THE PHYSIOLOGY OF THE GENUS BACILLUS

A Study of the Metabolism of Certain Members of the Genus *Bacillus* in Relation to Their Ability to Ferment Glucose, d- and l-Arabinose.

by

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TABLE OF CONTENTS

	Page No.
Introduction	1
Object of the Investigation	6
Experimental Procedure	8
Results of the Investigation	20
Discussion of the Results	151
Interpretation of graphical and tabular data	
Discussion of results on glucose	
Discussion of results on l-arabinose	
Discussion of results on d-arabinose	
General discussion of the results on sugars	
Summary	174
References	179

LIST OF TABLES

TABLE NO.		PAGE NO.
I	The various members of the genus <u>Bacillus</u> employed listed according to source, strain and incubation temperature - -	9
II	A summary of the accuracy of the daily carbohydrate control recoveries -	16
III	Comparison of pH produced and carbohydrate utilized by the members of the genus <u>Bacillus</u> - - - - - - - - - - - - - - -	21
IV	Interpretation of pH data from three sources to indicate dissimilation of glucose, d-arabinose and l-arabinose - -	132
V	A summary of strain reactions as they appear to be of value for species identification in indicating sugar dissimilation. (Summary of pH data in figures 1-130 and Tables III and IV.) -	148
VI	A grouping of the genus <u>Bacillus</u> according to the extent of glucose utilized and pH produced in glucose-peptone media	153
VII	A summary of the action of the <u>Bacilli</u> in l-arabinose peptone medium: pH compared to pH produced in peptone medium -	164
VIII	A summary of the metabolic activity of the genus <u>Bacillus</u> in d-arabinose media - - - - - - - - - - - - - - - - - -	168

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INTRODUCTION

The term genus Bacillus is applied to a rather large number of diverse species of bacterial organisms whose natural habitat appears to be the soil. The most outstanding characteristics of this group, so far as is known, are the ability to produce endospores and grow in the presence of oxygen, this latter characteristic differentiating it from the anaerobic spore-forming Clostridia.

Cohn (1875) was the first taxonomist to include the genus Bacillus in his classification of the small number of microorganisms known at that time (2). He, like all other early investigators until the time of Pasteur, was primarily a botanist. Using morphological characteristics as the sole basis of relationship between microorganisms, he introduced a crude taxonomic nomenclature which continues to be manifest in present day attempts to differentiate the members of this genus.

The fact that bacteria in general are, physiologically speaking, chemical entities had its inception with the works of Pasteur. Since chemical techniques were poor and means for their development slow, it was not until 1904, with the work of Chester (3), that any physiological methods for the differentiation of the members of the genus Bacillus were proposed.

It has been difficult for bacterial taxonomists to complete a rational system of differentiation for described members of the genus

Bacillus because of the inadequacy of the data. Complete descriptions of the morphological, cultural, serological, and physiological characteristics are lacking (1). Such characteristics as pigment production, size and location of endospores, size of bacterium, methods of spore germination, and colonial morphology are inadequate to describe and differentiate members of this large group of normal soil flora. The variations of these characteristics with constantly changing environmental conditions, whether natural or artificial, are too well known to require further amplification. As a result of the lack of adequate data, Bergey's Manual of Determinative Bacteriology (1) has necessarily based the differentiation of the members of the genus Bacillus primarily upon the aforementioned characteristics, fully realizing the limitations of such a taxonomic procedure.

Morphological and cultural descriptions of the Bacilli have probably received more attention than the other necessary criteria. The literature pertaining to these characteristics is quite large and beyond the scope of this survey. Lamanna (20, 21) has recently begun investigations concerned with the serology of the so-called "small" and "large" celled species. His data are too incomplete to be of much value at the present time.

Only a few investigators (4, 6, 9, 18, 19, 34, 37-42) have attempted fundamental physiological studies of the Bacilli and for this reason such physiological data are incomplete. With few exceptions (4, 9, 37-39) none of these has attempted a study of this group as a whole but have confined attention to widely separate species: B. rotans (34), B. subtilis (6, 19, 41), B. cereus (42), *B. megatherium* (19); or to special groups such as those which attack calcium n-butyrate, or cellulose and xylan (1).

