XXX. THE DETERMINATION OF GALACTOSE BY THE METHOD OF HAGEDORN AND JENSEN

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(Received 15 December 1936)

THE method of Hagedorn & Jensen [1923] was originally standardized for glucose and is widely used for the estimation of sugar in blood. The method depends upon the reduction of ferricyanide by the sugar, the excess ferricyanide being estimated iodimetrically. A water blank is carried out for each series of estimations and the sugar present calculated from the difference in thiosulphate titrations. The standardization has been extended to maltose [Hanes, 1929], invert sugar [Callow, 1930], fructose and mixtures of these sugars [Hulme & Narain, 1931], but not, so far as the author is aware, to galactose. During studies on the fermentation of galactose by bacteria, it became necessary to estimate solutions of 0.002–0.001 *M* in the presence of bacteria and nutrient salts; as the reducing power of galactose towards ferricyanide is less than that of glucose, it was necessary to standardize the method for this sugar. The method described below is suitable for the estimation of amounts of galactose from 0.1 to 0.4 mg. but is not reliable for amounts less than 0.1 mg. The bacteria are first coagulated by heating with zinc sulphate solution and are filtered off before the estimation of the sugar.

Standardization. The procedure is that described by Cole [1933] with a few modifications of detail.

The thiosulphate solution was prepared freshly every day from a stock solution about 0.1 *N*. This solution was initially standardized by two methods and then checked every few days. The ferricyanide solution was prepared from the recrystallized salt and other solutions were prepared from chemicals of Analar quality as supplied by British Drug Houses, Ltd.

The method as a whole was checked against known glucose solutions.

Standard solutions of galactose were prepared from a pure product for which I am indebted to Dr D. J. Bell of this Laboratory. This was prepared by the hydrolysis of pure β -methylgalactopyranoside, m.p. 177–178°, and recrystallized from 97% alcohol. $[\alpha]_D$ at equilibrium, +81.8° ($c=5$, $l=4$). The crystals were stored in a vacuum desiccator.

The coagulated bacteria were removed by filtration through small filter-papers previously washed with 3 ml. of boiling distilled water.

It is essential that a water blank be performed for each set of experiments and this blank must be taken through all the steps used for the sugar solutions. Omission of the initial heating and filtration leads to a considerable error in the result.

Subtract the ml. of thiosulphate required for the estimation from that required for the water blank. If this be equal to *D*, then the amount of galactose present in the sample taken is given by reference to Table I.

Table I. D (ml. 0.005N sodium thiosulphate): mg. galactose

	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.4	0.097	0.099	0.102	0.104	0.106	0.109	0.112	0.115	0.117	0.120
0.5	0.122	0.125	0.127	0.130	0.132	0.135	0.137	0.140	0.142	0.145
0.6	0.148	0.150	0.153	0.156	0.158	0.161	0.164	0.166	0.169	0.171
0.7	0.174	0.176	0.179	0.182	0.184	0.187	0.189	0.192	0.194	0.197
0.8	0.200	0.202	0.204	0.207	0.210	0.212	0.215	0.217	0.220	0.223
0.9	0.225	0.228	0.230	0.233	0.236	0.238	0.241	0.243	0.246	0.248
1.0	0.251	0.253	0.256	0.258	0.261	0.264	0.266	0.269	0.272	0.274
1.1	0.277	0.280	0.282	0.285	0.288	0.291	0.294	0.296	0.299	0.301
1.2	0.305	0.307	0.310	0.312	0.315	0.318	0.321	0.324	0.326	0.329
1.3	0.331	0.334	0.336	0.339	0.342	0.345	0.348	0.350	0.353	0.356
1.4	0.359	0.361	0.364	0.366	0.369	0.372	0.375	0.378	0.380	0.383
1.5	0.386	0.389	0.391	0.394	0.397	0.400	0.402	0.405	0.407	0.410
1.6	0.413	0.416	0.419	0.421	0.424	0.427	0.429	0.432	0.435	0.438

SUMMARY

A method is described for the estimation of amounts of galactose between 0.1 and 0.4 mg. in the presence of bacteria.

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