Technical Papers

Degradation of Radioactive Glucose[1]

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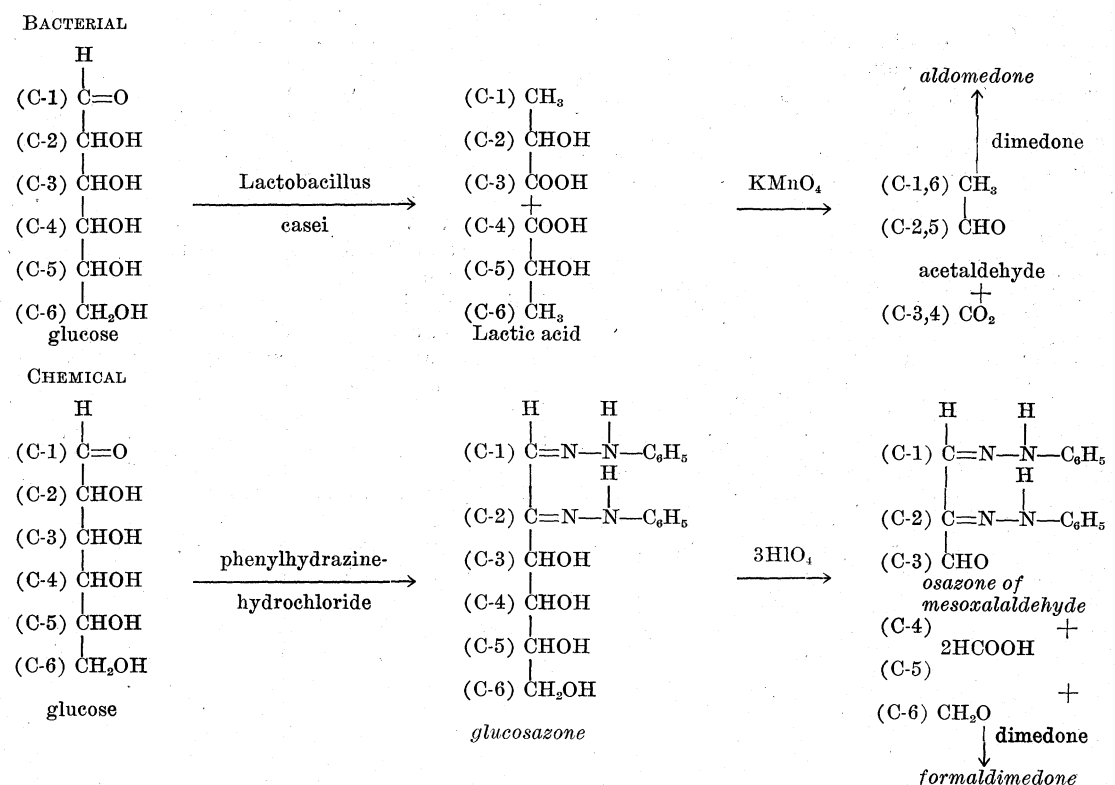
In the original method of glucose degradation used by Wood, Lifson, and Lorber (*1*), glucose is at first converted by *Lactobacillus casei* to lactic acid. By subsequent chemical degradation CO_2 derived from different positions of this lactate is precipitated and counted as $BaCO_3$. This method has been tested by other workers and found to be dependable. Since CO_2 derived from various positions of a glucose molecule is always counted as $BaCO_3$, a comparison of relative activity throughout a glucose molecule becomes an easy matter. Unfortunately this method is laborious and time-consuming. Moreover, the plating and counting of $BaCO_3$ present a number of difficulties.

to glucosazone and this is degraded with periodate. The products are bisphenylhydrazone of mesoxalaldehyde derived from C-1 + C-2 + C-3 of glucose, formic acid from C-4 and C-5, and formaldehyde from C-6. Formaldehyde is precipitated with dimedon and counted as such. Bisphenylhydrazone of mesoxalaldehyde is further oxidized by 1% KOH in absolute ethanol, yielding a precipitate of glyoxalosazone derived from C-1 + C-2.

Activity in C-1 + C-2 + C-3 is determined from counting bisphenylhydrazone mesoxalaldehyde. C-1 is presumed to be equal to C-6. C-1 + C-2 is determined from glyoxalosazone, and C-2 is equal to C-1 + C-2 − C-1. It is claimed by these authors that their method is rapid and that all the compounds formed are easily separated and readily plated with pyridine.

Since the present authors were faced with the problem of degrading a very large number of glucose samples, it was decided to try Aronoff and Vernon's



(The compounds counted are those in italics.)

FIG. 1. The scheme for glucose degradation.

