

4. Drain off the excess iodine solution, without blotting (no water being used) but the film is not permitted to become dry.

5. Add acetone (100 per cent.) drop by drop until no color is seen in the drippings from the slide, which is slightly tilted. This usually requires less than 10 seconds, and should be reduced to a minimum.

6. Air dry the slide.

7. Counter stain for 10-30 seconds with 0.1 per cent. aqueous solution of basic fuchsin.

8. Wash off excess stain by short exposure to tap water and air dry. If slide is not clear, immersion in xylol is recommended.

This method has yielded particularly good results in staining milk slides for *Bacillus Acidophilus* and in staining fecal specimens. By this method gonococci and diphtheria bacilli are particularly well differentiated and more easily identified than by the older methods. The same was found to be true for a number of common pathogens and saprophytes studied.

### 34 (1994)

**Permeability of the cell: the surface as contrasted with the interior.**

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Protoplasm is known to be permeable to some substances and not to others. The microinjection method appears to be the only method of determining whether this semi-permeability is a property of the entire mass of protoplasm or of its surface film only.

Kite<sup>1</sup> injected cells by the Barber pipette method<sup>2</sup> and claimed to have proved that semi-permeability is a property of all portions of protoplasm. His conclusions are open to criticism owing to the extreme difficulty of the method and to his having overlooked the extraordinary ability of protoplasm to form surface films over cut surfaces. The results which I<sup>3</sup> obtained are directly opposed to Kite's conclusions. Recently I have devised<sup>4</sup> a micro injection apparatus with which one can, with remarkable ease and accuracy, inject living cells by means of pipettes with a bore less than one micron in diameter.

A half molecular ammonium chloride solution in sea water is acid to neutral red. Starfish eggs stained with neutral red and immersed in this acid solution turn yellow, owing to the penetration of only the alkaline group of the dissociated salt. If, however, stained eggs be placed in an alkaline sodium bicarbonate solution, they give evidence of the penetration of only the carbonic acid group. These findings are being reported by Jacobs in the *Journal of General Physiology*. They confirm the observations of previous investigators that weak acids and bases freely penetrate living cells whereas strong acids and bases do not.

My experiments, described in a forthcoming number of the *Journal of General Physiology*, consisted in the injection of  $\text{NH}_4\text{Cl}$  and  $\text{NaHCO}_3$  into starfish eggs stained with neutral red. In the case where  $\text{NH}_4\text{Cl}$  was used the injected area immediately changed to a red color and then underwent cytolysis. The color change and accompanying cytolysis spread from this area till it reached the cortex of the egg which disintegrated from within outward. In some cases this spread was arrested by the formation of a surface film which converted the injected and disintegrated area into a vacuole. This experiment demonstrates that  $\frac{1}{2}$  M  $\text{NH}_4\text{Cl}$ , which causes an alkaline color change within eggs when its effect is transmitted only through the surface film, will, when injected into the interior of the eggs, produce

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<sup>1</sup> *Amer. Jour. Physiol.*, 1915, xxxvii, 282.

<sup>2</sup> Philipp, *Jour. Sc., Sec. B., Trop. Med.*, 1914, ix, 307.

<sup>3</sup> *Jour. Pharmacol. Exp. Therap.*, 1919, xiv, 75; *Proc. Soc. Exp. Biol. and Med.*, 1920, xviii, 66.

<sup>4</sup> *Anat. Rec.*, 1922, xxxiv, 1.

the acid color change and accompanying cytolysis characteristic of free HCl.

When  $\text{NaHCO}_3$  was introduced into a stained egg the injected area immediately turned yellow and cytolysis with liquifaction took place. The change to a yellow color and accompanying cytolysis spread throughout the cell. This showed that NaOH, which can not penetrate the surface film, will exert its characteristic effects if introduced directly into the interior of the cell.

The semi-permeability of a living cell is a function of its surface film. It is immaterial whether this film be that of the original cortex, a film newly formed over a cut surface, or a film that surrounds an artificially induced vacuole within the cell. As long as a surface film exists, neither the acid group of the  $\text{NH}_4\text{Cl}$  nor the alkaline group of the  $\text{NaHCO}_3$  can penetrate protoplasm. On the other hand, if injected beneath the surface film they freely permeate the protoplasm.

### 35 (1995)

**Fat transport in the body—changes in the lipid content of the blood and lymph during fat absorption in the dog.**

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After alimentary absorption of fat the content of both the total fatty acids and the phosphatides of the blood is increased. According to Bloor the phosphatides are synthesized from neutral fats by the blood corpuscles. It is also conceivable that phosphatide synthesis occurs during the passage of the fat components through the intestinal wall. To test this hypothesis the thoracic lymph and blood collected before and after introduction of olive oil into the duodenum of dogs previously starved for 18 hours was analyzed as follows: