "	4.28	3.52	0.823
	895	Sucrose (0.5 M)	4.28	4.19	0.978
52-224	764	Sea water	3.59	2.95	0.821
	875	" "	3.59	2.67	0.744
	725	Sucrose (1.6 M)	3.59	3.26	0.907
54-15	761	Sea water	7.95	7.55	0.949
	918	" "	8.75	7.06	0.806
	755	Sucrose (0.5 M)	8.75	8.35	0.955

decrease of pressure, the egg proper enlarges. The swelling is prominent, nearly, but never completely obliterating the perivitelline space. Swelling occurred in all the eggs studied, and also in those eggs used for staining and development experiments (Section *E*) in which no pressure determinations were made. After a varying period of time, the egg proper usually shrank again. This secondary decrease in volume was always associated with an increase in the perivitelline pressure, the processes resembling those occurring during the early stages of activation (4).

D. Inverse Relationship between Pressure and Volume

In order to establish the dependence of the volume of the egg proper upon the perivitelline pressure, evaluation of PV is carried out. P is the absolute

TABLE II

Pressure-Volume Relation of the Egg Proper

Experiment No. medium	Chorion volume	Pressure	$\frac{P}{P_0}$	Egg volume	$\frac{V}{V_0}$	Relative PV
	mm^3	*mm. Hg*		mm^3		
54-6 (sea water)	2.98*	758	1.000	2.43	1.000	1.000
	2.98	777	1.025	2.34	0.963	0.987
	2.98	804	1.061	2.34	0.963	1.021
	2.98	830	1.095	2.34	0.963	1.054
	2.98	852	1.124	2.34	0.963	1.082
	2.98	870	1.148	2.34	0.963	1.105
	2.98	880	1.161	2.34	0.963	1.118
	2.98	888	1.172	2.34	0.963	1.128
	2.98	902	1.190	2.34	0.963	1.146
(sucrose 1.9 M)	2.98	755	0.996	2.80	1.152	1.148
	2.98	756	0.997	2.80	1.152	1.149
	2.98	771	1.017	2.68	1.103	1.122
	2.98	839	1.107	2.58	1.062	1.175
	2.98	866	1.142	2.52	1.037	1.184
	2.98	875	1.154	2.52	1.037	1.197
	2.98	901	1.189	2.52	1.037	1.233
54-8 (sea water)	3.18	745	0.971	2.84	1.014	0.985
	3.18	754	0.983	2.84	1.014	0.997
	3.32*	767	1.000	2.80	1.000	1.000
	3.32	805	1.049	2.68	0.957	1.005
	3.32	854	1.113	2.68	0.957	1.066
(sucrose 0.95 M)	3.32	756	0.986	3.02	1.079	1.063
	3.32	760	0.991	2.98	1.064	1.055
	3.32	799	1.042	2.92	1.043	1.086
	3.32	838	1.093	2.92	1.043	1.139
	3.32	868	1.132	2.92	1.043	1.180
	3.32	900	1.173	2.92	1.043	1.224
54-9 (sea water)	3.32	750	0.960	3.12	1.102	1.059
	3.45	767	0.982	2.94	1.039	1.020
	3.65*	781	1.000	2.83	1.000	1.000
	3.65	789	1.010	2.83	1.000	1.010
	3.65	800	1.024	2.83	1.000	1.024
	3.65	803	1.028	2.83	1.000	1.028
	3.65	825	1.056	2.83	1.000	1.056
	3.65	836	1.070	2.83	1.000	1.070
	3.65	847	1.085	2.83	1.000	1.085
	3.65	860	1.101	2.83	1.000	1.101
	3.65	863	1.105	2.83	1.000	1.105

TABLE II—*Concluded*

Experiment No. medium	Chorion volume	Pressure	$\frac{P}{P_0}$	Egg volume	$\frac{V}{V_0}$	Relative PV
	mm^3	*mm. Hg.*		mm^3		
54-10 (sea water)	3.69*	740	1.000	3.30	1.000	1.000
	3.69	758	1.024	3.14	0.952	0.975
	3.69	794	1.073	3.02	0.915	0.982
	3.69	834	1.127	3.02	0.915	1.031
	3.69	850	1.149	2.98	0.903	1.037
	3.69	870	1.176	2.98	0.903	1.062
	3.69	880	1.189	2.92	0.885	1.052
	3.69	895	1.209	2.90	0.879	1.063
54-16 (sea water)	4.28*	750	1.000	4.19	1.000	1.000
	4.28	841	1.121	3.59	0.856	0.961
	4.28	889	1.185	3.59	0.856	1.016
	4.28	957	1.276	3.52	0.840	1.072
(sucrose 0.5 M)	4.28	895	1.193	4.19	1.000	1.193
	4.28	955	1.273	4.19	1.000	1.273
52-224 (sea water)	3.59*	764	1.000	2.95	1.000	1.000
	3.59	797	1.043	2.95	1.000	1.043
	3.59	806	1.055	2.89	0.980	1.034
	3.59	846	1.107	2.89	0.980	1.085
	3.59	875	1.145	2.67	0.905	1.121
(sucrose 1.6 M)	3.59	735	0.962	3.26	1.105	1.063

