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A method for the estimation of the hydrophilic colloid content of expressed plant-tissue fluids.

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The freezing-point depression of the freshly expressed plant juice is first obtained. Then, having determined the total solids by the refractometric method described by Gortner and Hoffman in the preceding note, a quantity of sucrose just sufficient to make a molar solution in the total water present is added. The freezing-point depression is again determined, and is usually found to have increased more than the theoretical amount (2.085°, allowing for the formation of sucrose hexahydrate).

It is assumed that the magnitude of the excess depression is a measure of the quantity of water held in such a way as to be unavailable for the solution of the sugar. This has been found to correspond in a general way with the content of hydrophilic colloids, as indicated by viscosity measurements, and proved by dialysis of the juice where this has been carried out, as well as by the preparation of colloidal solutions of known composition. Preliminary experiments with gum arabic indicate a close relationship between the "bound" water and the concentration of the added colloid.

It seems probable that the method may be applied to any biological fluid. A more detailed account of the experiments will be published in a botanical journal.

173 (1920)

Calcium phosphate metabolism showing the prevention of rickets by feeding clear grades of flour.

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In the milling of flour the ideal seems to have been production

of the whitest possible patent flour for human consumption, and the clear grades of flour, although they contain no shorts or bran, are too yellow in color for the American market. They contain much more phosphate than patent flour. The grain itself varies in phosphate content. Lack of available phosphate in the soil, or certain climatic conditions, may cause a reduction of phosphate content of grain. D. C. Mebane and myself showed that on a diet containing 45 per cent. of low phosphate rye (grown on peat soil) rickets was produced in white rats, whereas if this was substituted by high phosphate rye (from peat land in which the soil phosphate was made available by burning) no rickets developed. Soft winter wheat from the Ohio valley may be low in phosphate, and patent flour made from this wheat may contain as low as 0.075 per cent. P. Such flour was used in making a diet for white rats and produced severe rickets, whereas patent flour from hard spring wheat produced milder rickets, and Graham flour, no rickets. The percentage of phosphate in the patent flour depends, however, on the process of milling. At the Minnesota State Flour Mill some patent flour was made from the third middlings and contained 0.072 per cent. P, whereas the second clear flour contained 0.297 per cent. P. This, together with Graham flour, which I made by grinding hard winter tempered wheat containing 3.55 per cent. P, was used in making the following diets: Diet 96—NaCl 2 per cent., plaster of paris 2 per cent., yeast 1 per cent., spinach 1 per cent., lactalbumin 10 per cent., cotton seed oil 20 per cent., low phosphate flour 54 per cent., $NaHCO_3$ 10 per cent. Diet 98 was the same except there was no $NaHCO_3$ and the flour was 64 per cent. Diet 99 was the same as diet 98 except the second clear flour was used. Diet 100 was the same except that Graham flour was used.

	Diet.			
	96	98	99	100
P, per cent.....	0.120	0.133	0.287	0.340
Ca, per cent.....	0.607	0.621	0.645	0.635

Four rats of litter 23, twenty days old, weighing 27 ± 1 gm. were taken and placed on these diets. From the 33d to the 40th day of age they were kept in metabolism cages and the calcium phosphate metabolism determined with the following results:

Rat No.	Sex.	Diet.	Grams.				Milligrams per Day.						X-Ray 46 Days Old.
			Body Weight.			Food Intake per Day.	P.			Ca.			
			33 Days. Old.	40 Days Old.	Gain		In.	Out.	Ret.	In.	Out.	Ret.	
I	♂	96	33	34	1	3.49	4.2	3.1	1.1	21.2	19.7	1.5	Rickets
II	♀	98	36	42	6	3.82	5.1	3.5	1.6	23.7	22.7	1.0	Rickets
III	♀	99	47	65	18	7.50	21.4	13.8	7.6	48.4	38.1	10.3	No Rickets
IV	♂	100	52	73	21	7.36	25.0	15.8	9.2	46.7	32.7	14.0	No Rickets

It will be seen from the table that diets 99 and 100 containing the second clear and the Graham flours caused retention of P and Ca and prevented rickets, other things being equal. The X-ray plate suggested a very mild rickets in the rat eating Graham flour. Diet 96, containing high alkali, stopped the growth as well as produced rickets. We may conclude that it would be safer to feed infants Graham bread instead of white bread, but if the Graham bread is too laxative, bread may be made of second clear flour, which is not laxative, and rickets be prevented with more certainty than with the Graham flour.

Methods.—The rats were placed in wire cages sitting in six-inch silica dishes and at the end of the metabolism period the cages were lifted out, the dishes placed in a muffle and ashed at the lowest possible temperature in an atmosphere of O_2. The ash was then dissolved in dilute nitric acid and evaporated and redissolved and boiled.

Ca Analysis.—Take aliquot containing 10–50 mg. Ca in a 200 c.c. pyrex flask, add 1 drop brom-phenol blue, and 20 c.c. 2½ per cent. oxalic acid; add 20 per cent. Na_2CO_3 drop by drop until color changes to lavender; boil; stopper and shake one hour; filter; wash; transfer precipitate back to 200 c.c. flask with 100 c.c. H_2O; add 5 c.c. conc. H_2SO_4; heat to 75° and titrate drop by drop with 0.1 N

$KMnO_4$ (1 c.c. = 2 mg. Ca); add the filter paper to flask and titrate to find end point. Tenth normal oxalic acid (made from powdered oxalic acid dried over a mixture of hydrated and dehydrated oxalic acid) is used to standize the permanganate (as well as the NaOH for P analysis).

P Analysis.—Take aliquot containing 5–10 mg. P in a 200 c.c. pyrex flask; dilute to 100 c.c; add 10 c.c. HNO_3 and 1 drop brom-phenol blue; neutralize with ammonia; add 20 c.c. acid ammonium molybdate solution (usual formula); heat to 65°; shake 5 minutes; filter (the filtrate should turn methyl violet green). Wash until there is no titratable acidity in wash water; transfer paper and precipitate back to flask; run in 0.1 N NaOH until precipitate dissolves; boil 5 minutes; add ½ c.c. phenolphthalein and titrate to colorless with 0.1 N HCl. 1 c.c. alkali corresponds to 0.1194 mg. P.

174 (1921)

The agglutination reaction in the diagnosis of tuberculosis.

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Many attempts have been made in the past to make use of the agglutination reaction in the diagnosis of tuberculosis. The test has been found unsatisfactory largely because of the fact that the tubercle bacilli grow in adherent masses from which it has been difficult to prepare the homogeneous suspensions necessary for carrying out the test.

In the year 1918 Larson, Hartzell and Diehl [1] described a method of emulsifying and disrupting bacteria by subjecting them to the influence of carbon dioxide under high pressure, after which the pressure was suddenly released, causing a disruption of the organisms as a result of the rapid escape of the gas with which they were filled.

Tubercle bacilli grown on glycerine broth or glycerine agar are suspended in distilled water and placed in the apparatus where

[1] *Jour. Inf. Diseases*, 1918, xxii, 271–279.