The application and use of physiological characteristics as applied to the classification of the genus *Bacillus* has received attention a seemingly haphazard sort of way. This statement is justified on the basis of the fact that fundamental studies on the group as a whole have been lacking. Probably the most noteworthy published basis for this fact is manifest in the work of de Soriano (37-39) in an extensive attempt to classify more than one-half of the *Bacilli* on the basis of morphological and physiological descriptions in the literature. Finding descriptions inadequate, de Soriano carried out extensive physiological investigations of this genus in an attempt to establish reliable criteria of distinction.

In an attempt to clarify some of the physiological discrepancies existing in the literature to date, Fitzgerald (9) began a series of studies which led to the fundamental investigations of Coffee (4) on the physiology of this group. In general the works of these investigators confirm the following statements:

1. Cellular and colonial morphology must regress to a less important position in the differentiation of members of the genus *Bacillus*.
2. A number of reactions such as nitrate reduction and acetyl-methyl-carbinol production vary to such an extent within species that they maintain little taxonomic significance.
3. So few species of *Bacilli* fail to liquefy gelatin that this criterion is of value only when considered with other differentiating characteristics.

4. The effect of the organism on peptone, tryptone, and proteose-peptone is such that reliable observations of the activity of the organisms toward carbohydrate media cannot be made without numerous precautions. Since the necessity of these precautions is often unknown to the routine bacteriological worker, any reactions he may obtain are questionable.
5. Some indicator solutions, such as Andrades, employed routinely in bacteriological laboratories require too low a pH before color changes are detectable, and are not sensitive enough to detect acid production by certain of the Bacilli. Some of these indicator solutions appear to possess a bacteriostatic effect.
6. Bacilli differ in their ability to ferment d- and l- forms of a single sugar. This observation probably accounts for discrepancies reported by various authors concerning the ability of an organism to ferment a particular sugar.
7. The present method of classification of the genus Bacillus employed by Bergey's Manual (1), which is based primarily on cultural and morphological characteristics and the physiological distinctions proposed by de Soriano (37-39), is shown to be inadequate without further physiological description.

The investigations of de Soriano (37-39) are apparently the first extensive physiological studies of the genus Bacillus. In spite of the many excellent contributions of her investigations, they might be criticized (4) because Andrades indicator was employed to detect the production of acid from carbohydrate. Coffee (4) employed brom-thymol-blue and brom-cresol-purple in his investigations of the fermentability of 32 carbohydrates. He points out that discrepancy between results obtained by him, and those of de Soriano (37-39), might be due to his use of acid-indicators, which exhibit distinct color change per 0.2 pH unit from pH 5.2 - 7.6, while Andrades indicator shows no color change from pH 5.4 - 7.5.

Numerous investigators (4-7, 10-12, 16, 22, 29-32, 34, 45, 46) have demonstrated that although used routinely by bacteriologists, acid-indicators are inadequate to determine the utilization of carbohydrate from a carbohydrate-peptone medium by many organisms. This failure may often be due to a lack of "sparing action" of carbohydrate for the peptone of the medium, which sometimes results in an alkaline or neutral pH reaction, even though carbohydrate has been utilized.

A lack of "sparing action" of carbohydrate for peptone has been demonstrated in studies which compared the pH produced in a carbohydrate-peptone medium with the utilizability of the carbohydrate for the following organisms: B. rotans (34), the genus Mycobacterium (28), the tubercle bacillus (14), the hydrocarbon bacteria (15), the Brucella (25, 26, 49) and certain miscellaneous organisms (45).

Quantitative investigations such as these have not been made for many members of the genus Bacillus. If they were carried out, at least part of the existing taxonomic confusion might be eliminated, and knowledge of the physiology of the Bacilli, in general, would be expanded.

OBJECT OF THE INVESTIGATION

It is routine bacteriological procedure to consider an acidic pH reading, taken on a carbohydrate-peptone medium in which an organism is growing, as evidence that the bacterium in question utilizes the carbohydrate. In a carbohydrate-peptone medium an organism will utilize the carbohydrate in respiration as an energy source. The peptone is attacked as a source of growth constituents. Peptone is utilized as an energy food only in the absence of carbohydrate by most microorganisms. This mechanism is referred to by bacteriologists as the "sparing action" of carbohydrate for peptone. It has been shown (29, 31, 32) that a lack of "sparing action" of carbohydrate for peptone by species of the genus Bacillus makes it difficult and sometimes impossible to determine the utilization of various carbohydrates as measured by a lowering of the pH. The resulting pH of the medium, when there is no "sparing action", is dependent not only upon acid produced from carbohydrate dissimilation, but also upon concurrent acid and ammonia produced from peptone decomposition.

