

# THE CHANGE IN OSMOTICALLY INACTIVE FRACTION PRODUCED BY CELL ACTIVATION\*

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## I

### *The Osmotically Inactive Substance*

The study of cells approximately spherical in configuration, represented most closely by eggs of marine invertebrates, has led to the well established result (summarized by Lucké and McCutcheon (1932)) that these cells, as well as others (*e.g.*, erythrocytes (Hamburger (1898)) and leucocytes (Shapiro and Parpart (1937)) obey within limits the law of Boyle-van't-Hoff, so that the total cell volume varies inversely with the "activity" of the external solution with which it is in equilibrium osmotically. Stated symbolically,

$$PV = K \quad (1)$$

where  $P$  and  $V$  are the pressure and volume, and  $K$  is a constant. The cells which have been studied do not obey this simple relation unless a correction factor  $b$ , termed variously the "non-solvent space," "osmotically inactive substance," "dry substance," etc., is introduced, making the equation read

$$P(V - b) = K. \quad (2)$$

Microscopic examination of living cells reveals a variety of formed elements, which when centrifuged down can be observed to occupy a known portion of the cell volume. In the egg of the sea urchin *Arbacia punctulata*, Harvey (1932) gives the following: 1 per cent oil, 4.8 per cent mitochondria, 27.2 per cent yolk, 5.5 per cent pigment, and 0.4 per cent nucleus, totalling 38.9 per cent of the cell volume. If these formed elements took no part in the swelling and shrinking of the cell in anisotonic solutions, the value of  $b$  in Equation 2 would be at least 38.9 per cent of the cell's initial volume, but as Lucké, Larrabee, and Hartline (1935) have observed, the  $b$  value averaged 12 per cent in their measurements. The difference must reside in the fact that certain of the formed elements shrink and swell, along with the rest of the cytoplasm; for

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the nucleus this has been demonstrated to be the case (Beck and Shapiro (1936), in starfish germinal vesicle; Shapiro and Parpart (1937), in mammalian leucocytes).

The osmotically inactive fraction (o.i.f.) is of interest in that it is a quantitatively determined cell constant, and hence may afford an index to the progression of certain events within the cell. Also, in determining the "permeability constant" of the cell (a measure of the rate at which water or dissolved substances diffuse into or out of the cell (Lucké and McCutcheon (1932); Jacobs (1932)) it is essential to know the value of  $b$  to obtain the most precise values, particularly where  $b$  is large. In his calculations of the permeability constant for water in the egg of the worm *Chaetopterus pergamentaceus*, Shapiro (1941  $b$ ) found that the values were about 11 per cent higher when the osmotically inert material was not taken into account in the computations.

In the case of the sea urchin egg, the  $b$  value has been obtained only for unfertilized cells, and the implicit assumption made that it does not alter significantly when the cell changes from the resting to the actively dividing state. A variety of changes, chemical, physiological, and morphological, make their appearance when cellular activation (fertilization) occurs. There is an increase in oxygen uptake (Rubenstein and Gerard (1934), and many others), the dehydrogenase activity increases (Ballentine (1940)), mitotic phenomena are evident, lactic acid appears in small amount (Perlzweig and Barron (1928), Rapkine (1931), Runnström (1933), Hutchens *et al.* (1942)), echinochrome diffuses out of the cell (Shapiro (1946)), and so on. Mirsky (1936) observed that shortly after fertilization (between 3 and 10 minutes) about 12 per cent of the egg protein in *Arbacia* and in *Strongylocentrotus* coagulates and becomes insoluble. Unfertilized eggs contain 63 to 67 per cent of their dry weight as protein (Hutchens *et al.* (1942)). The net effect of these diverse changes upon the osmotically inactive fraction of activated cells has not hitherto been determined.

When the sea urchin egg is broken by centrifugal force into two viable parts known as the light and heavy half, the sum of the individual o.i.f.'s is greater than that of the parent cell. The figures arrived at by Lucké (1932) were 18,000  $\mu^3$  for the whole cell, and 8,000  $\mu^3$  and 15,000  $\mu^3$  respectively for the light and heavy half, an increase of about 28 per cent. Owing to the higher concentration of granules in the heavy half, it is to be expected that the o.i.f. will be greater, but it is of interest that the relative granule volumes are not those indicated by these figures, again pointing to the fact that the formed elements take part in osmotic swelling. The sum of the oxygen uptakes of the unfertilized half eggs, it may be added parenthetically, is about 29 per cent greater than that of the whole unfertilized egg (Shapiro (1935)). As will appear in the sequel, the problem of the osmotically inactive substance is integral with that of volume change on activation, hence we may turn our attention briefly to the latter point.

