Some Effects of Autoclaving Aqueous Solutions of Glucose

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There has been considerable interest directed toward the precursors of aromatic amino acids with respect to bacterial metabolism. Davis (1) suggested that shikimic acid could serve as a common precursor for phenylalanine, tyrosine, and tryptophane. In studying the growth response of *Lactobacillus arabinosus*, Snell (12) and Dunn *et al.* (2) reported that

phenylalanine and tyrosine were non-essential amino acids; however, Hegsted (5) and Hutchings and Peterson (6) stated that these two amino acids were required for the growth of this organism. Kulken *et al.* (8) reported that phenylalanine was necessary for growth, but tyrosine was a non-essential amino acid in the nutrition of *L. arabinosus*. In the experimental procedure described by all of these investigators, it was was noted that the medium was sterilized by autoclaving.

Previous work from this laboratory (10) has shown that phenylalanine was essential for *Streptococcus faecalis* A.T.C.C. No. 6057. However, if the medium was autoclaved, phenylalanine was a stimulatory amino acid. Further, it was demonstrated that autoclaving an aqueous solution of glucose would produce phenylalanine activity in this organism.

From the work of this laboratory and other investigations, it appeared that the autoclaving process produced some changes in the glucose molecule which could account for the phenylalanine activity.

The work described in this paper was carried out to shed some light on the chemical changes which take place in aqueous solutions of glucose during the autoclaving period.

Experimental Procedure

A solution of 8.33×10^{-4} moles per milliliter of glucose was used in all of the studies described. The solution was divided into two portions; one portion was used as a control and the other portion was autoclaved under a pressure of 15 pounds per square inch at 121° C. for a period of 8 minutes. The solutions were unbuffered when autoclaved and any pertinent experimental details accompanying physical or chemical measurements will be described in the results.

Comparison of the refractive indexes of the solutions both gave a value of 1.3348; however, the optical rotation of the two solutions were —52.54° per decimeter for the glucose and —60.0° per decimeter for the autoclaved glucose solution. A comparison of the physical and chemical measurements of the two solutions is shown in Table I.

TABLE I

	CONTROL GLUCOSE	AUTOCLAVED GLUCOSE
Absorption Spectra (λ max.)	None	228 mμ
Optical Rotation $(\alpha]_D^{27°})$	—52.54 °/dm.	—60.0 °/dm.
Refractive Index	1.3348	1.3348
Polarographic ($E_{\frac{1}{2}}$)		—1.28 volts
Chromatographic (R_f)	0.486	0.488
Periodic Acid Test (moles OH^-/ml.)	3.095×10^{-4}	3.430×10^{-4}
Bromine Test (moles Br_2 used/ml.)	0.710×10^{-4}	1.173×10^{-4}

Results and Discussion

When a mixture of glucose and fructose was substituted for the autoclaved glucose in the medium, a growth response was found which corresponded to the growth response of an autoclaved solution of glucose. Since glucose and fructose have a common ene-diol, there was a possibility that fructose was an intermediate to the precursor of phenylalanine. Inglis and Hanahan (3) demonstrated the presence of a ketose, probably fructose, when a solution of glucose buffered at *p*H 6.5 (phosphate buffer) was autoclaved for a period of 30 minutes. The results obtained were not consistent, and they suggested that the reaction had not reached an equilibrium at the end of these short periods of autoclaving. Further,

they reported that no ferricyanide ketoses could be detected in an unbuffered solution of autoclaved glucose, but they could not get 100 per cent recovery of the reducing sugars.

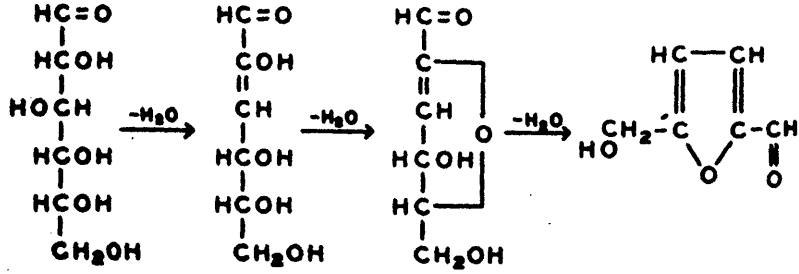
Since fructose could serve as an intermediate for shikimic acid, paper partition chromatography, and polarographic studies were carried out to check for the presence of fructose in the autoclaved glucose solution.