The object of the present investigation was to determine quantitatively the extent of glucose and d- and l- arabinose utilization in order to separate it from the other two factors influencing the pH change in the medium. The pH of the carbohydrate media employed was determined potentiometrically, using a glass electrode in order that the pH produced could be compared with the extent of utilization of these carbohydrates. Likewise, the pH produced by the same Bacilli in peptone alone was determined so that it could be compared with the pH produced in the carbohydrate-peptone media.

The fundamental observations concerned with the metabolism of the genus Bacillus (which may be derived from this study) may be of value in the taxonomic identification of various members of this group of organisms, and might lead to other interesting conclusions regarding the metabolism of these organisms in pure laboratory culture and also in mixed cultures in the natural habitat.

EXPERIMENTAL PROCEDURE

One hundred and thirty strains of the genus Bacillus, representing 41 species, were studied in these investigations. These strains and species are listed in Table I together with some indication of the source of each culture, where known.

Stock cultures of these organisms were propagated on Difco Stock Culture Agar and stored at approximately 3°C. when not in use. Before any strain of Bacillus was inoculated into the test medium it was first transferred five consecutive times on plain (sugar free) nutrient agar, pH 6.8-7.0. This step is considered necessary in most bacteriological work to insure that the culture's metabolism is normal.

Determination of pH was made by using a Beckman potentiometer equipped with a glass electrode. These hydrogen ion observations were made on individual strains in each of the following media at intervals of 1, 2, 3, and 7 days of incubation.

1. 1% peptone broth (Difco), pH 6.8-7.0.
2. 0.5% glucose--1% peptone broth (Baker C. P. grade glucose), pH 6.8-7.0.
3. 0.5% d-arabinose--1% peptone broth (Pfanstiehl d-arabinose), pH 6.8-7.0.
4. 0.5% l-arabinose--1% peptone broth (Pfanstiehl l-arabinose), pH 6.8-7.0.

TABLE I

The members of the genus Bacillus employed in this investigation are listed in the following table according to species, strain, source, and incubation temperature.

B. adhaerens A. T. C.	30°C.
B. agri U. S. D. A.	30°C.
B. albolactis V. P. I.	30°C.
B. albolactis Tex.	30°C.
B. alvei A. T. C.	37°C.
B. alvei Tex.	37°C.
B. alvei #622 Tex.	37°C.
B. alvei #622 Lockheed, Tex.	37°C.
B. anthracis Wis.	37°C.
B. anthracis Tex.	37°C.
B. anthracis (Wis.) Tex.	37°C.
B. anthracis Rall. Tex.	37°C.
B. anthracis (Smooth) Tex.	37°C.
B. aterrimus #353 Wis.	37°C.
B. aterrimus U. S. D. A.	37°C.
B. aterrimus Pesek, Wis.	37°C.
B. aterrimus R. C. Wis.	37°C.
B. brevis #604 A. T. C.	37°C.
B. brevis U. S. D. A.	37°C.
B. cereus #1 A. T. C.	30°C.
B. cereus #2 A. T. C.	30°C.
B. cereus Tex.	30°C.
B. cereus (Stock) Tex.	30°C.

B. cereus (Non-Motile) Tex.	30°C.
B. cereus #244 Tex.	30°C.
B. cereus Clark Tex.	30°C.
B. cereus Neb.	30°C.
B. cereus Smith-Thom Tex.	30°C.
B. cereus Wis.	30°C.
B. cereus A. T. C. Wis.	30°C.
B. circulans A. T. C.	30°C.
B. cohaerens #840 Tex.	37°C.
B. danicus U. S. D. A.	30°C.
B. danicus #1 A. T. C.	30°C.
B. firmus U. S. D. A.	30°C.
B. flexus V. P. I.	37°C.
B. freudenreichii A. T. C.	30°C.
B. fusiformis A. T. C.	35°C. (37°C.)
B. fusiformis Tex.	35°C. (37°C.)
B. fusiformis #339 Tex.	35°C. (37°C.)
B. fusiformis Wright Wis.	35°C. (37°C.)
B. globigii A. T. C.	30°C.
B. globigii #356 Wis.	30°C.
B. graveolens U. S. D. A.	37°C.
B. graveolens #615 Tex.	37°C.
B. graveolens Wright Wis.	37°C.
B. graveolens Neb.	37°C.
B. lacticola Tex.	37°C.
B. lacticola #876 Tex.	37°C.
B. lactic #618 Tex.	37°C.