## II

*Volume Change on Fertilization*

The possibility of a change in volume on fertilization engaged the attention of a number of earlier workers in the field, with discordant results in one case, on the same species. Loeb (1908) found no significant change in the volume of the egg of *Strongylocentrotus* as a result of fertilization. McClendon (1910) observed a decrease in mean egg diameter from 83 to 75 when *Arbacia punctulata* eggs were fertilized. These were eggs placed in a cane sugar solution, approximately isosmotic with sea water. On the other hand, he observed on fertilization an increase in diameter from 83 to 86, of eggs in ordinary sea water. It should be pointed out here that McClendon measured only twenty eggs each for the cane sugar experiment, and ten each for eggs in ordinary sea water. In the case of the latter the diameters showed a large variation (from 75 to 90, no units stated). Glaser (1914) concluded that on fertilization the eggs both of *Arbacia* and of the starfish *Asterias forbesii* show a decrease in diameter. In discussing this with Dr. Glaser, he pointed out that his measurements were confined to the "instant" of fertilization. Okkelberg (1914) held that the eggs of the brook lamprey decrease in volume after fertilization by about 13 per cent. In his calculations the eggs were assumed to be perfect ellipsoids of revolution.

It appears from the discussions by the various authors that they expected that all eggs should show the same qualitative volume change (either increase or decrease) on fertilization, without recognizing that since they were working in some instances on different genera, the direction of change might depend upon the particular cell being studied. Some evidence for this will be presented later.

## III

*Technic*

Eggs were obtained and handled as in earlier studies (e.g., Shapiro (1941)). The hypotonic sea waters were made up fresh from the laboratory's running sea water, which was mixed with distilled water. Owing to the variability of the o.i.f. in the eggs of different urchins, all eggs for any given series of measurements (as exemplified by the data of Fig. 1) were taken from a single female, and the values for unfertilized and fertilized eggs then compared. Two drops of egg suspension were placed in about 100 cc. of each of the hypotonic solutions, and were allowed ample time to attain equilibrium value (25 minutes or longer) at room temperature (about 25°C.). The diameters of twenty or more eggs were measured with a filar ocular micrometer at a magnification of approximately 140 diameters, the volume of each egg computed, and an average value obtained for egg volume at any given osmotic pressure. It might be noted in passing that averaging of egg diameters to obtain average egg volume, a shorter procedure, is nevertheless mathematically incorrect, since volume is not a linear, but a power function of the diameter. Lucké (1935) concluded that tempera-

ture change *per se*, when varied from 5.4° to 29.3°C., did not shift the position of osmotic equilibrium in the unfertilized *Arbacia* egg. The subsequent development of the swollen fertilized eggs, in hypotonic media, was followed after measurement. The eggs in 80 and 90 per cent sea water underwent from 85 to 100 per cent development in the different experiments; those in 70 per cent were retarded and more variable, whereas none were observed to cleave in 60 per cent sea water.

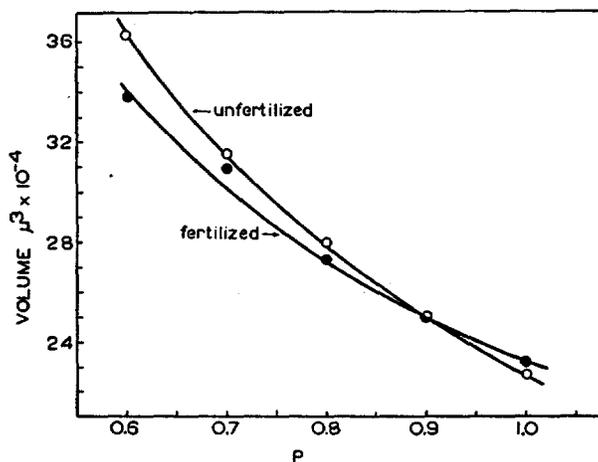


FIG. 1. Pressure-volume relationship for unfertilized and fertilized eggs of the sea urchin, *Arbacia punctulata*. The points represent equilibrium volumes of eggs swollen in various dilutions of sea water. The curves drawn in are theoretical ones calculated from  $P(V - b) = K$ .

