INFLUENCE OF GLUCOSE CONCENTRATION ON THE GROWTH OF STREPTOCOCCUS FAECALIS, ON pH, AND DISCOLORATION OF A NON-SYNTHETIC MEDIUM

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Charles Woodbury Fifield III

1955

This is to certify that the

thesis entitled

"The Influence of Glucose Concentration on the Growth of Two Species Fungalis on pH, and Discoloration of a Non-synthetic Medium."

presented by

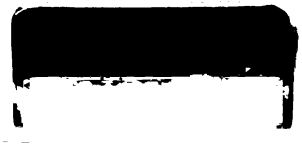
Charles W. Fifield

has been accepted towards fulfillment of the requirements for

M.S. degree in Microbiology

Major professor

Date March 8, 1955



INFLUENCE OF GLUCOSE CONCENTRATION ON THE GROWTH OF *STREPTOCOCCUS FAECALIS*, ON pH, AND DISCOLORATION OF A NON-SYNTHETIC MEDIUM

by

Charles Woodbury Fifield III

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

1955

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. W. L. Mallmann for his guidance and patience throughout the course of this work. Appreciation is also extended to Dr. H. J. Stafseth and Dr. E. S. Beneke for proof-reading the final draft of this thesis.

INFLUENCE OF GLUCOSE CONCENTRATION ON THE GROWTH OF *STREPTOCOCCUS FAECALIS*, ON pH, AND DISCOLORATION OF A NON-SYNTHETIC MEDIUM

by

Charles Woodbury Fifield III

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

Year 1955

Approved by _____

Using a non-synthetic medium consisting of 1.5 percent tryptose, 2.7 percent KH_2PO_4, 2.7 percent K_2HPO_4 and varying concentrations of glucose it was found that increase in glucose concentration caused an increase in browning and an increase in active hydrogen ion concentration in the autoclaved medium. The products of the reaction, however, were not toxic to Streptococcus faecalis in the non-synthetic medium used. If the pH of the glucose containing medium was adjusted after sterilization, equal growth was obtained in all concentrations of glucose used. Likewise equal growth resulted in the medium autoclaved in the presence of glucose and in the medium to which sterile glucose was added after autoclaving, if the above consideration was met.

Greater growth was obtained in the phosphate buffered medium than in the same medium in which the buffer was omitted. It is suggested that this is due to an enhancement of metabolic activity as well as to a buffering effect.

Oxygen uptake of the resting cells was identical in all but the lowest concentration of glucose used. This concentration affected O_2 uptake the same as the higher concentrations until it was completely utilized.

Alkaline degradation products of glucose in the low concentration used initiate growth as well as intact glucose.

A recommendation for the use of 0.25 to 0.5 percent glucose in the described non-synthetic medium is given.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

The formulae of many non-synthetic bacterial media used today have glucose concentrations in excess of 0.5 percent. These media generally are basically composed of a phosphate buffer and tryptose as a source of nitrogen. It has often been observed that the autoclaving of such media alters the composition in such a way as to have a deleterious effect on subsequent bacterial growth. This phenomenon has been referred to as the Maillard reaction, browning reaction, or caramelization and has been recognized as being extremely important in nearly all industries dealing with materials of a biological nature.

The present investigation describes studies involving the use of various concentrations of glucose as affecting (1) the growth of Streptococcus faecalis in a non-synthetic medium, (2) the oxygen uptake of the organism, and (3) the browning and pH of the medium.

Monod,[1] using a synthetic medium with mannite as the sole source of carbon and Escherechia coli as the test organism, showed that the total growth is a linear function of the concentration of the mannite, as long as the concentration does not exceed 0.5 percent.

In 1912 Maillard[2] demonstrated that solutions containing amino acids turned brown when heated with glucose. Hill

and Patton[3] suggested that optimal growth may not be obtained in microbiological assays due to the effects of the Maillard reaction. Using a casein hydrolysate medium prepared for tryptophane assay and using Streptococcus faecalis R as the test organism, they showed that better growth could be obtained in a medium in which the glucose had been autoclaved separately and added aseptically to the previously cooled base medium. Identical growth also resulted when sucrose, a non-reducing sugar, was substituted for glucose. Patton and Hill[4] found that a solution containing known amounts of L-tryptophane and D-glucose heated at pH 10 showed more browning but less tryptophane loss, as determined by microbiological assay, than a similar solution heated at a lower pH for a longer time. They were of the opinion that the decrease in growth was not due to formation of growth inhibitors as products of the browning reaction but to actual destruction of part of the tryptophane, and that nutrients of both a vitamin and amino acid nature may be damaged during autoclaving with glucose.

Stevens and McGinnis[5] pointed out that the decreased nutritive value of overheated soy bean oil meal for chicks was almost fully corrected by adding supplementary methionine and lysine. These amino acids when added to properly heated soy bean oil meal, however, did not improve the growth of

chicks. Lysine autoclaved for four hours at 120 C supplemented overheated soy bean oil meal for chick growth as effectively as unheated lysine.

Chen, Medler, and Harte[6] found that highly flourescent substances were formed from certain amino acids when heated with Deniges reagent (para-formaldehyde and sulfuric acid). Graham, Hsu and McGinnis[7] showed that flourescent substances were formed when glucose was used to furnish the aldehyde group instead of Denige's reagent. All fifteen amino acids tested showed flourescence. These investigators concluded that brown color formation, flourescence, and decreased amino nitrogen are associated with the destruction of methionine brought about by autoclaving in the presence of glucose.

