LXXVIII. A NOTE ON THE REDUCING SUBSTANCES FOUND IN ALCOHOLIC EXTRACTS OF BRAIN.

By ERIC GORDON HOLMES (*Grocers' Company Research Scholar*) and BARBARA ELIZABETH HOLMES.

From the Biochemical Laboratory, Cambridge.

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In a previous communication [B. E. and E. G. Holmes, 1925] reference was made to reducing substances which were extracted from brain tissue with dilute alcohol. For reasons stated in the original paper, it was believed that little, or none, of this reducing substance was glucose. Some of it appeared to be pentose, and it was observed that the reducing value increased after hydrolysis for one hour with 5 % of strong hydrochloric acid, but beyond this no evidence as to its nature was then available.

As a result of further work certain additional information is available, which, though so far insufficient completely to make clear the nature of the substances involved, yet seems worth recording in view of its bearing upon the Hagedorn and Jensen micro-method for sugar.

For the original experiments, rabbits were used. The yield of reducing substance obtainable from a rabbit's brain is extremely small, and it was necessary, for the sake of economy, to use sheep's brains for the work here described.

Details of the method of alcoholic extraction have already been given. In the present instance, as some time necessarily elapsed before the brains could be brought from the slaughter-house, they were treated with alcohol without freezing. Fifty sheep's brains (about 5 kg. of tissue, wet weight) were gradually worked up. The final aqueous extract, amounting to several litres, was concentrated *in vacuo* at 40°. Much inorganic material was removed by the addition of alcohol, which procedure was found to involve no material loss of reducing substance.

Finally, 800 cc. of alcoholic extract (alcohol about 80 %) were obtained, the reducing value of which, by the method of Hagedorn and Jensen [1923], was equivalent to about 1 g. of glucose before hydrolysis, and about 1·4 g. after hydrolysis.

At this stage, Sir F. G. Hopkins was good enough to undertake an investigation of the material. The alcohol was distilled off under reduced pressure and the residue dissolved in water. The aqueous solution gave a copious precipitate with phosphotungstic acid. This precipitate was filtered off and decomposed with baryta. The filtrate from the barium phosphotungstate

yielded, on evaporation, a crop of crystals. These were purified by recrystallisation from water and alcohol. They reacted positively to the Weyl and Jaffé tests, and formed a crystalline picrate. Although more exhaustive measures for identification were not employed, it seems quite clear that the material was creatinine.

Creatine has been described as occurring in brain by Beker [1913], Janney and Blatherwick [1915], and Harding and Eagles [1924], while Hammett [1923] has observed that brain contains an enzyme which can convert creatine into creatinine. Creatinine, and not creatine, was identified. Creatine is not precipitated by phosphotungstic acid, and would therefore remain in the first filtrate. On the other hand, the aqueous extracts are always acid in reaction, and were necessarily stored (in the refrigerator) for some time; the alcoholic extract, also, was acid, and was stored at room temperature. Creatine would, in these circumstances, tend to be converted into its anhydride.

Since our first paper was written, further experiments have been performed on the reducing properties of brain extracts. It was repeatedly observed that if, for any sample of extract, different volumes of fluid, *e.g.* 1 cc. and 2 cc., were used for successive sets of estimations, the results for amount of reducing substance (as glucose), calculated for 100 cc. of fluid, did not agree. This suggested the construction of a "reduction curve" for the extract, which was done by plotting cubic centimetres of fluid taken against reducing substance found, the latter calculated as glucose.

Such a curve is not a straight line, but tends to fall away as the amount of extract taken for each estimation increases. For instance, 0·25 cc. of a certain sample gave a value of 26·4 mg. of reducing substance per 100 cc., while 1·5 cc. gave a corresponding value of only 20·0.

The reduction curves which we determined for galactose, maltose, lactose, xylose and arabinose were all straight lines, though they differed, of course, from that of glucose.

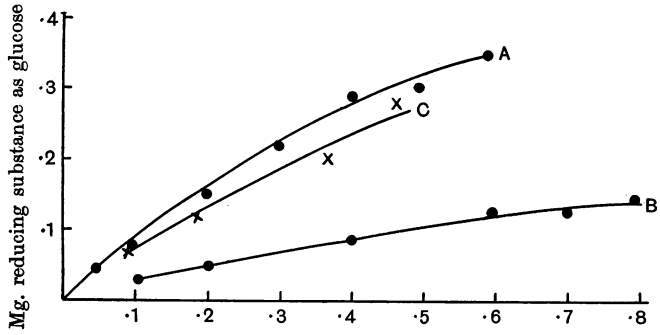

Fig. 1.

The curve given by pure creatinine, on the other hand, has the form shown in Fig. 1 (*A*), and falls away quite sharply as the concentration of creatinine increases. Creatine (Fig. 1 (*B*)) also behaves to the Hagedorn reagents as a

reducing substance, though less markedly than does creatinine. It seems reasonable to suppose that part, at least, of the reducing value of the extracts was due to creatine and creatinine, and that the rise on hydrolysis was occasioned by the conversion of the former into the latter. Fig. 1 (*C*) shows the reduction curve of creatine after being subjected to the treatment employed to hydrolyse the brain extracts.

A further circumstance no doubt contributed to the observed rise in reducing power of the brain extracts. In the earlier experiments a measured amount, *e.g.* 5 cc., of the hydrolysed extract was neutralised with soda from a burette, and 2 cc. of the neutralised (and therefore diluted) fluid was used for each estimation, the total reducing power being calculated from the volume of fluid that would have resulted, had the whole been neutralised. Evidently, this dilution alone would have caused an apparent rise in reducing value. Since a large rise persists even when this factor is eliminated by adding soda to each 2 cc. of hydrolysed extract separately, in the estimation flasks, it cannot have been by any means the chief factor.

The filtrate from the phosphotungstic acid still contains reducing substances, all of which, we have reason to believe, is not creatine. We hope shortly to publish certain other evidence on this point which is becoming available. The bearing of this work on the question of the application of micro-methods of sugar estimation to tissue extracts is sufficiently obvious. In this case, the shape of the reduction curve renders the application of a correction for creatinine extremely difficult, while the conversion of creatine into creatinine by heating with acid gives results which simulate the hydrolysis of a di- or poly-saccharide.

SUMMARY.

1. Creatine and creatinine both function as reducing agents towards the Hagedorn and Jensen reagents, creatinine more strongly than creatine.

2. Their reduction curves, unlike those of several carbohydrates tested, are not straight lines.

3. Creatinine has been identified as a constituent of alcoholic brain extracts. The conversion of creatine into creatinine gives results that simulate the hydrolysis of a di- or poly-saccharide.

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REFERENCES.

Beker (1913). *Z. physiol. Chem.* **87**, 21.
Hagedorn and Jensen (1923). *Biochem. Z.* **135**, 46.
Hammett (1923). *J. Biol. Chem.* **59**, 345.
Harding and Eagles (1924). *J. Biol. Chem.* **60**, 301.
Holmes, B. E. and E. G. (1925). *Biochem. J.* **19**, 492.
Janney and Blatherwick (1915). *J. Biol. Chem.* **21**, 561.