16243

# Do Chromosomes Manifest Osmotic Volume Change?

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In an earlier study[1] it was shown that the nucleus of the immature starfish egg in the germinal vesicle stage undergoes a kinetics of swelling and shrinking which is formally similar to that of the egg as a whole. Observations made also on equilibrium volumes indicated that the germinal vesicle contains a smaller percentage of osmotically inactive material than does the cytoplasm in this cell. The advantage of studying the nucleus in the germinal vesicle stage is that its size is large enough to realize sufficiently accurate diameter measurements under high dry magnification. When the egg matures, the nucleus becomes very much smaller, and direct measurements are rendered considerably more difficult by this method.

In the spermatozoon, the chromosomes are compressed into the smallest possible volume in an animal cell, nearly devoid of cytoplasm, hence an examination of the osmotic properties of the sperm affords the nearest approach to an intracellular study of the osmotic properties of the chromosomes themselves, in the intact living cell. Since spermatozoa are not spherical, and moreover are so small, it is necessary to use some method other than direct microscopic measurement, such as centrifuging, for estimation of cell volume. Apparently the first to study this quantitatively (though not with reference to the question of chromosome swelling) was Hamburger[2] who obtained an average value of 72.8% for the "Gerüstvolum" (literally, "framework volume"; operationally, the equivalent of "osmotically inactive fraction"[3]) of frog spermatozoa. In arriving at this value, he used low centrifugal forces (1800 or 3000 r.p.m., no radius stated) and placed the sperm in solutions of pure NaCl, a procedure known to be toxic to a variety of cells.[4] It was therefore decided to investigate the matter in a species where sperm are readily available in abundance, using a balanced ionic environment and a centrifugal force adequate to pack the cells quantitatively, and to test the applicability of the Boyle-van't Hoff law.

According to Harvey and Anderson[5] the Arbacia sperm has a pointed head with a flattened base (2 $\mu$ wide) adjacent to a short slightly narrower middle piece which seems to contain a pair of spherical bodies. The length of the head and middle piece is approximately 4 $\mu$. The length of the tail is about 45 $\mu$. Direct observations of swelling to about twice their original size were made of sperm placed in distilled water. Electron microscope observations revealed the tail to be composed of about 10 strands of uniform thickness, each about 50 m$\mu$ in diameter. Owing to the thickness of the heads, it was not possible to make out any discrete internal structure.

*Procedure.* Sperm were obtained from ripe testes of the sea urchin, *Arbacia punctulata,* by excision from the animal, and allowing the spermatic fluid to exude as a viscous creamy suspension into a stender dish. The material was pipetted into a test tube and centrifuged lightly in order to throw down any gross tissue particles. This still left the bulk of the sperm in suspension, occupying from 23.5 to 27.8% of the total volume. Hypotonic sea water solutions, varying in concentration from 1% to 80%, were made up fresh each day. Owing to the fact that the cell volume is only a fraction of the spermatic fluid, mixture with

[1] Beck, L. V., and Shapiro, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 170.

[2] Hamburger, H. J., *Osmotischer Druck und Ionenlehre,* 1904, Wiesbaden, J. F. Bergmann, Vol. 3, p. 4, Die Volumanderungen der Spermatozoen unter den Einfluss hypertonischer und hypisotonischer Salzlosung.

[3] Lucké, B., and McCutcheon, M., *Physiol. Rev.,* 1932, **12**, 68.

[4] Loeb, J., *Pfluger's Arch.,* 1903, **97**, 394.

[5] Harvey, E. B., and Anderson, T. F., *Biol. Bull.,* 1943, **85**, 151.

the hypotonic sea waters yielded a range of resultant effective osmotic concentrations of 42.4 to 88.5% sea water.

The spermatic fluid was pipetted in 0.1 ml aliquots into small shell vials, an equal quantity of hypotonic sea water was added to each vial, and the contents mixed thoroughly. The shell vial was covered with a cover slip, and from 50 minutes to 2 hours allowed for the attainment of osmotic equilibrium at room temperature (about 25°C). Specially drawn capillary tubing was used, of uniform bore (approx. 0.39 mm, checked by microscope measurements of diameters at both ends) and outside diameter, 0.56 mm. The tubing was cut up into lengths of about 43 mm, and from 30 to 35 mm of diluted sperm suspension drawn up by capillarity, following which the opposite end was sealed off in a microflame. The perfection of each seal was checked by microscopic examination. Centrifuging (15 to 17 minutes duration) was carried out in an air turbine, at approximately 20,000 times gravity, in a steel rotor made available through the courtesy of Dr. A. K. Parpart. Column lengths were measured under a binocular dissecting microscope with the aid of a metric steel rule. At this centrifugal force erythrocytes are completely packed; for Arbacia eggs it has been estimated that a minimal centrifugal force of 4330 × gravity is required to pack the cells completely.[6] The exact force at which complete packing is attained will be related to the physical properties of the cell in question, and to the medium.[7]

*Condition of the Sperm.* The physiological state of the sperm cells was studied after swelling by microscopic observation of their motility and of fertilizing power. In all the dilutions employed, numerous highly active sperm were observed. As the packed sperm were allowed to rest in the capillary tube after centrifuging, the cell column length increased slightly with time, apparently due to sperm at the free end of the column migrating into the supernatant fluid. When swollen sperm were returned to a suspension of eggs in normal sea water, high percentages of membrane formation and cleavage ensued, demonstrating that the sperm had not been irreversibly affected by the swelling process.