On the other hand several investigators have indicated that the heating of a medium in the presence of glucose is essential for maximum growth of certain organisms. Orla-Jensen[8] reported this phenomenon after studying various streptococci and lactobacilli. Smiley, Niven and Sherman,[9] working with a synthetic medium for Streptococcus salivarius, found that if glucose was added aseptically to the medium after autoclaving, growth was greatly delayed or never initiated. It was suggested that a hydrogen acceptor was required in the initial carbohydrate metabolism. If such an acceptor were not present, carbohydrate metabolism might

INFLUENCE OF GLUCOSE CONCENTRATION ON THE GROWTH OF *STREPTOCOCCUS FAECALIS*, ON pH, AND DISCOLORATION OF A NON-SYNTHETIC MEDIUM

by

Charles Woodbury Fifield III

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Microbiology and Public Health

Year 1955

Approved by _____

Using a non-synthetic medium consisting of 1.5 percent tryptose, 2.7 percent KH_2PO_4, 2.7 percent K_2HPO_4 and varying concentrations of glucose it was found that increase in glucose concentration caused an increase in browning and an increase in active hydrogen ion concentration in the autoclaved medium. The products of the reaction, however, were not toxic to Streptococcus faecalis in the non-synthetic medium used. If the pH of the glucose containing medium was adjusted after sterilization, equal growth was obtained in all concentrations of glucose used. Likewise equal growth resulted in the medium autoclaved in the presence of glucose and in the medium to which sterile glucose was added after autoclaving, if the above consideration was met.

Greater growth was obtained in the phosphate buffered medium than in the same medium in which the buffer was omitted. It is suggested that this is due to an enhancement of metabolic activity as well as to a buffering effect.

Oxygen uptake of the resting cells was identical in all but the lowest concentration of glucose used. This concentration affected O_2 uptake the same as the higher concentrations until it was completely utilized.

Alkaline degradation products of glucose in the low concentration used initiate growth as well as intact glucose.

A recommendation for the use of 0.25 to 0.5 percent glucose in the described non-synthetic medium is given.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

INTRODUCTION

The formulae of many non-synthetic bacterial media used today have glucose concentrations in excess of 0.5 percent. These media generally are basically composed of a phosphate buffer and tryptose as a source of nitrogen. It has often been observed that the autoclaving of such media alters the composition in such a way as to have a deleterious effect on subsequent bacterial growth. This phenomenon has been referred to as the Maillard reaction, browning reaction, or caramelization and has been recognized as being extremely important in nearly all industries dealing with materials of a biological nature.

The present investigation describes studies involving the use of various concentrations of glucose as affecting (1) the growth of Streptococcus faecalis in a non-synthetic medium, (2) the oxygen uptake of the organism, and (3) the browning and pH of the medium.

Monod,[1] using a synthetic medium with mannite as the sole source of carbon and Escherechia coli as the test organism, showed that the total growth is a linear function of the concentration of the mannite, as long as the concentration does not exceed 0.5 percent.

In 1912 Maillard[2] demonstrated that solutions containing amino acids turned brown when heated with glucose. Hill

and Patton[3] suggested that optimal growth may not be obtained in microbiological assays due to the effects of the Maillard reaction. Using a casein hydrolysate medium prepared for tryptophane assay and using Streptococcus faecalis R as the test organism, they showed that better growth could be obtained in a medium in which the glucose had been autoclaved separately and added aseptically to the previously cooled base medium. Identical growth also resulted when sucrose, a non-reducing sugar, was substituted for glucose. Patton and Hill[4] found that a solution containing known amounts of L-tryptophane and D-glucose heated at pH 10 showed more browning but less tryptophane loss, as determined by microbiological assay, than a similar solution heated at a lower pH for a longer time. They were of the opinion that the decrease in growth was not due to formation of growth inhibitors as products of the browning reaction but to actual destruction of part of the tryptophane, and that nutrients of both a vitamin and amino acid nature may be damaged during autoclaving with glucose.

Stevens and McGinnis[5] pointed out that the decreased nutritive value of overheated soy bean oil meal for chicks was almost fully corrected by adding supplementary methionine and lysine. These amino acids when added to properly heated soy bean oil meal, however, did not improve the growth of

chicks. Lysine autoclaved for four hours at 120 C supplemented overheated soy bean oil meal for chick growth as effectively as unheated lysine.

Chen, Medler, and Harte[6] found that highly flourescent substances were formed from certain amino acids when heated with Deniges reagent (para-formaldehyde and sulfuric acid). Graham, Hsu and McGinnis[7] showed that flourescent substances were formed when glucose was used to furnish the aldehyde group instead of Denige's reagent. All fifteen amino acids tested showed flourescence. These investigators concluded that brown color formation, flourescence, and decreased amino nitrogen are associated with the destruction of methionine brought about by autoclaving in the presence of glucose.

On the other hand several investigators have indicated that the heating of a medium in the presence of glucose is essential for maximum growth of certain organisms. Orla-Jensen[8] reported this phenomenon after studying various streptococci and lactobacilli. Smiley, Niven and Sherman,[9] working with a synthetic medium for Streptococcus salivarius, found that if glucose was added aseptically to the medium after autoclaving, growth was greatly delayed or never initiated. It was suggested that a hydrogen acceptor was required in the initial carbohydrate metabolism. If such an acceptor were not present, carbohydrate metabolism might

be blocked. Evidently the breakdown of glucose supplied the necessary hydrogen acceptor.

Evans[10] reported that glucose breaks down into many compounds when oxidized under alkaline conditions. Products of carbohydrate metabolism such as pyruvic and lactic acids as well as acetaldehyde were among the products which appeared as the result of the alkaline breakdown.