B. *laterosporus* V. P. I.	30°C.
B. *lautus* V. P. I.	37°C.
B. *macerans* U. S. D. A.	42°C. (37°)
B. *megatherium* U. S. D. A.	37°C.
B. *megatherium* #1 A. T. C.	37°C.
B. *megatherium* #2 A. T. C.	37°C.
B. *megatherium* Tex.	37°C.
B. *megatherium* #240 Tex.	37°C.
B. *megatherium* Wis.	37°C.
B. *megatherium* A. T. C. Wis.	37°C.
B. *megatherium* Neb.	37°C.
B. *mesentericus* A. T. C.	30°C.
B. *mesentericus* U. S. D. A.	37°C.
B. *mesentericus* Tex.	37°C.
B. *mesentericus* A. T. C. Wis.	37°C.
B. *mesentericus* Frost, Wis.	37°C.
B. *mesentericus* Neb.	37°C.
B. *mycoides* #1 V. P. I.	30°C.
B. *mycoides* #101 V. P. I.	30°C.
B. *mycoides* (Beaded) Tex.	30°C.
B. *mycoides* A. T. C. #80 Tex.	30°C.
B. *mycoides* A. 2 Tex.	30°C.
B. *mycoides* A. 6 Tex.	30°C.
B. *mycoides* #23 Tex.	30°C.
B. *mycoides* #65 Tex.	30°C.
B. *mycoides* #405 Tex.	30°C.

B. mycoides #410 Tex.	30°C.
B. mycoides #417 Tex.	30°C.
B. mycoides #420 Tex.	30°C.
B. mycoides #421 Tex.	30°C.
B. mycoides #422 Tex.	30°C.
B. mycoides #425 Tex.	30°C.
B. mycoides #426 Tex.	30°C.
B. mycoides #427 Tex.	30°C.
B. mycoides #430 Tex.	30°C.
B. mycoides Wright, Wis.	
B. mycoides Neb.	30°C.
B. mycoides (left-hand Sarles) Wis.	30°C.
B. niger A. T. C.	30°C.
B. niger U. S. D. A.	30°C.
B. niger #228 Wis.	30°C.
B. niger 'Old' Wis.	30°C.
B. niger 'S' Wis.	30°C.
B. panis A. T. C.	30°C.
B. panis U. S. D. A.	30°C.
B. paraalvei Tex.	30°C.
B. polymyxa V. P. I.	30°C.
B. polymyxa Tex.	37°C.
B. prausnitzii Neb.	37°C.
B. robur #946 Tex.	
B. rotans A. T. C.	22°C.
B. rotans Tex.	22°C.

B. rotans (probably Wis.)	22°C.
B. ruminatus #952 Tex.	37°C.
B. serositides Neb.	37°C.
B. silvaticus #957 Tex.	37°C.
B. simplex Tex.	30°C.
B. simplex #335 Tex.	30°C.
B. sphaericus #348 Tex.	30°C.
B. subtilis #231 A. T. C.	30°C.
B. subtilis Tex.	30°C.
B. subtilis Koch-Novy Tex.	30°C.
B. subtilis Marburg Tex.	30°C.
B. subtilis Gram neg. Tex.	30°C.
B. subtilis #231 Tex.	30°C.
B. subtilis Marburg Wis.	30°C.
B. subtilis Marburg New York	30°C.
B. subtilis Marburg Neb.	30°C.
B. subtilis 'L' Neb.	30°C.
B. subtilis #600 Neb.	30°C.
B. subtilis V. P. I. (Milk)	30°C.
B. tumefaciens Tex.	30°C.
B. tumescens Tex.	37°C.
B. tumescens #995 Tex.	37°C.
B. vulgatus A. T. C.	30°C.
B. vulgatus U. S. D. A.	30°C.
B. vulgatus Neb.	30°C.
B. vulgatus Tex.	30°C.
B. globigii Wis. (Probably Vogel)	30°C.
Delong strain pectin fermenter, Wis.	30°C.

The results of the hydrogen ion concentrations were recorded to the nearest 0.1 pH unit. (The instrument was capable of greater accuracy but it was felt that readings closer than 0.1 pH units were not reproducible and had no meaning.)

