and turtle heart muscle as shown in a previous paper.[2] No distinct volume changes were observed during the recovery.

Pieces of the body wall, containing the longitudinal muscle fibers, showed spontaneous volume changes of about 0,000 02 of the total muscle volume, although the motor activity of the muscle was so weak that it could not be recorded with the isometric levers at my disposal.

Since the retractor muscle develops a smaller tension than a striated muscle, but has a volume change about as large as that of a striated muscle, a markedly higher volume change occurs in the retractor than in frog muscle per unit of tension developed. For the body wall muscles of Phascolosoma the volume change per gm. tension must be at least 100 times greater than for a frog sartorius. Meyerhof[1] assumes that the volume change is caused by the chemical processes involved in the energy output for contraction, and that the size of the volume changes is more or less parallel to the working metabolism. Since it is generally assumed that less energy is needed for the activity of smooth muscle than for the contraction of striated muscle, it is hard to explain why the volume change in smooth muscle is so much larger. Furthermore, the fact that the direction of the volume changes is not constant and can be influenced to a certain extent by the physical conditions (initial length or tension) indicates that other factors are much more important as the cause of the volume changes than the working metabolism of the muscles.

8762 C

Permeability to Water and Staining Rate of Developing and Resting Sea Urchin Eggs.*

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It was shown some years ago by R. S. Lillie[1] that fertilized *Arbacia* (sea urchin) eggs are more permeable (about four times) to water than unfertilized eggs, since they swell and shrink more

2 Fischer, E., *Am. J. Physiol.*, 1935, **113**, 42.

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1 Lillie, R. S., *Am. J. Physiol.*, 1916, **40**, 249.

rapidly in hypotonic and hypertonic sea water. The change in permeability to water at fertilization thus more or less parallels the change in rate of oxygen consumption. It had also been shown earlier by Lyon and Shackell[2] that at fertilization the egg of *Lytechinus* (sea urchin) increases its permeability to certain dyes but not to others. The immature eggs were found to be even more permeable to certain dyes than the fertilized egg, showing that maturation is associated with a decrease in permeability and that low permeability to dye thus correlates with the absence of cell division in the unfertilized egg.

Since the unfertilized egg is in a state of developmental inhibition, from which it is released at fertilization, it is of some interest to examine further the properties of the developing immature egg, before it enters the state of inhibition, for comparison with the developing fertilized egg.

A number of experiments on the eggs of the sea urchin *Strongylocentrotus franciscanus* show that immature eggs, like fertilized eggs, stain deeply much more rapidly in sea water containing Methylene Blue, Gentian Violet, or Methyl Violet than do unfertilized resting eggs. This is true in dilutions of the dyes in which the first cell division takes place as well as in stronger concentrations which are more quickly toxic. The phenomenon may be demonstrated more accurately if the jelly is removed since this may stain heavily. In this species as in *Lytechinus* the immature eggs appear to stain somewhat more rapidly than the fertilized eggs. These results show almost beyond doubt that the permeability to these dyes is much less in the resting unfertilized stage, although it must be granted that the effect of the biochemical state of the cell on entered dye may also be involved. The three types of eggs of the sand dollar *Dendraster excentricus* show essentially the same relations to the three dyes.

The permeability to water of the three types of eggs of *S. franciscanus* was investigated in some 16 series of measurements. In each series, an egg of each type which was very nearly circular as seen from above was selected from the same female, and the three types were selected with the same initial diameter in sea water. This was done by selecting unfertilized and fertilized eggs which were slightly smaller than average. By means of a micro-lip-pipette it was possible to transfer an egg with a very small amount of sea water into dilute (50%) sea water and suck new medium about it in about 10 seconds. The diameter was periodically meas-

[2] Lyon, E. P., and Shackell, L. F., *Science*, 1910, **32**, 249.

ured as swelling progressed. The cells were presumably very nearly spherical, but since no side view observations were made to check against flattening, the results are given in terms of diameters as seen from above without attempting to convert to volumes. Sev-