Englis and Hanahan[11] demonstrated that phosphate plays an active part in initiating various changes in glucose on autoclaving. Not only was the glucose altered but a distinct discoloration of the solution occurred.

Dubos and Davis[12] reported that autoclaved glucose gave rise to toxic caramelization products which inhibit the growth of small inocula of tubercle bacilli.

EXPERIMENTAL

In experiments dealing with the effect of heat on a medium, it is important to include not only the time and temperature of sterilization but the approximate total time the medium is subjected to heat. The autoclave used in these experiments was a 17" x 26" single wall type made by the American Sterilizer Company and was in perfect working order. The time required for the temperature to reach 121 C from room temperature was six minutes. The time of sterilization was measured as soon as 121 C was reached. Eleven minutes elapsed after the period of sterilization before the medium was removed from the autoclave.

The term, "joint sterilization", is used frequently in this thesis. It is a term adopted from Orla-Jensen[8] to mean that the sugar was autoclaved in the presence of the other constituents of the medium.

The base medium used in the following experiments, unless otherwise stated, consisted of:

Bacto-Tryptose	1.5%
K_2HPO_4	0.27%
KH_2PO_4	0.27%

The organism used was Streptococcus faecalis B 33 A. The temperature of incubation in all cases was 37 C.

Preliminary Studies

The ingredients of the medium including glucose were autoclaved separately and in various combinations in 100 ml amounts in 250 ml Erlenmeyer flasks at 121 C for 10 minutes. The hydrogen ion concentration was measured before and after autoclaving using a Beckmann Model G pH meter. The amount of discoloration was measured with a Beckmann Model B. Spectrophotometer using a wave length of 610 millimicrons. Table I. The amount of glucose, when used, was 2 percent.

The results (Table I) indicate that a solution of pure glucose showed a little, if any, discoloration after autoclaving. This is in agreement with the results of many workers. The formation of small amounts of acid lowered the pH considerably. A solution of 1.5 percent tryptose remained relatively stable as to pH and discoloration. A combination of the phosphate buffer and glucose was stable as to pH but showed very slight discoloration. A solution of glucose and tryptose showed a significant drop in pH but almost the same discoloration as the tryptose alone or the solution of glucose and buffer. Considerable discoloration was evident in the glucose, buffer, tryptose solution.

Growth Studies

Growth curves obtained by using the minimal inoculum technique were employed to determine the effect of the

TABLE I

pH AND LIGHT TRANSMISSION READINGS (610 MILLIMICRONS) OF THE CONSTITUENTS AND VARIOUS COMBINATIONS OF THE CONSTITUENTS OF THE BASE MEDIUM AND GLUCOSE BEFORE AND AFTER AUTOCLAVING AT 121C FOR 10 MINUTES

Solution	pH Before Autoclaving	pH After Autoclaving	% Light Trans. Before Autoclaving	% Light Trans. After Autoclaving
Distilled Water	6.55	6.55	100.	100.
Buffer (Phosphate)	6.75	6.75	--	--
Glucose	6.30	4.50	99.	99.5
Tryptose	7.10	7.05	93.	90.
Buffer + Glucose	6.75	6.74	99.5	96.
Buffer + Tryptose	6.75	6.75	92.	91.
Glucose + Tryptose	6.98	6.68	92.	89.
Glucose + Buffer + Tryptose	6.74	6.58	92.	84.

browning reaction on the growth of S. faecalis when various concentrations of glucose were used. Ninety-nine ml of the base medium together with the glucose were placed in 250 ml Erlenmeyer flasks. In each case the pH and light transmission readings of the medium were recorded before autoclaving at 121 C for 7 minutes and again after the medium had cooled. A wave length of 610 millimicrons was used for the light transmission readings. The pH of the medium was not adjusted after autoclaving. The results in Table II show that the higher the glucose concentration the greater the drop in pH and the darker the medium.

Two drops of a 16-hour culture were placed in a dilution bottle containing 99 ml of sterile physiological saline. Four-tenths ml of this suspension was pipetted into a second 99 ml saline dilution bottle and from the latter one ml was transferred into each flask containing the medium.

The seedings of each culture were between 49 and 56 organisms per ml. Duplicate plates were made from each sample at the time of seeding and after 2, 4 and 6 hours of incubation. All plates were incubated 24 hours. Table III.

From the results shown in Table III, it was not possible to ascertain whether the decrease in growth in the higher concentration of glucose resulted from the browning or Maillard reaction or from the acidity produced from the breakdown of glucose by the autoclaving process.

TABLE II

pH AND LIGHT TRANSMISSION READINGS OF THE MEDIUM CONTAINING GLUCOSE

BEFORE AND AFTER AUTOCLAVING AT 121C FOR 7 MINUTES

% Glucose in Base Medium	pH Before Autoclaving	pH After Autoclaving	% Light Transmission Before Autoclaving	% Light Transmission After Autoclaving
0	6.81	6.81	100.	100.
0.025	6.81	6.81	99.0	98.8
0.25	6.81	6.81	98.0	97.8
0.5	6.81	8.79	97.5	94.6
1.5	6.81	6.71	94.5	91.0
3.0	6.76	6.58	94.3	85.5
5.0	6.73	6.48	93.0	83.3

TABLE III

THE GROWTH OF S. FAECALIS FROM MINIMAL NUMBERS IN MEDIA AUTOCLAVED IN THE PRESENCE OF VARYING CONCENTRATIONS OF GLUCOSE.