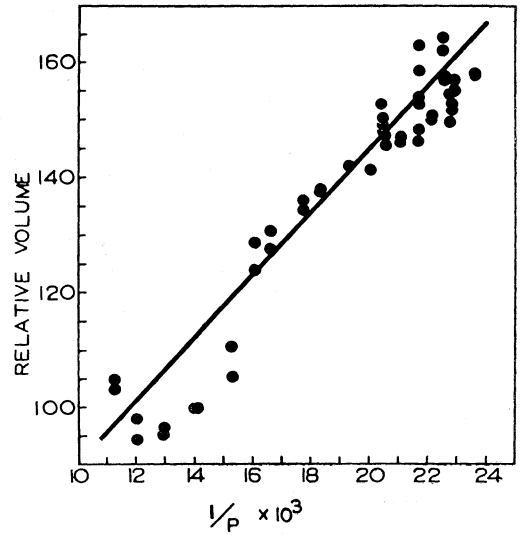


FIG. 1.
Showing the applicability of the Boyle-van't Hoff law to equilibrium volumes of sea urchin sperm swollen in various dilutions of sea water.

*Results.* Fifty-six determinations were carried out, the results of which are summarized in Fig. 1, which is a plot of relative sperm volume against the reciprocal of the osmotic pressure of the medium. A rectilinear relation indicates the applicability of the Boyle-van't Hoff law,

$$P\ (V - b) = k,$$

where P, V, b, and k represent respectively osmotic pressure, cell volume, osmotically inactive fraction, and a constant. Extrapolation of the curve to the zero ordinate (not shown) yields an average value for "b," the "osmotically inactive fraction," of 33%, a value in the same region as that observed for other cells (*e. g.*, in unfertilized Chaetopterus eggs, "b" is 38.8%.[8]) The best average curve, fitted visually, has been drawn through the data. Application of the Criterion of Least Squares shows that the line so drawn lies close to one computed by least squares. Although the plotted experimental points in Fig. 1 show a fair amount of scatter, it is essential to note that if lines with the greatest

[6] Shapiro, H., *Biol. Bull.*, 1935, **68**, 363.

[7] *Cf.*, for example, Johnson, F. H., and Harvey, E. N., *J. Cell. and Comp. Physiol.*, 1937, **9**, 363.

[8] Shapiro, H., *J. Cell. and Comp. Physiol.*, 1941, **18**, 143.

and least slope reasonably possible be drawn that "b" will have a maximum possible value of approx. 48%, and a minimum value of approx. 18%.

*Discussion.* Since the chromosomes appear to constitute the bulk of the content of the spermatozoon, it appears from this result that they must partake in the osmotic swelling of the cell. If the chromosomes did not swell, the value obtained for "b" would be very high, of the order of 90% or more, judging from the standard cytological pictures of sperm heads. In so reacting to hypotonic solutions, the chromosomes display an osmotic pattern shared by other formed elements within the nucleus and the cytoplasm, viz., the nucleolus, and the diverse types of granules which undergo osmotic volume changes. The value of 72% obtained by Hamburger for frog sperm may be due to a species difference or to the differing circumstances, alluded to in the introduction, in which the experimental determinations were carried out.

Churney[9] studied the swelling of the germinal vesicle in immature eggs of *Arbacia punctulata* and *Nereis limbata*. Unfortunately, no calculations of osmotically inactive fraction were given. He concluded that "the experiments failed to reveal any appreciable amount of osmotically inactive material in either of the nuclei." An interesting observation was also briefly reported in a footnote to the effect that "the giant chromosomes of the salivary gland cells of *Sciara coprophila* and of *Chironomus sp.* swell and shrink reversibly in anisotonic solutions." Quantitative data were not furnished.

*Summary.* A. The applicability of the Boyle-van't Hoff law to the swelling of spermatozoa of the sea urchin, *Arbacia punctulata,* in hypotonic solutions of effective concentrations varying from 42.4 to 88.5% sea water was investigated. Cell volumes were determined by centrifuging in small capillaries in an air turbine at 20,000 × gravity.

B. In the range of dilutions employed, the Boyle-van't Hoff law appeared to be followed. The "osmotically inactive fraction" ("b") averaged 33%. Thus the sperm head, which is chiefly chromosomal material, has a non-swellable volume which does not differ greatly from that of certain other cells. The results indicate that the chromosomes take part in the osmotic swelling of the cell.

9 Churney, L., *Biol. Bull.*, 1942, **82**, 52.

16244

## An Investigation of Transmethylation from N[1]-Methylnicotinamide

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With the discovery that nicotinamide is methylated in the animal body to give N[1]-methylnicotinamide[1] it became of interest to know whether this product is a methyl donor in the biological synthesis of N- and S-methyl compounds. One approach to this problem has been the study of the lipotropic activity of N[1]-methylnicotinamide when added to a methyl-deficient diet. Najjar and coworkers[2,3] have presented data to show that N[1]-methylnicotinamide has some lipotropic action in the rat, whereas Handler and Dubin[4] found in a similar experiment that it had no activity in preventing the development of fatty livers.

We wish to report here an experiment of

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1 Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1943, **150**, 395.

2 Najjar, V. A., and Deal, C. C., *J. Biol. Chem.*, 1946, **162**, 741.

3 Najjar, V. A., and Ratcliffe, I. M., *Bull. Johns Hopkins Hosp.*, 1947, **80**, 142.

4 Handler, P., and Dubin, I. N., *J. Nutrition*, 1946, **31**, 141.