With few exceptions the incubation temperatures employed in this investigation were those which were considered optimum for the growth of the organism. Those strains having an optimum growth temperature of 42°C. were, however, incubated at 37°C.

The utilization of carbohydrate was determined quantitatively according to the method of Stiles, Peterson and Fred (43). This is a direct method for measuring the amount of carbohydrate remaining in an inoculated tube after incubation. It is a modification of the volumetric technique of Schaffer and Hartman. In principle it consists of oxidizing with iodine the cuprous oxide formed by the sugar with cupric sulphate, and titrating the excess iodine with thiosulphate. The iodostarch endpoint is very sensitive and easily observed (43).

Carbohydrate utilization by this method was determined at intervals of 1, 2, 3, and 7 days of incubation to coincide with the time of the pH observations. Sets of 8 tubes of each medium being tested were inoculated with each organism and incubated. A separate tube was employed for each utilization determination and for each pH reading.

Although this method of inoculation necessitated more handling than a sampling procedure, it precluded the possibility of irregular results from contamination and evaporation. The quantitative determinations per tube were made on triplicate samples. All tubes of media were pipetted with an automatic overflow pipette so that each 5 ml. contained 25 mg. of a particular carbohydrate.

Each day that sugar utilization determinations were made upon inoculated tubes of media, similar determinations were made upon incubated, but uninoculated, tubes of the same medium. These control determinations permitted a record of the accuracy of the determinations each day and at the same time served as the basis for calculation of the amount of carbohydrate utilized by an organism at any particular period of incubation.

A summary of the accuracy of the daily control determinations may be found in Table II.

No appreciable difference was noted in the percentage daily recovery of any of the control carbohydrates tested during the 1-7 day incubation period at 30°C. or 37°C. Neither was there any detectable change in the pH of either the glucose of l-arabinose media. A change did take place in the pH of d-arabinose during the 1-7 day incubation period. In general the pH of this medium dropped from the initial value of 6.8-7.0 to a low of 6.5. There was a slight change in the pH of d-arabinose after sterilization at 10 lbs. pressure for 15 minutes. This change became more pronounced during incubation.

TABLE II

A Summary of the Accuracy of the Daily Carbohydrate Control Recoveries

Number Carbohydrate Samples	% Daily Recovery Controls	Number Carbohydrate Samples	% Daily Recovery Controls	Number Carbohydrate Samples	% Daily Recovery Controls
Glucose	103.1	l-Arabinose	104.2	d-Arabinose	81.6
76	98.7	66	102.1	26	86.3
	102.0		104.1		84.8
	102.9		95.9		85.8
	99.0		95.9		80.7
	99.6		97.3		83.1
	99.6		89.7		81.9
	99.3		95.3		85.9
	96.9		99.0		
	100.9		99.0		
	104.0		93.0		
	100.0		100.2		
	101.9		100.7		
	110.6		101.0		
	100.1		100.3		
	98.5		100.1		
	100.9		99.0		
	97.8		97.6		
	97.7				
	95.8				
	98.0				
	98.5				
	90.6				
	87.1				
AVERAGE:	99.3		98.51		83.4

The low percentage recovery of d-arabinose may be due to impurities or to destruction of this carbohydrate during sterilization.

Method of Calculation and Interpretation of Carbohydrate Utilization Data:

The average percentage recovery of the control determinations made on 1, 2, 3, and 7 days of incubation was used as a basis for calculating the extent of carbohydrate utilization. The following tabular form illustrates the manner in which the data representing glucose utilization by *B. albolactis* Tex. were calculated:

CONTROL DETERMINATION (GLUCOSE)

Incubation Period (days)	Total mg. Glucose Det'n. per Tube	Total mg Glucose per Tube by Wt.	% Glucose Recovery	Date Det'n. Made
1	24.68	25.01	98.7	2-25-44
2	24.92	25.01	99.6	2-26-44
3	24.92	25.01	99.6	2-27-44
7	24.80	25.01	99.3	3-2-44
Average	24.83		99.3	

B. albolactis Tex.

Incubation Period (days)	Total mg. Glucose Det'n. per Tube	Ave. 4 Days Control Glucose Det'ns. (mg.)	Mg. Glucose Utilized	Date
1	19.60		5.23	2-25-44
2	18.08		6.75	2-26-44
3	17.20		7.63	2-27-44
7	12.90		11.93	3-2-44
		24.83		

<u>Effect of Metabolic Products in Peptone Medium upon $Na_2S_2O_3$:</u>

To determine the effect of metabolic products produced by <u>Bacilli</u> growing in peptone broth upon $Na_2S_2O_3$ used in titrating excess iodine, a comparison was made of the total reduction of copper by cultures grown on this medium. The results, summarized in the table below, indicate that reducing substances were not produced in quantities sufficient to reduce the copper reagent.