pH NOT ADJUSTED AFTER AUTOCLAVING

Hours of Incubation	Number of Bacteria per ml					
	0% Glucose	0.025% Glucose	0.5% Glucose	1.5% Glucose	3% Glucose	5% Glucose
0	54	47	54	50	49	56
2	150	600	525	525	375	350
4	1900	9600	9900	9200	5900	4900
6	26500	285500	302000	221500	148000	89500

A second growth curve was determined using a procedure identical to the first with the exception that after autoclaving, the medium in all flasks was adjusted aseptically to pH 6.8. For each concentration of glucose autoclaved jointly with the medium, a control was used containing glucose sterilized by filtration. A 40 percent solution of glucose was filtered through a Seitz sterilizing filter. Care was taken to maintain a constant volume of 100 ml in all flasks. Results of this experiment up to an incubation time of six hours are shown in Table IV. The flasks were allowed to incubate beyond the six-hour sampling period and were watched for the first signs of visible turbidity. Turbidity occurred in all flasks at the same time (9 1/2 hours). Since certain inherent errors are present in the plate count method when determining large numbers of cells, light transmission readings were taken with the spectrophotometer. To avoid the error introduced by various color densities in the autoclaved medium, a standard consisting of a sample of the uninoculated medium for each glucose concentration was used. The reading for each sample was adjusted to 100 percent light transmission. The turbid samples were diluted ten times for greater accuracy. Table V.

Since phosphate, according to Englis and Hanahan,[11] catalyzes the breakdown of glucose in the presence of heat,

TABLE IV

THE GROWTH OF S. FAECALIS FROM MINIMAL NUMBERS IN A MEDIUM AUTOCLAVED JOINTLY WITH VARYING CONCENTRATIONS OF GLUCOSE AND IN A MEDIUM IN WHICH FILTERED GLUCOSE WAS USED*

Time in Hours	0%	0.025%		0.25%		0.5%		1.5%		3%		5%	
		A*	F*	A*	F*	A*	F*	A*	F*	A*	F*	A*	F*
0	45	41	39	40	42	49	46	44	40	41	40	42	38
2	45	175	180	120	200	200	250	200	190	230	190	180	190
4	1000	6000	3300	5000	4900	4700	4600	5300	6000	4400	5300	4500	5000
6	13000	120000	140000	150000	170000	190000	150000	160000	140000	130000	140000	150000	140000

* A = Autoclaved glucose
F = Filtered glucose

TABLE V

PERCENT LIGHT TRANSMISSION READINGS AFTER 9 1/2 HOURS OF INCUBATION OF S. FAECALIS IN MEDIA AUTOCLAVED WITH AND WITHOUT GLUCOSE

% Glucose in Medium	Percent Light Transmission	
	Glucose Autoclaved with Medium	Glucose Added After Autoclaving Medium
0	99.1	99.1
0.025	98.4	97.4
0.25	98.1	97.9
0.5	97.5	98.8
1.5	99.0	98.0
3.	99.6	98.8
5.	99.0	99.1

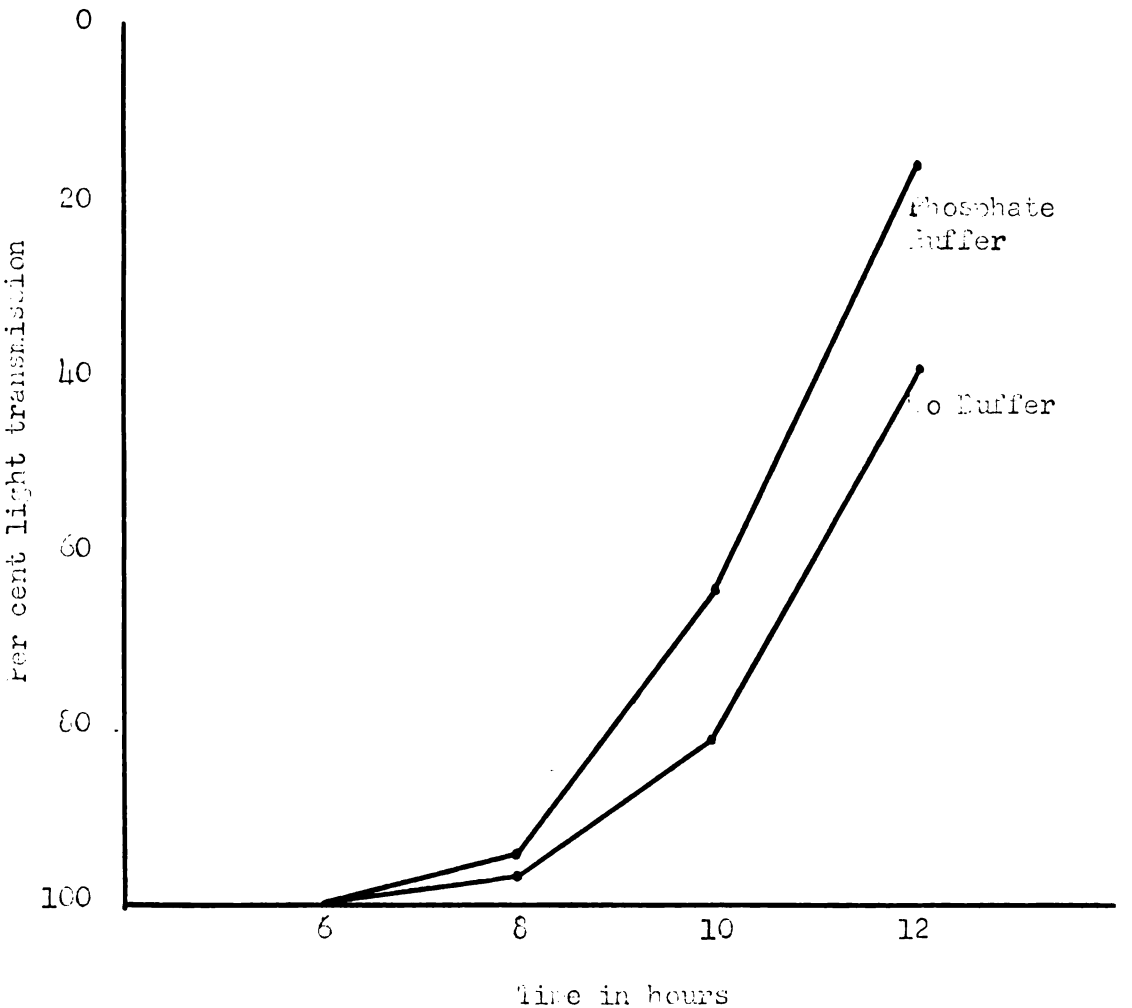
it seemed reasonable to determine whether or not the absence of the phosphate buffer would affect cell numbers.

