point is found at which the loop just disappears from view. The distance of the loop below the meniscus is then measured with a centimeter scale laid along the tube, giving the first reading. A second reading is obtained after the addition of a measured amount of the diluent, and the data required for substitution in the equation for the corrected reading is at hand. The readings and calculations require but two or three minutes. In the zone of most accurate measurement, with suspensions of such opacity that the loop disappears between 1 and 4 cm. below the surface, repeated readings may be made with a variation of about one millimeter, an error of less than 10 per cent. The wire loop, which alone comes in contact with the bacteria, may be flamed after each determination, thus reducing the danger of contamination to a minimum.

A more complete explanation of the method, with a description of the more convenient and accurate instrument shown at the meeting will appear in a forthcoming number of the *Journal of Experimental Medicine*.

23 (1483)

Some studies on the surface layer in the living egg cell.

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The results recorded here were obtained through the use of Barber's mechanical pipette holder somewhat modified for microdissection purposes.

The cells experimented upon were the egg cells of the starfish and of the sea urchin. The eggs, which are somewhat over 1/10 of a millimeter in diameter, were placed in a drop of sea water hanging from the roof of a moist chamber. The microscopically fine tips of the glass dissecting needles projected into the moist chamber and up into the hanging drop. By manipulation of the screws of the mechanical pipette holder the cells in the hanging drop could be dissected with considerable accuracy and an estimate ascertained of their physical consistency. Detailed accounts

of Barber's apparatus and its application to microdissection have already been published.[1]

The egg cells studied consist of a decidedly fluid interior surrounded by a more solid surface layer of appreciable thickness. This surface layer is most solid on its external surface. Internally its consistency seems to merge insensibly into that of the fluid interior. The inner surface of this layer adheres to the touch. This is demonstrated by introducing a microdissection needle into an egg and pushing the needle through until its tip comes into contact with the inner boundary of the surface layer on the side of the egg opposite the puncture. On withdrawing the needle the layer adheres to the needle tip and strands are drawn into the interior of the egg.

If the surface layer be torn while the egg is kept under compression the fluid interior will bulge out through the tear. The cytoplasm, on coming into contact with the surrounding water, tends to establish a definite surface film which prevents the cytoplasm from mixing with the water. If the internal pressure be not too great this film persists and, in time, strengthens into a definite ectoplasmic layer. The bulge then slowly retracts until the original contour of the egg is reëstablished. If the neck of the protruding mass of cytoplasm be small it may pinch off a spherule of cytoplasm which to all appearances is normal. If the internal pressure be too great a succession of films may form as, one after the other, they succumb while the escaping cytoplasm disperses and disintegrates in the surrounding water and the film which finally holds out may enclose only a fraction of the original cell but what it encloses will be normal protoplasm.[2]

Churning of the contents of a mature unfertilized sea-urchin egg causes the ectoplasmic layer to revert to the fluid condition of the interior. The surface film of such an egg is very thin and very easily tears upon which the entire egg disintegrates. On standing, however, the surface film steadily strengthens until the normal condition is reëstablished.

That the distribution of substances throughout the egg cell is

[1] Barber, *Philippine Journal of Sc.*, Vol. X, Sec. B, Tropical Medicine, 1914; Chambers, *Biol. Bull.*, Vol. 34, 1918.

[2] Chambers, *Amer. Journ. Physiol.*, Vol. 43, 1917.

not uniform can be demonstrated by the following experiment on the starfish egg: If the surface of a mature unfertilized egg be torn while the egg is kept under compression almost all of the internal cytoplasm may be made to flow out to form a spherule of cytoplasm which pinches off from the rest of the egg. What is left behind is a collapsed remnant consisting mainly of protoplasm which originally enveloped the egg. This remnant consisting largely of the more solid ectoplasm tends only slowly to round up. The extruded mass, which is very fluid, immediately assumes a shape approximating that of a sphere. This may be termed an endoplasmic sphere. The remnant containing the original ectoplasmic substance of the egg is readily fertilizable and undergoes segmentation. The endoplasmic sphere is unfertilizable. If, on the other hand, the endoplasmic sphere remains for some time connected by means of a bridge of protoplasm with the remnant containing the original ectoplasmic substance it is fertilizable. The ability of the endoplasmic sphere to approximate normal conditions of segmentation is a function of the length of time that it remains in organic continuity with the original ectoplasmic mass. Possibly there exists a substance necessary for development which normally accumulates in the surface layer of an egg. This substance is diffusible and will distribute itself over new protoplasmic surfaces. If a bridge of protoplasm connects the ectoplasmic remnant with the endoplasmic sphere this substance will diffuse into the sphere thereby rendering it fertilizable.

The nature of the surface film produced by cutting an egg cell differs in an unfertilized egg from one which has been fertilized. Before fertilization the needle may be pushed vertically into the side of the egg and moved through the egg from one side to the other without cutting the egg in two. The cytoplasm closes behind the needle thus obliterating the furrow. Shortly after fertilization, however, such a procedure cuts the egg cleanly in two. The sides of the furrow produced by the needle do not fuse although contiguous. The character of the surface film which forms over a cut is thus changed upon fertilization. This change prepares the egg for the ensuing segmentation process by causing the formation of a type of surface film which prevents contiguous blastomeres from fusing with one another.