Incubation Period (days)	No. tubes titrated	No. strains represented	Ave. Titr. value: cc. Na_2SO_3: Inoc. Peptone Broth	Ave. Titr. value: cc. Na_2SO_3: Uninoc.P.B.	Titr. Diff. cc 0.005 N $Na_2S_2O_3$
1	74	25	22.27	22.36	-0.09
7	74	25	22.41	22.30	+0.11
1	62	21	19.39	19.31	+0.08
3	61	22	19.70	19.75	-0.05
7	62	22	19.71	19.80	-0.09

The identification of all the reducing substances produced was outside the scope of this study. A comparison of the titration values of peptone broth inoculated with 47 strains of <u>Bacilli</u> and uninoculated peptone broth reveal a maximum average difference of 0.2 ml. of $Na_2S_2O_3$, or an amount equivalent to 0.46 mg. of glucose. Thus, all <u>Bacilli</u> which utilize more than 0.46 mg. of glucose are considered positive utilizers of glucose under conditions of the experiment.

All sugars do not reduce copper to the same extent it is reduced by glucose. The ratio of this reduction to the reduction by glucose varies with each sugar. With the exception of maltose and lactose, the reducing power of many other sugars is so nearly equal to that of glucose that in most bacteriological work the differences may be neglected (43). In this investigation, these differences for d- and l-arabinose have been taken into consideration in determining the extent of their utilization. The ratio 1.06 for arabinose was determined by the authors (43) of the procedure used.

It follows that after applying the ratio for arabinose, 0.46 mg. of glucose is equivalent to 0.48 mg. of either d- or l-arabinose. Thus all of the <u>Bacilli</u> which utilize more than this amount of arabinose can be assumed to be positive utilizers of this carbohydrate.

RESULTS OF THE INVESTIGATION

The results of the pH and carbohydrate utilization determinations are recorded in Table III and are given graphically in Figures 1-130.

In Table IV will be found an interpretation of the pH values recorded in Table III together with comparable data from the investigations of Coffee (4), and the reactions from other literature listed in Bergey's Manual (1). Plus (+) marks are employed to indicate acidic pH values. Negative, alkaline, or neutral pH values are recorded as minus (-).

Table V presents a summary of the data in Tables III and IV and in Figures 1-130. In this table the reactions of the various strains of Bacilli employed are summarized into a reaction apparently characteristic of the species represented. Only those reactions which appear to have taxonomic value are listed in this table. The 'legend' for the interpretation of these reactions accompanies the table. Further discussion may be found under "Discussion of Results".

TABLE III

Comparison of pH Produced and Carbohydrate

Utilized by Members of the Genus Bacillus

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. adhaerens A.T.C.	1	7.0	5.8	3.7	6.5	1.3	6.3	1.2
	2	7.7	5.2	7.4	6.9	-	5.9	2.5
	3	7.6	5.1	8.7	7.0	0.6	6.5	4.6
	7	8.3	5.1	28.1	8.2	0.6	6.0	5.6
B. agri U.S.D.A.	1	6.6	5.5	4.9	6.1	0.7	6.3	0.7
	2	6.8	5.2	6.0	6.5	-	6.0	1.0
	3	6.6	5.1	6.4	6.5	0.1	5.7	1.1
	7	7.4	5.4	9.2	7.7	0.4	5.6	3.0
B. albolactis V.P.I.	1	7.1	4.9	4.2	6.5	1.8	7.0	0.2
	2	7.4	5.0	6.8	7.2	-	7.2	1.0
	3	7.3	5.6	6.7	7.3	2.3	7.4	0.6
	7	-	5.5	7.8	8.4	2.6	7.8	1.0