The medium consisted of tryptose 1.5 percent, and glucose 2 percent, and distilled water. The intact base medium with 2 percent glucose was used as a control. The sugar was sterilized by Seitz filtration and was added after the medium had been autoclaved for 10 minutes at 121 C. The pH of the control was adjusted to pH 7 after autoclaving. The inoculations of the 100 ml samples were carried out as previously described. Light transmission readings were made with a Cenco-Sheard-Sanford Photolometer with a No. 25 Kodak Wratten Filter. The results presented in Graph 1 show that the absence of the phosphate buffer from the base medium caused a considerable decrease in growth even at ten and twelve hours. The hydrogen ion concentration was measured throughout the growth period (Graph 2). The buffering action of the phosphate medium remained intact until the tenth hour of incubation. The unbuffered medium had a lower pH at all times.

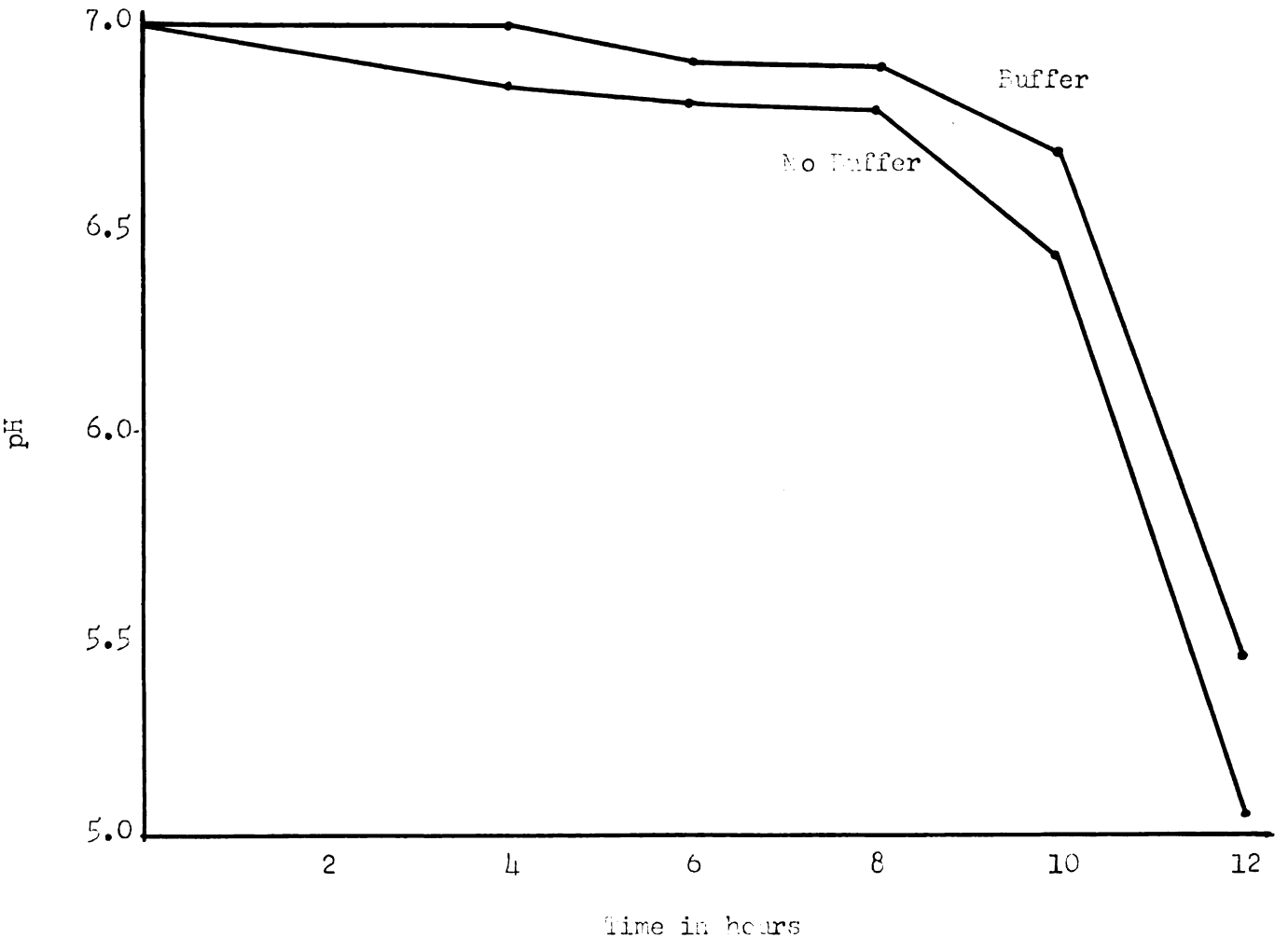
Oxygen Uptake Studies

The oxygen uptake of resting cells of Sarcina lutea in glucose concentrations of 0.2 to 0.5 percent fell along a similar curve according to Gerard.[13] To determine if the