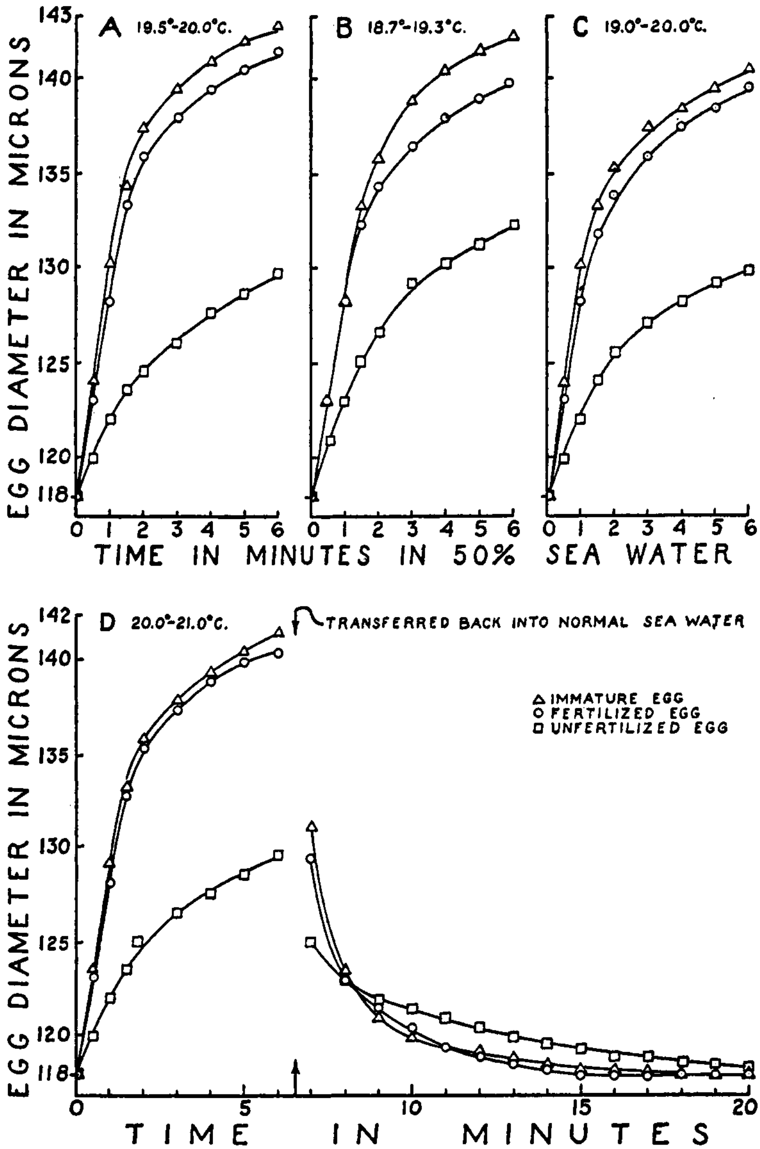

FIG. 1.

Rate of swelling of immature, unfertilized, and fertilized eggs of *S. franciscanus* in dilute (50%) sea water (A. B. C.). Also shrinking after return to normal sea water (D).

eral typical results are shown in Fig. 1. All results indicate that immature eggs, like fertilized eggs, swell more rapidly than the unfertilized eggs. Under the conditions of the experiments in 50% sea water the immature eggs usually swelled slightly more rapidly than the fertilized eggs, and the equilibrium diameter was usually somewhat greater.

In 7 series the eggs were returned to normal sea water after swelling for 6 minutes in dilute sea water. A typical result is shown in Fig. 1D. These 7 experiments indicate that in exosmosis as in endosmosis the permeability to water of the immature egg as well as of the fertilized egg is considerably greater than the permeability of the resting unfertilized egg.

The immature eggs used throughout this work were almost as large as the average mature egg, but they were entirely immature since the large germinal vesicle was present well within the cell. If the germinal vesicle is too close to the surface (in eggs on the verge of maturation) it is apt to burst under osmotic stress after which the egg is often fertilizable. Such border line types of eggs were avoided. Complete maturation normally precedes the resting stage in the sea urchin egg.

Eggs were used from 9 different females of *S. franciscanus* in April and May, 1936. At the start of swelling, the various immature eggs had been in sea water 20 minutes to 2 hours, the unfertilized eggs 20 minutes to 2.5 hours, and the fertilized eggs 35 minutes to 3 hours. In all cases the fertilized eggs were placed in dilute sea water at approximately the same developmental stage, namely, when the hyaloplasmic layer was first definitely secreted. Depending on temperature, this was of the order of 10 minutes after fertilization. Temperature ranged from 17°C. to 22°C. over all, but did not vary more than 2°C. within a series.

It has been shown by Goldforb[3] that the permeability of unfertilized *Arbacia* eggs increases as they age, and by Lillie[4] that the permeability of the fertilized eggs varies initially with time after fertilization.[5] The present results do not cover these variables and therefore no attempt is made to show the exact ratios of the permeabilities of the three types of eggs. It is rather the orders of magnitude which are indicated, with relative similarity between immature and fertilized eggs under the conditions of the experiments.

[3] Goldforb, A. J., *J. Gen. Physiol.*, 1935, **19**, 149.

[4] Lillie, R. S., *Am. J. Physiol.*, 1918, **45**, 406.

[5] See also Dorfman, W. A., *Protoplasma*, 1932, **16**, 56.

Summary and Conclusions. Immature eggs of the sea urchin *S. franciscanus,* like fertilized eggs, stain more rapidly in solutions of Methylene Blue, Gentian Violet, and Methyl Violet than do resting unfertilized eggs. Like the fertilized eggs they are also more permeable to water. The immature and fertilized eggs are developing while the unfertilized egg is in a state of developmental inhibition. Increase in water permeability, as well as staining rate, at fertilization (when the egg is released from inhibition) is thus the converse (in direction) of the change when the immature egg enters the resting stage.

8763 P

Concentration of Gonadotropic Substance from Pregnancy Urine.*

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Katzman and Doisy[1, 2] reported that the gonadotropic substance found in the urine of pregnant women (P.U.) can be concentrated conveniently by adsorption on benzoic acid or by precipitation with phosphotungstic acid. Hellbaum *et al.*[3] have shown that the same substance is precipitated quantitatively from the urine by tannic acid. However, the purification of the tannic acid precipitate is rather difficult.

We have found that cresol extracts P.U. efficiently and its use offers definite advantages over the methods mentioned in simplicity, ease of manipulation and recovery of reagents. It removes the active material from the urine as completely as does tannic or benzoic acid and the product is a powder which is a convenient starting material for further purification.

The urine used in these experiments was from a stock supply which had been collected and kept in the coldroom for several days. The cresol was the U.S.P. grade of J. T. Baker Co.

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1 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

2 Katzman, P. A., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1188.

3 Hellbaum, A. A., Fevold, H. L., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1566.