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. albolactis Tex.	1	6.9	5.3	5.3	5.9	1.3	6.5	1.0
	2	7.3	5.1	5.7	7.3	-	6.8	0.2
	3	7.1	4.9	5.8	7.1	2.0	7.4	0.6
	7	8.0	5.0	17.1	8.3	3.4	7.6	0.3
B. alvei A.T.C.	1	6.2	5.2	3.7	6.4	1.3	6.2	1.0
	2	7.4	5.2	3.1	7.0	-	6.4	0.8
	3	7.7	5.2	4.8	7.2	1.1	7.0	0.1
	7	8.4	5.3	3.6	8.0	1.6	7.3	0.9
B. alvei Tex.	1	6.0	5.5	2.2	6.4	1.3	6.2	0.9
	2	7.0	5.4	2.2	7.2	-	6.5	1.3
	3	7.5	5.4	2.0	7.3	1.1	7.1	0.2
	7	8.4	5.5	1.1	8.2	2.4	8.0	1.7

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. alvei #622 Tex.	1	6.1	5.4	2.2	6.2	1.1	6.3	0.6
	2	7.0	5.4	2.7	7.1	-	6.0	0.3
	3	7.5	5.4	2.4	7.3	1.5	7.4	0.5
	7	8.5	5.5	0.0	8.2	2.1	7.9	0.7
B. alvei #622 Lockhead Tex.	1	6.2	5.0	0.8	6.1	1.5	6.3	0.9
	2	7.0	5.7	3.1	7.2	-	6.6	1.0
	3	7.5	5.7	2.7	7.6	0.8	7.5	0.8
	7	8.5	5.5	7.7	8.2	1.7	7.8	1.5
B. anthracis Wis.	1	6.9	5.4	2.2	5.8	-0.8	5.8	0.1
	2	6.4	5.4	4.2	6.4	-	6.0	0.2
	3	6.5	5.4	3.7	6.5	0.4	6.4	0.4
	7	7.6	5.4	4.4	7.4	0.8	6.7	1.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. anthracis Tex.	1	6.4	6.7	5.4	5.9	-0.4	5.8	0.2
	2	6.4	5.5	4.0	6.7	-	6.1	1.2
	3	6.8	5.3	4.8	6.7	1.0	6.4	0.5
	7	7.3	5.3	3.7	7.2	1.3	6.8	0.1
B. anthracis (Wis.) Tex.	1	6.0	5.4	4.7	6.1	-0.4	5.9	0.2
	2	6.4	5.3	4.1	6.6	-	6.0	0.4
	3	6.8	5.3	5.4	6.6	0.3	6.6	0.5
	7	7.6	5.4	4.4	7.5	1.1	7.1	0.2
B. anthracis Rall. Tex.	1	6.2	5.6	5.7	6.0	-0.3	6.0	0.7
	2	6.5	5.3	4.3	6.5	-	7.0	0.6
	3	6.5	5.4	6.4	6.5	0.8	6.4	0.4
	7	7.4	5.4	4.9	7.1	1.3	6.8	-0.2

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. anthracis (smooth) Tex.	1	6.7	5.4	4.2	5.8	-0.5	5.9	-0.2
	2	7.0	5.5	4.1	6.5	-	6.1	0.5
	3	8.0	5.7	5.4	6.5	1.1	6.5	0.6
	7	8.4	5.8	3.8	7.5	2.0	7.0	0.3
B. aterrimus #353 Wis.	1	6.4	5.5	2.1	7.5	1.4	6.1	1.5
	2	8.1	5.5	5.2	7.8	-	6.2	3.2
	3	8.1	5.5	8.4	8.1	1.7	6.3	5.8
	7	8.3	6.3	15.2	8.3	1.7	6.7	13.1
B. aterrimus U. S. D. A.	1	6.6	5.5	4.9	6.8	0.0	5.5	0.8
	2	6.9	5.6	3.9	7.6	-	5.2	4.0
	3	7.8	-	6.0	7.9	1.1	5.2	4.8
	7	8.7	5.7	19.6	8.3	1.5	5.4	8.3

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. aterrimus Pesek Wis.	1	6.7	5.5	6.4	7.0	0.0	6.1	1.6
	2	7.2	5.5	9.1	7.7	-	5.9	3.4
	3	8.0	5.6	3.8	7.8	0.6	6.0	10.2
	7	8.4	6.0	21.6	8.2	1.8	6.8	13.0
B. aterrimus R. C. Wis.	1	6.8	5.5	5.7	7.1	0.6	6.1	1.3
	2	7.9	5.5	10.2	7.9	-	6.0	3.4
	3	8.2	5.6	10.4	8.0	0.5	6.4	7.0
	7	8.4	6.2	16.7	8.2	1.9	6.5	13.8
B. cereus #1 A. T. C.	1	7.5	5.1	13.4	6.9	0.3	6.8	-0.2
	2	7.7	5.0	13.7	7.4	-	7.0	0.4
	3	7.6	5.1	13.0	7.4	1.4	7.2	0.3
	7	8.3	5.1	22.7	8.3	1.4	7.7	2.4