#### IV

#### RESULTS

The results are summarized in Table I and in Figs. 1 and 2.  $K_1$  and  $K_2$  refer to relative volumes and osmotic pressures; the constants calculated in Table I are expressed in terms of the volume of the unfertilized egg as 1,000. The ordinates in Figs. 1 and 2 give the cell volumes in cubic micra. The magnitude of the o.i.f. is indicated by the departure of  $K_1$  and  $K_2$  from unity.

The o.i.f. shows on fertilization a large increase, varying from 136 per cent to 519 per cent. There is an average volume increase of some 2.7 per cent on fertilization, and the osmotic pressure at which the volumes of fertilized and unfertilized eggs are equal is in the region of 86 per cent sea water. Lucké *et al.* (1935) found the o.i.f. for unfertilized eggs to range in different individuals from 6 to 20 per cent; in this series it varied from 5.5 to 9.6 per cent. The variability of this cell constant in different urchins may possibly be a reflection of the variation in nutritional status of the animals. When brought into

the laboratory, they may be kept in the aquarium in running sea water for days or weeks at a time, without being fed, and yet contain at the end of these times ripe ovaries, with eggs which show normal cleavage, development, and osmotic properties.

TABLE I  
*Osmotically Inactive Fraction in Unfertilized and Fertilized Sea Urchin Eggs*

Date	$b_1$ (un-fertilized)	$b_2$ (fertilized)	$\frac{b_2}{b_1}$	Osmotic pressure where volumes are equal (calculated; sea water = 1.000)	Volume increase on fertilization	$K_1$	$K_2$
1947						<i>per cent</i>	
July 15.....	0.096	0.292	3.04	0.897	1.4	0.898	0.722
July 28.....	0.055	0.341	6.19	0.813	5.4	0.945	0.713
July 30.....	0.083	0.196	2.36	0.877	1.4	0.917	0.818
Average.....	0.078	0.276	3.86	0.862	2.73	0.920	0.751

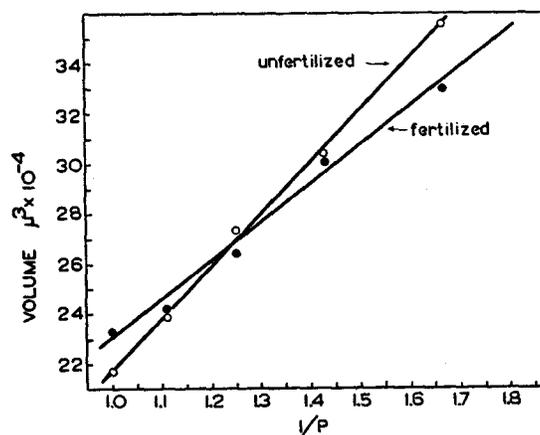


FIG. 2. Plot showing applicability of the Boyle-van't-Hoff law to another set of data. The intercept of each line at zero pressure (not shown) with the  $y$ -axis gives the osmotically inactive volume. The curve represents a close approximation to a least squares fit.

It should be pointed out that the conclusion arrived at in this paper as to the increase in cell volume of the sea urchin egg on fertilization is based not merely on the measurements in sea water alone, but is supported by the positions of the other points on the  $P$ - $V$  plot which demonstrate that there are two distinct curves, each obeying Boyle's law, with different values for  $b$  and  $K$ .

In an earlier paper (Shapiro (1941 *b*))  $b$  values were calculated for individual

eggs of the worm *Chaetopterus pergamentaceus*, and were found to average 38.8 per cent for unfertilized and 29.9 per cent for fertilized. This was the reverse of the result obtained in the present paper for *Arbacia*. A preliminary experiment was run on *Chaetopterus* eggs following the technic used on *Arbacia* in the present paper, *viz.* averaging volumes of about twenty eggs at each osmotic pressure, and a confirmatory result was obtained:  $b$  declined on fertilization in the worm eggs. Since low percentage of cleavage (30 per cent) was obtained, further work is required with these eggs in order to establish with certainty this difference between *Arbacia* and *Chaetopterus*. The qualitatively different results in the two forms are of course entirely possible, and it is of interest that such is the case. It may be pointed out moreover at this juncture that the direction of the change in  $b$  in *Chaetopterus* and *Arbacia* parallels the direction of respiratory change (oxygen uptake). *Chaetopterus* exhibits a decline in rate of oxygen uptake on fertilization (Whitaker (1933)), and *Arbacia* shows an increase (various investigators). The parallelism between the increase in

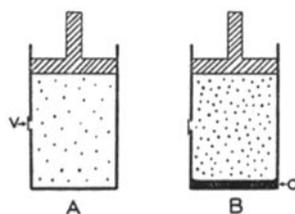


FIG. 3. A mechanical analogue of the changes undergone on activation of the sea urchin egg. For description, see text.

oxygen uptake and the increase in o.i.f. which occurs when the *Arbacia* egg is broken into halves by centrifugal force has been pointed out earlier in this paper. What significance this may have, and whether it is a generally occurring phenomenon, requires further investigation. It has been shown (Shapiro (1941 *b*)) that permeability changes and respiratory changes in the same cell do not necessarily go hand in hand.