In an attempt to eliminate these drawbacks Aronoff and Vernon (*2*) suggested a method based on an entirely different principle. Glucose is at first converted

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method. We have, however, experienced a difficulty in the step involving the degradation of bisphenylhydrazone of mesoxalaldehyde with 1% alcoholic KOH. The reported (*3*) melting point of glyoxalosazone is 178°–180° C, but the crystals obtained

TABLE 1

Product counted	Av atomic number
Glucosazone	3.96
Bisphenylhydrazone mesoxalaldehyde	4.1
Aldomedone	3.5
Formaldimedone	3.5
($BaCO_3$	17)

by us in this step only occasionally had this value. In the majority of cases their melting point was 123°–125° C. We could not, therefore, duplicate this step.

It is clear, therefore, that the step in Aronoff and Vernon's technique of obtaining the C-1 + C-2 value by degrading bisphenylhydrazone of mesoxalaldehyde with alcoholic potash is not dependable. Until more is known about the products of this reaction, the technique of Aronoff and Vernon, as proposed by these authors, cannot be used.

The other steps of their method, however, have been found satisfactory, and because of the advantages of this method we did not wish to drop it. The method, as claimed by the authors, is rapid, and the various products, with the exception of glyoxalosazone, are easily obtained and plated.

In a search for some other method of precipitating and counting C-1 + C-2, we decided to isolate these two carbons as acetaldehyde and precipitate it with dimedone (*4*). Such a precipitation is quantitative and rapid, and the crystals are readily plated in pyridine. The technique for glucose degradation becomes now a combination of some of the steps of Wood, Lifson, and Lorber (*1*) and some of those of Aronoff and Vernon (*2*), plus a new step involving precipitation of acetaldehyde as aldomedone. Fig. 1 presents the scheme of such a degradation.

Glucose is fermented with *L. casei,* and the lactic acid formed is oxidized with $KMnO_4$. Acetaldehyde produced from C-1 + C-2 or C-6 + C-5 is precipitated with dimedone and counted as such. Glucosazone, bisphenylhydrazone mesoxalaldehyde, and formaldehyde are produced and counted according to Aronoff and Vernon. Table 1 gives the average atomic number of various compounds counted. Since this number is about the same for all the compounds counted, the backscattering effects are similar. For this reason the activities of such compounds are directly comparable.

TABLE 2

DISTRIBUTION OF C^{14} IN VARIOUS POSITIONS OF GLUCOSE AS PERCENTAGE OF TOTAL ACTIVITY

Glucose sample	Degradation technique used	Carbon atoms of glucose		
		3 and 4	2 and 5	1 and 6
1	Wood, Lifson, and Lorber	34.5	32.5	33
1	Vittorio, Krotkov, and Reed	33.7	32.1	34.2
2	Wood, Lifson, and Lorber	52	25.9	22.1
2	Vittorio, Krotkov, and Reed	50.9	26.1	23.0

Two samples of glucose, with uniform and nonuniform distribution of C^{14} in various positions, were degraded using both our proposed modification and the original scheme of Wood, Lifson, and Lorber. The results are given in Table 2, which shows that the results obtained by both methods agree within about 2.5%.

References

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Competition of the Aliesterase in Rat Serum with the Pseudo Cholinesterase for Diisopropyl Fluorophosphonate[1]

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The molar concentration of pseudo cholinesterase in certain mammalian sera can be determined by the use of a competitive reversible inhibitor, Nu-683, the dimethyl carbamate of (2-hydroxy-5-phenylbenzyl) trimethylammonium bromide, and an analog of prostigmine (*1*).

The concentration of inhibitor that causes 50% inhibition of the pseudo-cholinesterase activity (I_{50}) is determined at various concentrations of serum; a plot of I_{50} against the relative enzyme concentration should give a straight line with a slope equal to one half the molar concentration of pseudo cholinesterase in the serum solutions. Typical results of this kind as obtained with rat serum are indicated in Fig. 1.

Extensive investigations with other inhibitors of this type have failed to reveal any enzymes other than the cholinesterases that are inhibited by very low concentrations of these inhibitors. Moreover, a comparison of the experimental results (*1*) with the theoretical predictions for a reversible competitive inhibitor (*2*) shows directly that Nu-683 must be acting as a selective inhibitor of the pseudo cholinesterase in the serum preparation (Fig. 2).

There is no similar theoretical criterion by which the selectivity of an irreversible inhibitor can be established. However, it was observed that the results with the irreversible inhibitor diisopropyl fluorophosphonate (DFP) frequently give a higher value for the apparent cholinesterase concentration than that obtained by the above technique with Nu-683. Thus it appears that the results obtained with DFP must be at fault for some reason.

One source of error was revealed by experiments

[1] This work was carried out under the guidance of B. Mendel and with the technical assistance of M. de Jonge.