Filter paper chromatograms using the method of Partridge (11) and the modified developing method of Trevelyan (13) showed the autoclaved and unautoclaved glucose R_f's to be identical while that of the fructose was found to be different. Using a phenol saturated with water-system solvent and a phenylhydrazine-acetic acid developer, the autoclaved glucose showed an R_f of 0.486, unautoclaved glucose an R_f of 0.488, and fructose an R_f of 0.625. The color test for fructose of Maurmeyer *et al.* (9) confirmed the results of Englis and Hanahan (3).

Solutions for the polarographic analysis were buffered to a *p*H 7.6 using a primary potassium phosphate-potassium hydroxide buffer. The supporting electrolyte was a 0.1 molar solution of lithium chloride. Nitrogen was bubbled through the solution to remove any oxygen present. The curves were obtained by using a recording potentiometer. A half-wave potential of −1.28 volts was obtained for the autoclaved glucose, but no half-wave potentials were detected for the glucose or fructose. Differences in the curves were shown by running the solutions on the same polarogram.

Although numerous theories have been presented in the literature (7), no exact interpretation, other than the half-wave potentials, of current-voltage curves for irreversible reaction can be given.

Wolfrom *et al.* (14) found that upon heating an aqueous solution of glucose small quantities of 5-hydroxymethylfurfural are formed. They suggested that the mechanism of the reaction was:



Since 5-hydroxymethylfurfural could be detected by absorption spectra, similar studies were initiated on the autoclaved glucose solution. The absorption spectrum of this solution is shown in Figure 1.

An absorption maximum at 285 millimicrons (14) was shown by 5-hydroxymethylfurfural, but the autoclaved glucose solution showed a maximum at 228 millimicrons. Evans and Gillam (4) have shown that absorption maximum in the region of 230 millimicrons is characteristic of α-β unsaturated aldehydes.

From this, it appeared that a portion of the glucose was being converted into the intermediates suggested by Wolfrom *et al.* (14). The determination of which intermediate or the relative proportion of the two intermediates was attempted. It would be possible to distinguish the mono-dehydration product of glucose from the di-dehydration product by a

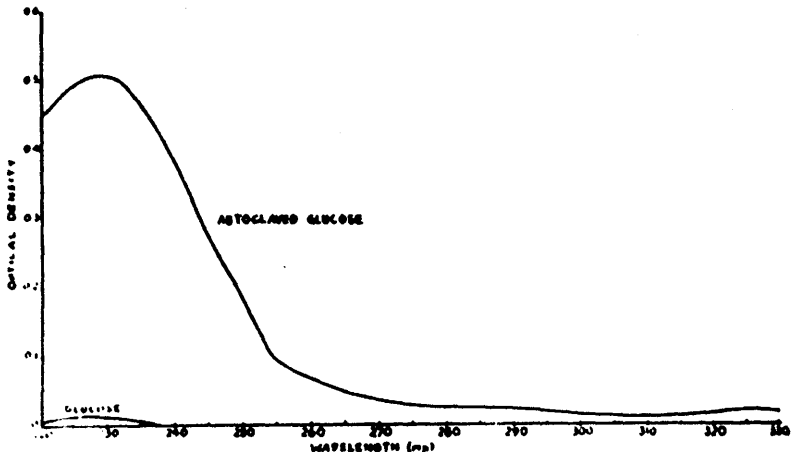


FIGURE 1. Absorption Spectrum of Glucose and Autoclaved Glucose.

selective bromination and the periodic oxidation of the solutions. For example, the bromination could be used to determine the sum of the concentrations of both unsaturated aldehydes, but the periodic oxidation would not react with the compound which contained the butylene oxide ring.

A selective bromination showed that 0.463 x 10^{-5} moles of bromine were added to the autoclaved glucose when compared to the controlled solution.

However, the periodic oxidation showed an increase in the hydroxyl groups when compared to the controlled solution of glucose. For every 2 molecules of glucose, one more hydroxyl group was present in the autoclaved glucose solution.

While this combination of reactions could not distinguish the relative amounts of the two unsaturated aldehydes, it indicated that more than one reaction was taking place in the autoclaving process. Apparently, glucose can form the ene-diol compound as well as the unsaturated aldehydes during the autoclaving process. This possibility could explain the results of the chemical and physical tests on the solutions.

From this work it appears that the process of autoclaving glucose with or without the presence of other compounds could cause some changes in the glucose molecule which could account for some of the physiological activity noted in the case of aromatic amino acids and that these changes do not involve one single reaction.

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