6. The patient remained for four months in a condition of nitrogenous equilibrium, and otherwise in good health, on a diet containing about 6.5 grm. of nitrogen and 3,000 calories, which were ultimately reduced to 2,500 calories to prevent a constant gain in weight.

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The formation of gluconic acid by the olive-tubercle organism and the function of oxidation in some microörganisms.

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The olive-tubercle organism, *Bacterium savastanoi*, recently described by Erwin F. Smith,[1] when grown in the presence of glucose and an excess of calcium carbonate, converts the greater part of the glucose into calcium gluconate. The amount of energy liberated thereby is exceedingly great in comparison to the weight of the organisms. This is to be explained by the fact that the energy requirements of microörganisms are very much greater than those of higher forms, partly because of the disproportion between the body surface and the body volume of microorganisms, and partly because microörganisms exist in a medium which is an excellent conductor of heat.

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On the fertilizing and cytolytic effect of soap.

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It has been shown by experiments on the eggs of sea-urchins, starfish, and annelids that the artificial membrane formation is the act which causes the unfertilized egg to develop. The agencies which cause the artificial membrane formation, as a rule, injure the egg. For the eggs of the starfish and certain other annelids

[1] Erwin F. Smith: Recent Studies of the Olive-Tubercle Organism. U. S. Department of Agriculture, Bureau of Plant Industry, Bulletin No. 131, Part IV, Washington, 1908.

this injury is rather slight, and these eggs are able to develop into larvæ without any further treatment. In the egg of the Californian sea-urchin this secondary, injurious effect connected with the artificial membrane formation is more severe and demands a further treatment of the egg. This consists in preventing the eggs from developing for about from two to three hours after the membrane formation, by depriving them of oxygen or by preventing oxidations in the egg through the addition of a trace of potassium cyanide. During this time the egg is able to recuperate from the injurious effects of the membrane formation and is able to develop perfectly normally into a pluteus if transferred into normal sea-water. In my first experiments with this method, four years ago. not more than ten per cent. of the eggs could be caused to develop in this way. I have recently found that by a slight improvement of the method all the eggs can be caused to develop into larvæ, The segmentation is as a rule as normal as if the eggs were fertilized by sperm. A second method of overcoming the injurious effect caused by the artificial membrane formation consists in putting the eggs for from 10 to 40 minutes into hypertonic sea-water. This method also causes all the eggs to develop.

These experiments showed that the process of membrane formation is the real cause which starts the development of the unfertilized egg; and the question therefore arose what the nature of this process is. My recent experiments have shown that the agencies which cause hemolysis also cause the membrane formation and the development of the unfertilized egg. I have thus been able to show that saponin, solanin, digitalin, bile salts, fatty acids, alkalis, hydrocarbons, ether and alcohols and the blood serum of not too closely related forms cause the membrane formation of the unfertilized egg and its subsequent development. There remained only one cytolytic substance which seemed to form an exception, namely, soap, but experiments which I have recently carried out have shown that it is possible to cause the membrane formation and subsequent development of the egg with sodium oleate.

If the unfertilized eggs of the sea-urchin are put into a mixture of 50 c.c. N/2 sodium chloride + 0.2 c.c. N/10 sodium oleate, the eggs form no membrane, nor do they undergo cytolysis; but if they are transferred into sea-water they form membranes and a smaller percentage of them undergoes cytolysis. If the eggs remain a short time only in the soap solution, they all form membranes, but few cytolyze after being transferred into sea-water; if they remain for a longer time, they all form membranes but cytolysis follows very soon after the membrane formation.

The question arises, why do the eggs form their membrane only after they are transferred into sea-water? This is due to the alkaline reaction of the sea-water. If we make the sea-water faintly acid by the addition of hydrochloric acid no egg forms a membrane or undergoes cytolysis after being transferred into sea-water, and if we make the solution of sodium oleate in sodium chloride slightly alkaline by the addition of sodium hydroxide the eggs form membranes while they are in the soap solution.

If we allow the soap solution to act only long enough to cause the membrane formation, but not long enough to cause cytolysis, the eggs can be caused to develop larvæ. We may from all these experiments draw the inference that the development of the resting egg is caused by a superficial or mild cytolysis, and that the spermatozoon must carry a cytolytic substance into the egg, possibly a trace of higher fatty acid.

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On the depression of the freezing point of water due to dissolved caseinates.

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The question whether or not proteins possess, in solution, a definite osmotic pressure has been the subject of much controversy. The original investigations of Graham[1] appeared to indicate that colloids in general exert a high osmotic pressure. Subsequent investigators, however, attribute these results to an admixture of crystalloids and the investigations of Sebanejew,[2] Tamman,[3]

[1] Graham: *Phil. Trans. Roy. Soc.*, 1861, cli, 183.

[2] Sebanejew: *Berichte d. deut. chem. Gesell.*, 1890, xxiii, 87; 1891, xxiv, 558; xxvi, 385. Sebanejew and Alexandrow: *Journ. of the Russian Phys.-chem. Soc.*, 1891, p. 7; quoted after *Maly's Jahresber. f. Tierchem.*, 1891, xxi, 11.

[3] Tamman: *Zeit. f. physikal. Chem.*, 1896, xxi, 180.