Graph 1. Growth of S. faecalis in phosphate buffered medium (control) and in non buffered medium



Graph 2. The pH of medium without buffer and of buffered control at various incubation times of *S. faecalis*.



same were true of S. faecalis, but using a greater range of concentrations, the oxygen consumption was measured with the Warburg apparatus. The cells were grown in Brain Heart Infusion Broth for 18 hours. The organisms were harvested by centrifugation, washed three times with physiological saline and stored in the refrigerator until used. The concentration of the cells was adjusted so that a ten-fold dilution would allow a light transmission reading of 7 percent in the Lumetron apparatus with a 660 millimicron filter.

The Warburg flasks at the start of the determination contained the following:

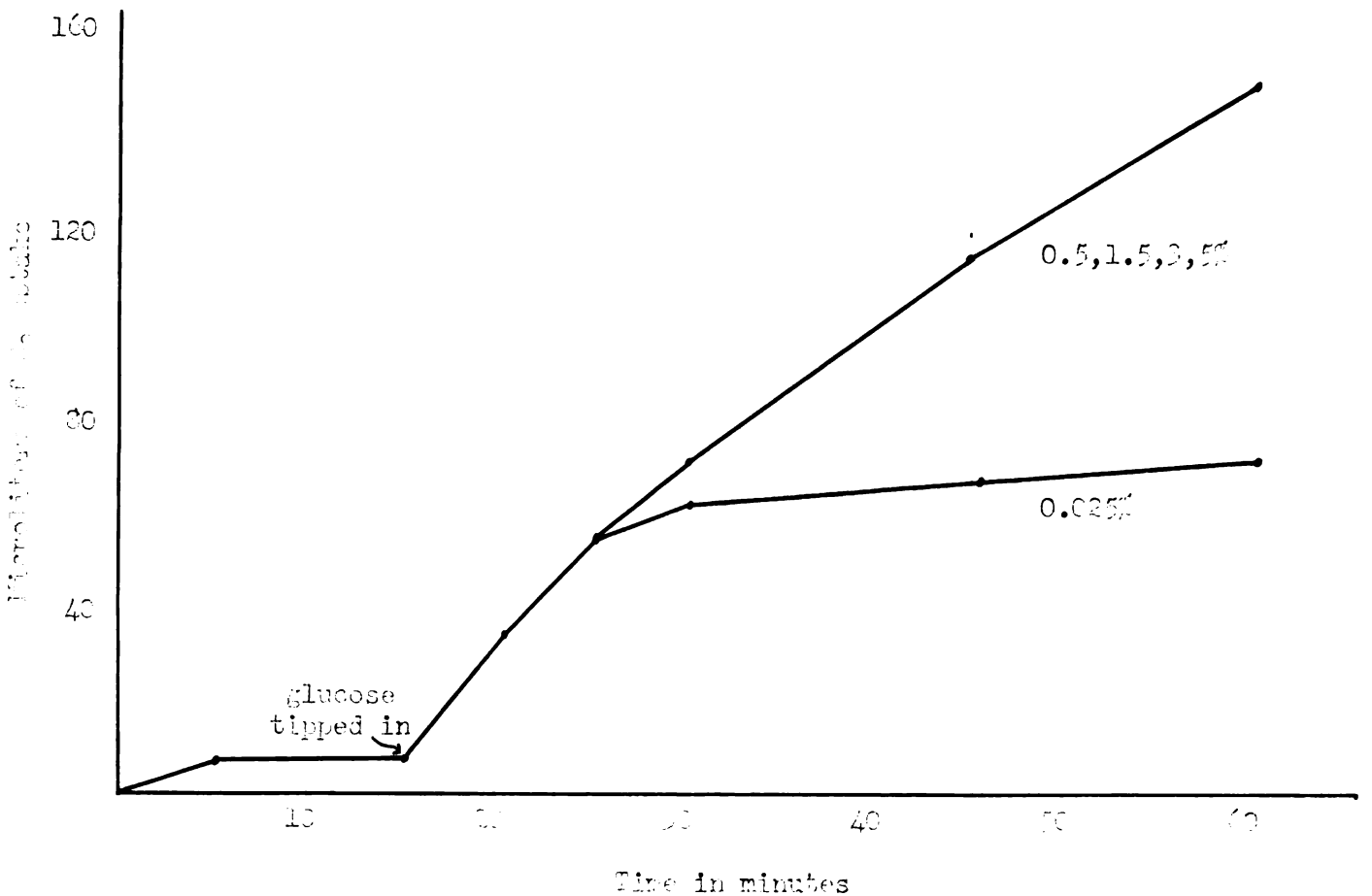
1 ml Sorenson's Buffer pH 7.0
0.5 ml Saline cell suspension
0.5 ml Glucose solution in sidearm
0.2 ml 10 percent KOH in center well

The temperature of the water bath measured 36.5 C. Endogenous respiration was measured up to 15 minutes at which time the contents of the sidearm were tipped in and the oxygen uptake of the glucose system measured. The results presented in Graph 3 show that the oxygen uptake was the same for all concentrations of glucose studied with the exception of 0.025 percent.