pressure, and is the sum of the atmospheric pressure and the internal hydrostatic pressure. It prevails throughout the entire content held within the inelastic chorion, although a significant portion of it originated in the perivitelline colloid. In such a system, the volume of the egg proper V, if it is dependent on the perivitelline pressure, should vary approximately inversely as the pressure. At present, this relation can only be tested by evaluating PV during the normal course of increasing pressure, and, on occasion, when the perivitelline pressure was lowered by osmotic means. Since changes of osmotic gradients may produce effects of their own, the evidence would be stronger if the perivitelline pressure could be influenced independently by other means. Although no such method is yet available, at least one can be readily excluded. That involves changing the atmospheric pressure surrounding the whole egg, a procedure which will not materially occasion any transient pressure gradients between the egg proper and its immediate environment, the perivitelline contents, which retains its colloid osmotic pressure.

The results of the PV evaluation of 6 experiments are shown in Table II.

The criterion of using for volume determinations only those eggs which were spherical imposed a severe restriction on the amount of available data, because the period which these evaluations covered corresponded closely to the time of appearance of the blastodisc. The latter appeared first as a conical elevation and then as a discoidal collection at one pole of the egg proper which itself lost the spherical configuration. The values of P and V occurring when the chorion has become inelastic as indicated by the cessation of volume changes, have been taken as the basis of normalization. They have been desig-

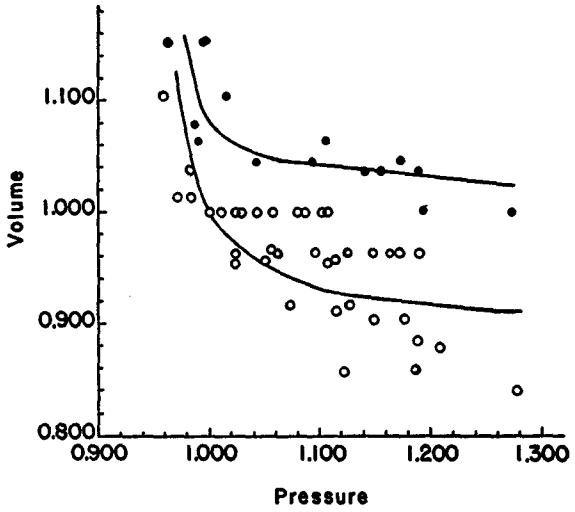

FIG. 4. Pressure-volume relation of the egg proper in sea water (hollow circles) and in sucrose (solid circles). Data are listed in Table II. Curves are traced by eye. In both media, the relationship is approximately a rectangular hyperbole. In sucrose, the curve is displaced upwards, indicating an increase of the incompressible fraction.

nated as P_0 and V_0 and have been assigned values of unity. In Table II, these values correspond to the row marked by an asterisk. All other values of P and V are fractions thereof (P/P_0, V/V_0); the column "relative PV" is the ratio PV/P_0V_0. Probably all the changes which apparently indicate some elasticity of the chorion can be attributed to experimental error in determining the diameter of the egg. In the experiments, the diameter and radii could be determined to 0.05 mm., approximately 3 per cent of the average diameter of 1.7 mm. Such an error in either direction will affect the volume by about 10 per cent, and is undoubtedly an important source of error.

In spite of this and other reasons to be discussed, it is evident from Table II that PV is relatively constant. The mean deviation is 8.53 per cent under various extreme conditions which are present or can be induced in the *Fundulus* egg. In Fig. 4 the pressure-volume relation of the data is shown. Clearly,

it has the shape of a rectangular hyperbola. The tendency towards attaining an asymptote along the pressure axis rather rapidly can be explained by the incompressibility of the egg interior. This factor, which is chiefly responsible for the deviation from ideality, is also evident from Table II. In it, larger deviations are seen to occur more often in the later stages of activation when the volume change has become limited. Fig. 5 illustrates the variation of PV as a function of P. The hollow circles indicate the relation in sea water. For an ideal gas there would be no deviation, and a horizontal line passing through

