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.

40 (378)

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

By **T. BRAILSFORD ROBERTSON** and **THEODORE C. BURNETT.**

[*From the Rudolph Spreckels Physiological Laboratory of the University of California.*]

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.

Dreser,[1] Koeppe[2] and others indicate that when they are carefully freed from associated inorganic substances the cryoscopic depression due to dissolved proteins is negligible, while Reid[3] finds that proteins purified by repeated recrystallization, resolution and recrystallization frequently possess, in solution, no measurable osmotic pressure; and he concludes that provided every precaution be taken to exclude impurities (among which he includes inorganic constituents) from the protein solution it will be invariably found to possess no osmotic pressure whatever and that the osmotic pressures observed in solutions incompletely purified are due, not to protein, but to the associated impurities.

It appears to us that many of the above-quoted observations and conclusions are vitiated by the fundamentally erroneous conception that the inorganic constituents which are found associated with proteins are invariably present as impurities and not in a state of chemical combination. The manner in which this assumption vitiates conclusions regarding the molecular weight (estimated from the depression of the freezing-point or directly from the osmotic pressure) of proteins will be clear from the following considerations: Bases and acids have been demonstrated to form definite salts of a constant composition with casein, serum globulin and protamin, and there can be no doubt whatever that similar compounds are formed with other proteins. In solutions of casein and of serum globulin it can be shown that as the neutral point is approached the alkali-binding power becomes less and from a variety of data it can be shown that this phenomenon is due to a polymerization of the protein molecule according to equations of the type: $HXOH + HXOH = HXXOH + H_2O$[4] so that at or in the neighborhood of the neutral point molecular aggregates are formed of such dimensions that, in the cases of the proteins mentioned, the solution assumes the character of a suspension and the protein is precipitated; addition of acid or alkali shifts the equilibrium in the direction of the lower complexes and the protein goes into solution again in the form of a salt. Similar phenomena may be safely as-

[1] Dreser: *Arch. f. exper. Path. und Pharm.*, 1892, xxix, 314.
[2] Koeppe: *Arch. f. d. ges. Physiol.*, 1896, lxii, 571.
[3] Reid: *Journ. of Physiol.*, 1904, xxxi, 438.
[4] T. Brailsford Robertson: *Journ. of Physical Chem.*, 1908, xii, 473.

sumed to occur in other protein solutions, although the polymerization of the protein, which occurs when the uncombined protein is set free, may not result in actual precipitation. The elaborate precautions which have been taken by many observers to free the protein under investigation from accompanying inorganic substances, have, therefore, defeated their own ends by converting the protein into molecular aggregates so enormous as to possess a necessarily immeasurably small osmotic pressure.

Since it appears probable, therefore, that the dissolved *salts* of proteins may exert a measurable osmotic pressure in solution, and hence, cause an appreciable lowering of the freezing-point of water in which they are dissolved, we have undertaken a series of determinations of the lowering of the freezing-point of water, which is brought about by dissolved (neutral) caseinates.

The solutions are made up as follows: Alkali of a given concentration is shaken up with excess of casein until no more casein will dissolve and the solution is then filtered through rapid-filtering paper. The resulting solution is a solution of the "neutral caseinate" of the base and is neutral to litmus.[1] The cryoscopic depression is estimated in the usual way. The following are the results which have so far been obtained:

Experimental error of determination $\pm$ 0.0025°.

Base.	Concentration of base "saturated" with casein.	Δ	Indicating a concentration of
NH_4OH	$m/50$	0.045	$m/41$
"	$m/33.3$	.055	$m/33.6$
KOH	$m/50$	.0325	$m/57$
"	"	.0375	$m/49.3$
"	$m/33.3$	.0425	$m/43.7$
"	"	.0475	$m/38.9$
"	$m/20$	.05	$m/37$
"	"	.075	$m/24.6$
"	$m/15$	.1	$m/18.5$
LiOH	$m/59.5$	.03	$m/61.6$
"	$m/39.6$	.045	$m/41$
"	$m/23.8$	.07	$m/26.4$
"	$m/17.8$	.08	$m/20.3$

Since these solutions are neutral and no inorganic substance is introduced save the base employed to dissolve the casein it is evident that the compounds of bases with casein cause, in solution in

[1] T. Brailsford Robertson: *Journ. of Biol. Chem.*, 1907, ii, 336.

water, a definite cryoscopic depression. In harmony with deductions from titration- and conductivity-data[1] the results are such as indicate that casein behaves towards bases, essentially as a monobasic acid possessing a molecular weight, in solutions neutral to litmus, of approximately 2,000.

41 (379)

The daily excretion of bacteria in the feces of healthy men.

By **W. J. MACNEAL, LENORE L. LATZER** and **JOSEPHINE E. KERR.**

[*From the Laboratory of Physiological Chemistry, Department of Animal Husbandry, University of Illinois, Urbana, Ill.*]

During the past year we have examined at intervals of about two weeks the fecal bacteria of each individual in a group of twelve men who were the subjects of a prolonged metabolism experiment. These men were fed a mixed diet, in quantity according to their choice at the beginning of the experiment. Altogether we have examined bacteriologically 266 stools.

The quantity of bacteria in each of these stools was estimated by two different methods of microscopic counting and in about half of them the quantity of bacterial dry substance was also estimated by the gravimetric method of Strasburger.

In the individual examinations the largest number of bacteria observed was 816×10^9 bacterial cells per gram fresh feces, $2{,}642 \times 10^9$ bacterial cells per gram dry feces. The smallest number of bacteria counted was 124×10^9 per gram fresh feces, 983×10^9 per gram dry feces. By the gravimetric method the largest quantity of bacterial dry substance observed was 42.53 per cent. of the fecal dry substance or 13.2 per cent. of the moist feces. The smallest quantity of bacterial dry substance observed was 14.03 per cent. of the fecal dry substance or 2.6 per cent. of the moist feces. The average of all examinations was 375×10^9 bacterial cells per gram fresh feces; $1{,}587 \times 10^9$ bacterial cells per gram dry feces; bacterial dry substance in fecal dry substance,

[1] T. Brailsford Robertson: *Journ. of Physical Chem.*, 1908, xii, 479, etc.