Growth of S. faecalis in Glucose Degradation Products

In view of the findings of Smiley, Niven and Sherman[9] pertaining to degraded glucose an attempt was made to

Graph 3. Oxygen uptake of *S. faecalis* in varying concentrations of glucose at 36.5 C



determine the effect of small quantities of these products on the growth of S. faecalis in the non-synthetic medium.

Degradated glucose was prepared in a manner described by Smiley, Niven and Sherman[9] whereby a N/10 solution of NaOH containing 10 percent glucose was autoclaved at 121 C for 20 minutes. The deep colored solution which had become acid was neutralized with NaOH. The concentration of the degraded glucose used in the base medium was 0.25 percent. Samples containing the degradation products autoclaved with the base medium as well samples to which these products were added after sterilization were included. A comparison was made between these media and media containing glucose in the same concentration. Growth curve procedures were as previously outlined. Table VI shows that the organisms can utilize the degradated glucose as well as the intact glucose molecule. Likewise the autoclaving of the degradated glucose with the medium had no effect on growth.

TABLE VI

GROWTH OF S. FAECALIS FROM MINIMAL NUMBERS IN A MEDIUM CONTAINING 0.25 PERCENT GLUCOSE DEGRADATION PRODUCTS AS COMPARED TO GROWTH IN A MEDIUM CONTAINING 0.25 PERCENT GLUCOSE

Hours of Incubation	Number of Bacteria per Ml			
	Filtered Glucose Added After Autoclaving Medium	Glucose Autoclaved With Medium	Degradated Glucose Added After Autoclaving Medium	Degradated Glucose Autoclaved With Medium
0	59	60	51	53
2	160	210	240	360
4	15,000	18,000	15,000	18,000

DISCUSSION

The results in Table I show that a combination of the phosphate buffer and glucose was stable as to pH but showed slight discoloration. This is in keeping with the work of Englis and Hanahan,[11] although they reported more discoloration. This may be attributed to the fact that they used a higher concentration of phosphate and a longer heating period. Englis and Hanahan[11] showed that phosphate is a factor in glucose breakdown and discoloration during heating.

A reaction between glucose, phosphate buffer and tryptose seemed to enhance browning. Before an explanation of this phenomenon could be attempted, a considerable number of chemical analyses would have to be undertaken. There was no notice of a direct or inverse relationship between drop in pH and the extent of browning.

Many laboratories are in the habit of raising the pH of a medium above the specified level with the result that after autoclaving the desired level is reached. This practice eliminates sterilizing the ingredients separately or adjusting the pH aseptically after autoclaving. Usually the medium becomes darker. Obviously, if a medium drops in pH and becomes discolored, it is reasonable to assume that some ingredient or ingredients have been altered. In many

cases this is not important, for growth is affected little, if any. However, in physiological studies and in differential media, especially those depending on a carbohydrate as the differentiating substance, it is of the utmost importance. Table I shows that a solution of buffer and tryptose remains stable after autoclaving as to pH and discoloration. If glucose sterilized by filtration was added to this autoclaved solution, the resulting medium would have, for the most part, the same composition as before sterilization. If the medium was refrigerated until used, the glucose would be present as glucose and not as the breakdown products of glucose. The practice of adding sterile carbohydrate to an autoclaved medium to prevent breakdown is not new. The results presented here serve only to strengthen that argument.

The fact that joint sterilization of various concentrations of sugar affects pH and discoloration of the medium is shown in Table II. The mechanism of browning is complicated and, as yet, is not clearly understood. It has been postulated that the hydrogen ion concentration increases because of a combination of the aldehyde groups of the carbohydrate and the amino groups of the nitrogenous substance which results in setting free a corresponding amount of carboxyl groups.

Table III represents the results of growth measurements in a medium in which the pH was not adjusted after autoclaving. There was a marked decrease at four and six hours in the total number of viable cells in the media that contained the higher concentrations of glucose. When the pH was adjusted after autoclaving so that all media were the same (pH 6.85), the growth in all concentrations of glucose was the same. Table IV. Likewise there was no difference in growth between the medium autoclaved jointly with glucose and the medium autoclaved separately. At the time of turbidity the bacterial density was significantly the same for all glucose concentrations as shown by Table V. The base medium without glucose showed less growth at six hours but was comparable to the media containing glucose at 9.5 hours indicating the presence of an available carbon source in the tryptose; but in all probability, it was not as readily available as glucose, at least during the lag phase.

Two facts are obvious: (1) the products of the browning reaction are not toxic but the difference in growth in the media not adjusted as to pH was due to a higher initial acidity as a result of heating the glucose and base medium together, and (2) low concentrations of glucose, much lower than recommended for most non-synthetic media, will support the growth of S. faecalis up to the time of turbidity as efficiently as the higher concentrations.

Monod[1] found that a straight line relationship existed between total cell numbers of E. coli and the concentration of glucose used if that concentration was below 0.5 percent. He stressed the fact that this relationship would not hold unless there was but one source of carbon. It is presumed that his counts were made at 24 hours. The longest incubation time of the experiments described in this paper was 12 hours. It is the opinion of many investigators that the lag phase is the phase most affected by a medium. By using a minimal inoculum, the marginal differences in growth rates in the various media are increased.