#### V

##### *Calculation of the Point of Intersection*

From inspection of Fig. 2 it is evident that the osmotic pressure at which the cell volumes of unfertilized and fertilized eggs from the same batch become equal can be determined analytically. Let the constants for unfertilized and fertilized be indicated respectively by the subscripts 1 and 2; then Boyle's law can be expressed as

$$P(V - b_1) = K_1 \quad (3)$$

$$P(V - b_2) = K_2 \quad (4)$$

where  $P$ ,  $V$ ,  $b$ , and  $K$  are respectively the osmotic pressure, cell volume, osmotically inactive fraction of the cell volume, and the Boyle's law constant. The solution gives the  $x$  and  $y$  coordinates of the intersection of the two functions as

$$\begin{aligned} x &= \frac{b_1 - b_2}{K_2 - K_1} \\ y &= \frac{K_2 b_1 - K_1 b_2}{K_2 - K_1} \end{aligned} \quad (5)$$

$x$  being the reciprocal of the osmotic pressure ( $1/P$ ), and  $y$  the cell volume, corresponding to the coordinates in Fig. 2. The calculated and observed points of intersection all fell at relative osmotic pressures less than unity (*i.e.*, in hypotonic solutions).

## VI

## DISCUSSION

What is the significance of these results? The production of insoluble protein on fertilization would lead to a slight decrease in the dissolved molecular content of the cell, and the resulting volume change would be a slight decrease in volume, owing to osmotic adjustment. However, the data show on fertilization an increase in volume for the sea urchin egg, which means, if one considers all volume changes as the consequence of osmotic effects alone, an increase in the number of free particles in solution in the cell. It is probable that this occurs; *e.g.*, the small increase in lactic acid referred to above derives from the breakdown of large carbohydrate molecules; there is also a liberation of echinochrome possibly from some echinochrome-protein complex (Shapiro (1946)), and other enzymatic processes and oxidative changes may lead to an enhancement of the osmotically active content of the cell.

The views incorporated above may be condensed into a simple, admittedly incomplete, analogue. Consider the piston and cylinder shown in Fig. 3.  $A$  represents the unfertilized egg, and the constitution of the vapor inside the cylinder is indicated by the dotted array. On fertilization ( $B$ ), the valve  $v$  is opened, and a minute amount of an activator is admitted, which results in a number of physicochemical changes, such that the condensate  $C$  appears (analogous in part to precipitated protein) and an increase in the number of gaseous molecules (corresponding to an alteration in the cell's osmotic pressure). The  $P$ - $V$  points for the cylinder now fall along a different curve, corresponding to the new Boyle's law constant (*cf.* Fig. 1).

## VII

## SUMMARY

1. Resting and activated eggs of the sea urchin *Arbacia punctulata* were swollen in hypotonic sea water (60, 70, 80, and 90 per cent), and allowed to attain equilibrium volumes (Figs. 1 and 2).

2. Both fertilized and unfertilized eggs obey the Boyle-van't-Hoff law, but the value for  $b$ , the "osmotically inactive fraction" or non-swellable volume, was different for the two, averaging in the cases studied 7.3 per cent for unfertilized and 27.4 per cent for fertilized.

3. On activation, the eggs of the sea urchin undergo a definite increase in total cell volume, of approximately 2.7 per cent.

4. Some evidence is adduced for the possibility that the alteration in cell volume and in o.i.f. may depend upon the species in question.

5. A parallelism between change in  $b$  and alteration of respiratory metabolism in *Arbacia*, *Chaetopterus*, and *Arbacia* fragments is pointed out. This requires further investigation in other species to establish generality.

6. Equations for the calculation of the point at which osmotic pressures and cell volumes are identical for unfertilized and fertilized eggs are included.

7. A mechanical analogue of the phenomena is introduced (Fig. 3).

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