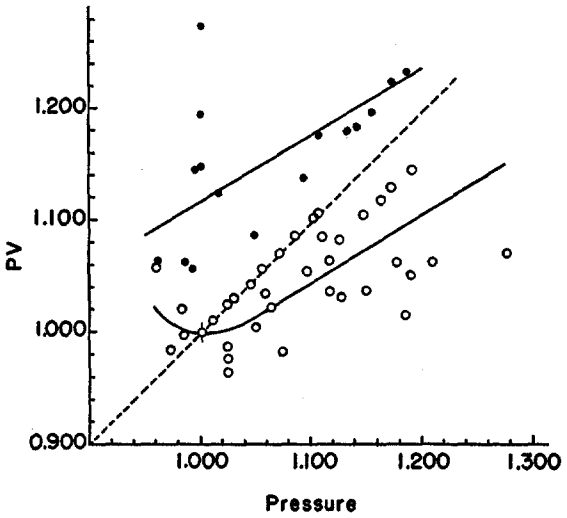

FIG. 5. Relation of PV to pressure. Hollow circles for values in sea water; solid circles, in sucrose. The dotted line has a slope of 1 and depicts the PV-P relation of an incompressible body. All values in sea water pass through the point of intersection of the normalized unity. The relation in sucrose is displaced upwards.

the point of intersection of unity would be present. For a completely incompressible body a line with a slope of 1 and passing through the same point would be the case, as indicated by the dotted line in the graph. The results of the measurement on *Fundulus* eggs fall somewhere in between the two limits, and must indicate that the egg proper does not behave as an incompressible body. At the pressures prevailing, this fact further illustrates that the plasma membrane is not always impermeable to water, because some egg content must be lost in order to account for the apparent compressibility (4). The incompressible fraction is analogous to the osmotically inactive fraction of the *Arbacia* egg (10) which must be subtracted from the volume in order for the PV relation of that cell to behave according to the Boyle, and van't Hoff laws.

After the egg had swollen in hypertonic sucrose and when sucrose penetrates

the chorion, an interesting shift of the *PV* curve occurs (Fig. 4). Satisfactory data for these conditions, free of all complications, are difficult to secure. But in all 3 sets of data obtained, there is a displacement of the *PV* curve upwards, indicating a larger volume in sucrose than in sea water at corresponding pressures. In other words, shrinking of the egg proper when immersed in hypertonic sucrose solution differs quantitatively from the change occurring in sea water in having a larger incompressible fraction. This change is also shown in Fig. 5, in which the *PV-P* relationship in sucrose is far removed from the normalized reference. The difference of the pressure-volume relationships in sea water and in sucrose can be explained by a difference in the stages of activation. In sea water, the egg proper starts from an unactivated state and changes into a more or less incompressible body of reduced volume. As stated previously, during the change some egg contents might have been lost. A similar process must take place when the egg proper shrinks in hypertonic sucrose solution when the perivitelline pressure returns. But the larger volume occurring in such eggs indicates that at corresponding pressures, less egg content can be expressed at this stage than during the process of activation. In other words, the plasma membrane has become less permeant as a result of changes taking place during activation. Hypertonic sucrose solution in the external environment, producing a drop in perivitelline pressure, apparently increases the membrane permeability non-specifically. Results in the following section and in the accompanying paper (3) will show further evidence of this effect.

E. Effect of Sucrose on Staining with a Non-Penetrating Dye, and on Development

To test the possibility that the effect of sucrose is to produce a non-specific increase of the membrane permeability, a solution of brom-creosol purple was used as an indicator. A batch of 20 fertilized eggs having well formed perivitelline spaces was immersed in sea water containing 0.04 per cent brom-creosol purple. Another batch was placed in a solution of 50 per cent sea water and 0.96 M sucrose, containing the same concentration of dye. The indicator was purple in both solutions because of the alkaline sea water, but changed color to greenish yellow at pH 6.8. Being a sulfonated dye, it normally does not enter a cell from without. After immersion for 12 hours in each of the solutions, the eggs were washed in a large volume of sea water to eliminate the purple colored dye in the perivitelline space. Eggs from each solution were then examined against a white background of a thin layer of transilluminated paraffin wax. It could be easily determined that the eggs soaked in sea water and indicator alone were not dyed because the yolk retained its original color, while the eggs immersed in the solution with a hypertonic concentration of sucrose possessed a greenish yellow yolk sac. The difference

in color was especially prominent when an egg from each solution was placed side by side and both examined within the same field with a dissecting microscope.

