XX. A NOTE ON THE METABOLISM OF NITROGENOUS SUGAR DERIVATIVES.

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It is very generally accepted that carbohydrates are of fundamental importance in the metabolism of nitrogenous foodstuffs. Loewi [1902], using protein decomposition products which no longer gave the biuret reaction, showed carbohydrate to be necessary for the maintenance of a nitrogen equilibrium. Lesser [1904] subsequently demonstrated the inability of fats to replace the carbohydrate. It has been observed further that glycosuria following excision of the pancreas in dogs is attended by increased excretion of nitrogen [Falta, Grote and Staehelin, 1910], and Taylor and Cathcart [1910] found phloridzin diabetes to be followed by disturbances of nitrogen metabolism. Finally, Falta and Gigon [1908] have concluded that carbohydrate is indispensable for the utilisation of protein, and it has been assumed that some compound is formed between the carbohydrate and the nitrogenous material [Lüthje, 1906].

In an attempt to determine the nature of this synthesis Spiro [1907] showed that the injection of glycine and fructose together gave rise to the appearance in the urine of a dicarboxylic acid normally absent. On the other hand much *in vitro* work has been done and various definite compounds of sugars and nitrogenous substances have been obtained. Lobry de Bruyn [1904] prepared compounds of sugar and ammonia, Schoorl [1900] a urea derivative of glucose, and Morrell and Bellars [1907] have synthesised a product from glucose and guanidine. It may be noted however that in some cases at least the reaction does not seem to be one of simple condensation [Irvine, 1909]. Little seems to have been done on the metabolism of substances of this nature.

Fabian [1899] found that glucosamine is with difficulty oxidised in the body and is in great part excreted unchanged. The extreme stability of this

compound and its great resistance to *in vivo* oxidation are somewhat unexpected; but in light of recent evidence brought forward by Irvine and Hynd [1912] for considering glucosamine to be not a simple derivative of glucose, but a ring compound containing a betaine grouping, Fabian's results are largely explained.

For the purposes of studying the metabolism of compounds such as those mentioned above, those referred to offer certain disadvantages; Lobry de Bruyn's products are somewhat indefinite, the urea derivative difficult to obtain in quantity, and most other compounds of this nature are highly unstable.

For the following experiments, glucose *p*-phenetidine, the condensation product of glucose and *p*-phenetidine, was selected on account of the ease of its preparation and its comparative stability to hydrolytic agents. At the boiling point, however, in solution it is readily hydrolysed by 0.2% hydrochloric acid.

The preparation of glucose *p*-phenetidine was carried out according to the method devised by Irvine and Gilmour [1909].

The toxicity of the compound is also disputed [St Mostowski, 1909] (quoted from the above-mentioned authors).

White rats were used, the faeces and urine being collected separately in cages similar to those described by Schäfer [1912]. To prevent loss of nitrogen from the urine in the form of ammonia, known volumes of concentrated sulphuric acid were generally added to the receiving vessel previous to collection. The glucose *p*-phenetidine was administered either in the food (bread and milk *ad libitum*), or less often by intra-peritoneal injection. Quantities of from 0.1 to 1 g. (usually 0.5 g.) were given daily. The intake and output of nitrogen were determined both before and during the experiment, but no alteration in the nitrogen equilibrium was observed. The presence of glucose *p*-phenetidine in the food caused no variation in the amount consumed *per diem* which could not be accounted for by the normal difference from day to day.

The urine of those animals receiving the compound was without exception of a much darker colour than that of the controls or of themselves before or after the feeding. Discontinuance of feeding at once restored the normal light yellow colour. The colour is not caused by hydrolysis taking place in the urine by means of the acid present, for when the urine was collected alone the colouration persisted¹.

¹ Oxidation of glucose *p*-phenetidine by means of hydrogen peroxide in neutral solution produces a brownish black syrup of unknown composition.

Injection of 0.1 g. produced similar results, and moreover in each case a substance displaying active reducing properties was excreted in the urine. Greater reduction of Fehling's solution, Barfoed's solution and Nylander's reagent occurred after previous boiling of the urine with mineral acid. In one case following injection, a compound was obtained from the urine by means of phenylhydrazine, which in microscopic appearance resembled the phenylosazone of glucose, but owing to the small quantity confirmation could not be obtained.

It was mentioned above that the toxicity of glucose *p*-phenetidine was disputed, St Mostowski denying the general conclusion that it is poisonous (we are unaware however of the quantities administered by this author).

In these experiments it is shown that in quantities up to 1 g. *per diem* it produces no ill effects in rats of from 220 to 250 g. body weight. Experiments with a view to determining the effects of larger doses were not performed owing to scarcity of material.

Injection of *p*-phenetidine however is followed by marked results. The *p*-phenetidine cannot be given by feeding owing to the animals refusing to eat all food containing it.

In a cat 4 c.c. of an 8% solution caused a large and rapid fall of blood pressure which returned to normal within a minute and a half and remained steady and regular until death ensued ten minutes later. Post-mortem examination revealed extensive intra-vascular clotting.

It seems probable that much of the glucose *p*-phenetidine escapes hydrolysis or is rendered innocuous by conversion into some other substance, from the facts that in doses of 4 c.c. of an 8% solution or 0.32 g. per 2.5 kilo body weight, *p*-phenetidine is highly toxic, and that glucose *p*-phenetidine is harmless when given in amounts of 1 g. per 220 g. or 4.5 g. per kilo body weight, equivalent, if complete hydrolysis took place, to about 2 g. *p*-phenetidine.

SUMMARY.

1. After administration of glucose *p*-phenetidine by mouth or by injection, a reducing substance is excreted by the urine.
2. No effects on nitrogen metabolism are produced.
3. In amounts up to 1 g. per 220 to 250 g. body weight *per diem* glucose *p*-phenetidine is not toxic.
4. Some of the glucose *p*-phenetidine escapes oxidation in the body or is converted into a substance of non-poisonous nature.

5. In amounts of 0.32 g. per 2.5 kilo body weight (or 0.108 g. per kilo) *p*-phenetidine is highly toxic.

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