The absence of the phosphate buffer from the base medium caused a considerable decrease in growth even at ten hours as shown in Graph 1. The results presented in Graph 2 show that the buffer reaches its maximum at about twelve hours after which the pH drops rapidly. It is evident that the non-buffered medium has some buffering capacity although not as great as the phosphate buffered medium, especially at ten hours.

Since there is greater growth in the buffered medium, even at seven hours it would seem that the increase in growth must be explained on some other ground than a mere neutralization of the active hydrogen ions. The results of Mallmann and Gallo[14] proved this point. They used

representative organisms from many groups and showed that the addition of potassium or sodium phosphate to nutrient media enhanced physiological activity by playing a part in the direct metabolism of the organism tested.

Slanetz and Rettger[15] observed that when a solution of K_2HPO_4 and NaH_2PO_4 was added to a plain peptone medium, so that the concentration was 1 percent, and inoculated with various members of the Enteric group, it exerted a regulatory effect on the hydrogen ion concentration, but in the same medium to which glucose had been added the buffer exerted little check on the pH drop. They obviously failed to realize that the limit of the buffer was probably reached, due to the acid from glucose, before their first reading at 24 hours was taken.

Darby and Mallmann[16] found that when 0.4 percent K_2HPO_4 and 0.15 percent KH_2PO_4 were employed in a lactose-tryptose medium for *E. coli* 161, the buffer caused a much greater growth in the late logarithmic phase and a slightly greater increase during the lag phase.

The results of the oxygen uptake studies on *S. faecalis* are similar to those of Gerard[13] on *S. lutea*. The oxygen uptake of the resting cells is not dependent on concentration of glucose. The system may be regarded as an enzyme versus glucose reaction. The curves representing the various

sugar concentrations fell on the same position on the graph until about 25 minutes when the curve representing 0.025 percent glucose returned to the endogenous rate. It is assumed that the available glucose had been utilized. There is, then, at least within the range of concentrations studied, no threshold concentration needed for respiration. Had there been a noticeable difference in oxygen consumption with the various concentrations of glucose it might be reasonable to suspect a similar difference in multiplying cells, however the fact that the oxygen uptake was the same for the four highest concentrations does not necessarily indicate that multiplying cells would be affected to the same degree.

Since Smiley, Niven and Sherman[9] noticed that jointly autoclaved glucose was necessary for the growth of S. salivarius but that the alkaline degradated glucose initiated growth equally as well, it was a matter of interest to learn whether degradated glucose autoclaved jointly or separately would affect the growth of S. faecalis in the non-synthetic base medium used in these studies. Since there seemed to be no difference in growth in these experiments, it remains to make analogous studies using a synthetic medium.

CONCLUSION

1. A combination of glucose, phosphate buffer and tryptose in solution was discolored when sterilized, to a greater degree than other combinations of the ingredients of the medium used.

2. Increase in glucose concentration caused an increase in browning and an increase in active hydrogen ion concentration in the autoclaved medium.

3. The products of the browning reaction were not toxic in the non-synthetic medium used. If the pH of the glucose containing medium was adjusted after sterilization, equal growth was obtained in all concentrations of glucose used. Likewise equal growth resulted in the medium autoclaved in the presence of glucose and in the medium to which sterile glucose was added after autoclaving, if the above consideration was met.

4. Greater growth was obtained in the phosphate buffered medium than in the non-buffered medium. It is suggested that this is due to an enhancement of metabolic activity as well as to a buffering effect.

5. Oxygen uptake of the resting cells was identical in all but the lowest concentration of glucose used. This concentration affected O_2 uptake the same as the higher concentrations until it was completely utilized.

6. Alkaline degradation products of glucose in the low concentration used initiate growth as well as intact glucose.

7. A recommendation for the use of 0.25 to 0.5 percent glucose in the described non-synthetic medium is given.

BIBLIOGRAPHY

1. Monod, J. 1941. Academie des Sciences, Comptes Rendus, 212, 771-773.

2. Maillard, L. C. 1912. Academie des Sciences, Comptes Rendus, 154, 66.

3. Hill, E. G., and Patton, A. R. 1947. Science, 105. 481.

4. Patton, A. R., and Hill, E. G. 1948. Science, 107, 68.

5. Stevens, J. M., and McGinnis, James. 1947. J. Biol. Chem., 171, 431.

6. Chen, J. L., Medler, J. D., and Harte, R. A. 1948. J. Amer. Chem. Soc., 70, 3145.

7. Graham, W. D., Hsu, P. Y., and McGinnis, J. 1949. Science, 110, 217.

8. Orla-Jensen, A. D. 1933. J. Soc. Chem. Ind., 52, 374-379.

9. Smiley, K. L., Niven, Jr., C. F., and Sherman, J. M. 1943. J. of Bact., 45, 445-453.

10. Evans, Wm. Lloyd. 1929. Chem. Revs., 6, 281-315.

11. Englis, D. T., and Hanahan, D. J. 1945. Jour. Am. Chem., 67, 51-54.

12. Dubos, R. J., and Davis, B. D. 1946. Jour. Expt'l. Med., 83, 409-423.

13. Gerard, R. W., and Falk, I. S. 1931. Biol. Bull. Marine Biol. Lab., 60(3), 213-226.

14. Mallmann, W. L., and Gallo, F.. 1930. Mich. Acad. Science, 14, 617-639.

15. Slanetz, C. A., and Rettger, L. F. 1928. J. of Bact. 15, 297-317.

16. Darby, C. W., and Mallmann, W. L. 1939. J. of Amer. Water Works, 31, 689-706.