If a hypertonic sucrose solution does alter the characteristic of the surface of the egg proper, the extent of its deleterious effects must be estimated. For this purpose, a batch of eggs was fertilized in sea water. When the perivitelline space was well formed, 60 of them were transferred to 0.95 M sucrose in sea water in which the perivitelline space all visibly decreased, and the chorion collapsed. After half an hour, the period usually allowed in the pressure experiments, half of the eggs in sucrose were transferred back to sea water, and the other half left to develop in hypertonic sucrose solution. Of the 30 eggs which underwent transient immersion in sucrose 29 of them hatched. The same result was observed in another 30 untreated ones. Of the 30 eggs remaining in sucrose, none of them hatched, and most of them died after the heart beat was present. Two striking features of the embryos in sucrose were the small size of their yolk sacs and the markedly sludged blood flow, both being consistent with a state of dehydration.

DISCUSSION

From a continuous record, the rate of penetration of sucrose through the chorion can be obtained. It can be assumed that no sucrose is present in the perivitelline space in the beginning, and that the rate of the return of the pressure is dependent directly on the amount of sucrose entering through the chorion. From these, the rate of entry can be determined by the rate of the pressure return. For instance, for one egg in 0.5 M sucrose, 7 minutes were required for half of the original pressure and 18 minutes for all of the pressure to return. Such evaluation, however, was soon abandoned because much variation was present. Thus, with any one concentration, different eggs had quite different rates; similarly, for any one egg, different concentrations did not produce any orderly sequence of rates of penetration. Variations like these must therefore indicate that the chorion is a rather heterogeneous structure with varying numbers of pores of different sizes.

The dependence of the volume of the egg proper upon the perivitelline pressure is clearly shown in the results. Further support is provided by the fact that an inverse relation between pressure and volume occurs in both sea water and sucrose, and at different stages of activation. Better agreement with ideality may be obtained if a correction is made for the incompressible fraction. Such a correction has been avoided, however, because unlike the case of the *Arbacia* egg (10), the permeance properties of the *Fundulus* egg change with time. Without more knowledge of the time course of this change, such corrections appear superfluous in the presence of other experimental errors.

Part of the mechanism responsible for a change of permeance properties in the *Fundulus* egg may be examined by contrasting the various phenomena of activation with those of the lifting of the fertilization membrane in some echinoderm eggs. For the *Fundulus* egg, both the volume and surface area decrease during activation when the perivitelline colloid swells, separating the egg proper from the inelastic chorion. Recent experiments (11) suggest that the lifting of fertilization membrane is also attributable to imbibition of a released colloidal substance external to the ovum. The fertilization membrane, however, is not rigid; swelling of the colloidal material causes it to expand without affecting the volume of the egg proper. This constancy of the volume and surface area of the ovum, and a finding that water permeability increases on fertilization (7) serve as illuminating contrasts to the changes in the *Fundulus* egg. In addition, recent experiments have shown that the ionic permeability of the *Fundulus* egg decreases about fivefold (2, 3) upon activation, while in the starfish egg no significant difference occurs before and after fertilization (14). Thus, the available evidence, direct as well as contrasting, supports the suggestion that the acquisition of the impermeable properties of the *Fundulus* egg during activation is at least in part related to a decrease of the surface area. Were the decrease of surface area to affect mainly the diameter of the pores of the plasma membrane then the change in permeance properties is not surprising (*cf.* reference 4). Probably such a mechanism also exists in the trout and the salmon eggs, and perhaps in other teleost eggs as well.

However, some other changes, the nature of which is unclear, must function in conjunction with this mechanism. That the perivitelline pressure does not account for all the changes of permeance is clearly shown by the shift of the pressure-volume relation in sucrose and late in activation. If only the size of the pores were involved, then the pressure-volume relation in sea water during activation and in sucrose late in activation would be superimposable. But the presence of a larger incompressible fraction late in activation must reflect a more lasting alteration of the plasma membrane as a result of activation. The volume changes observed are undoubtedly accomplished by water filtration through pores. A more or less permanent narrowing of the pores following activation may account for the shift of pressure-volume relation. However, these interpretations still cannot explain the absence of D_2O diffusion across the trout egg (6, 13) and the salmon egg (11). On the other hand, the suggestion that these plasma membranes are composed of a complete lipide phase (13, 15) does not agree with the values of membrane capacity (3).

SUMMARY

Upon activation, an internal hydrostatic pressure develops within the *Fundulus* egg, and compresses the egg proper to a reduced volume. When the

perivitelline pressure is abolished by a highly hypertonic sucrose solution, the egg volume increases. As sucrose penetrates the chorion, the volume again decreases. The relation between P and V in these conditions is inverse, and approximates a rectangular hyperbola. The limiting factor causing most of the deviation is shown to be the incompressible fraction. It is concluded that the volume of the egg proper is controlled by the perivitelline pressure, and that the effect of hypertonic sucrose solution is exerted by lowering the pressure and thereby increasing membrane permeability non-specifically. It is also shown that some permanent alterations occur within the plasma membrane during activation that reduce the permeance, and thereby, increase the incompressible fraction.

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