in two normals was not changed after glutose. Glutose did not protect rabbits from insulin shock. Of four individuals on high fat diet, three showed no reduction of ketosis after glutose, while one did. Thus most of the evidence points toward glutose being inert in the body.

It is of great interest in this connection that yeast did not form a hexosephosphate with glutose under conditions that led to ready hexosephosphate formation with glucose. *B. coli* was found to yield both acid and gas on glutose broth.

Methods of preparation and estimation will be described in a subsequent communication.

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The ionic nature of amylase.

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By studying the distribution of trypsin and pepsin between suspended particles of gelatin and the fluid surrounding them, Northrop[1] has been able to show that these enzymes behave like univalent ions, the former being a cation and the latter an anion. We have carried out some preliminary experiments in which Northrop's procedure has been used to investigate the ionic nature of amylase.

Particles of iso-electric gelatin were suspended in solutions, the pH of which was varied by the addition of HCl or NaOH. On the alkaline side of the iso-electric point a small amount of KCl was added to furnish an ion (Cl^-) which could easily be titrated. The suspensions were stirred constantly for 2 hours. The solution containing the enzyme[2] was then added, and stirring was

[1] Northrop, J. H., *J. Gen. Physiol.*, 1924, vi, 337; *Ibid.*, 1925, vii, 603.

[2] "Taka-Diastase" (Parke-Davis) was used in these experiments. In a few instances a 5 per cent aqueous solution was used. It was found more convenient, however, to employ a 20 per cent solution of this preparation in 60 per cent alcohol.

TABLE I.

pH	Cl- gel. / Cl- filtrate	Amylase (gel.) / Amylase (filtrate)	Trypsin (gel.) / Trypsin (filtrate)
7.2	1/3.3	1/5	1.7
7.2	1/2.0	1/11	
7.2	1/3.3	1/5.1	
7.1	1/2.1	1/14	
6.4	1/2.8	1/17	1.6
5.6	1/2.0	1/6	
5.5	1.3	1/1.3	
4.3	1.45	15.	
4.3	1.35	14.	
4.1	5.6	1.2	
3.9	3.5	41.	1/3.9
3.9	2.6	19.	1/2.3
3.8	1.3	7.5	
3.8	4.5	1.2	
3.5	4.4	3.4	
3.5	4.4	3.3	1/5.1
3.35	6.1	1.1	1/2.8

continued for 2 hours more; the temperature was maintained between 0° and 4° C. The gelatin was then separated from the surrounding fluid by filtration, and the concentration of amylase in the filtrate and in the melted gelatin was determined by a viscometric method.[3]

In the accompanying table the ratios of the amylase concentration in the gel to that in the filtrate at various hydrogen ion concentrations are compared with the corresponding ratios of the Cl^- ions[4] in the 2 phases. Since the preparation used contained a small amount of trypsin, the ratios for this enzyme were also determined in a few instances.[5] The figures in the table represent single experiments.

As can be seen from the table, the distribution of amylase between the gelatin and the filtrate is extremely variable from pH 3.8 to 5.6. It would seem that forces other than those of the Donnan Equilibrium play a part here. This is in harmony with the observations of Northrop on pepsin that adsorption occurs near the iso-electric point.

A certain regularity is, however, found in the ratios obtained farther from the iso-electric point. On the acid side, the concentration of amylase is always greater in the gel, while on the alkaline side more amylase is constantly found in the filtrate. Although the data are not sufficient to warrant conclusions as to the valence of amylase, the distribution seems to indicate that this enzyme, like pepsin, is a negatively charged ion.

The few determinations of the trypsin ratios are in agreement with Northrop's observations on this enzyme.

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[3] Davison, W. C., *Johns Hopkins Hosp. Bull.*, 1925, xxxvii, 281.

[4] Chlorine ions were determined by titration in the filtrate; the Cl^- concentration in the gel being calculated by difference.

[5] Trypsin determinations were made by the method of Northrop and Hussey, *J. Gen. Physiol.*, 1923, v, 353.