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. cereus #2 A. T. C.	1	6.4	5.5	4.7	6.0	0.4	5.1	0.3
	2	6.8	5.4	4.2	6.4	-	5.5	3.0
	3	6.7	5.5	3.4	6.5	0.6	5.4	2.3
	7	7.6	5.5	3.0	6.7	1.6	6.1	1.2
B. cereus Tex.	1	6.5	5.6	7.7	6.4	0.6	6.3	0.2
	2	6.8	5.6	11.0	6.8	-	8.3	2.0
	3	6.9	5.7	16.4	7.4	1.0	6.1	1.3
	7	7.8	6.0	28.2	8.2	1.7	6.8	4.3
B. cereus Stock Tex.	1	7.4	5.1	6.7	6.7	0.7	6.8	0.0
	2	7.4	5.0	5.7	7.2	-	6.9	0.3
	3	7.4	5.0	7.8	7.4	0.7	7.2	0.1
	7	8.2	5.1	15.4	8.4	1.1	7.5	3.0

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. cereus Non-motile Tex.	1	7.4	5.1	7.0	6.8	0.5	6.8	0.2
	2	7.4	5.0	9.8	7.2	-	6.9	2.0
	3	7.4	5.0	8.8	7.2	0.5	7.4	0.0
	7	8.1	5.1	19.0	8.1	0.5	7.8	1.6
B. cereus #244 Tex.	1	7.4	5.4	5.2	6.7	0.3	7.0	0.3
	2	7.4	5.1	6.8	7.2	-	7.0	0.8
	3	7.5	5.4	7.6	7.3	0.3	7.2	0.5
	7	8.2	5.3	11.9	8.2	1.2	7.7	0.3
B. cereus Clark Tex.	1	7.5	5.1	17.7	6.7	0.5	7.0	0.2
	2	7.7	5.0	15.5	7.1	-	7.0	2.1
	3	7.8	5.0	17.8	7.3	0.4	7.5	0.5
	7	8.3	5.2	25.0	8.5	1.2	7.9	1.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. cereus Neb.	1	7.4	5.2	17.7	6.9	-		
	2	7.5	5.1	11.8	7.2	2.3		
	3	7.6	5.1	-	7.3	0.8		
	7	8.3	5.3	22.0	8.0	0.9		
B. cereus Smith-Thom Tex.	1	7.0	5.9	1.4	6.6	0.8	6.4	1.2
	2	7.8	5.7	4.9	7.0	-	6.3	3.1
	3	8.1	6.2	5.5	7.1	0.6	6.3	5.2
	7	8.3	6.3	12.4	8.2	0.9	6.2	7.6
B. cereus Wis.	1	6.6	5.2	6.5	6.3	0.4	6.8	0.2
	2	7.0	5.0	6.8	7.7	-	6.0	0.9
	3	7.0	5.1	6.3	6.8	0.4	5.6	1.9
	7	7.5	5.1	9.3	7.4	2.1	5.5	4.6

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. cereus A. T. C. Wis.	1	7.5	5.0	7.7	6.8	-0.2	6.9	0.5
	2	7.8	5.0	8.6	7.3	-	7.2	1.5
	3	7.8	5.0	10.5	7.4	0.5	7.3	0.7
	7	8.3	5.1	16.1	8.3	1.7	7.5	0.6
B. brevis #604 A. T. C.	1	7.3	7.1	1.5	7.5	0.4	7.4	1.2
	2	8.0	7.7	1.3	8.1	-	7.5	-
	3	8.2	7.9	-	8.4	1.6	8.1	1.4
	7	8.7	8.6	3.8	8.9	1.8	8.4	2.9
B. brevis U. S. D. A.	1	7.2	7.2	1.0	7.2	1.0	6.9	1.6
	2	7.7	7.6	2.6	8.1	-	7.2	2.0
	3	7.8	7.8	-	8.5	1.0	7.5	0.3
	7	8.7	8.5	3.2	8.7	2.0	8.2	1.3

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. circulans A. T. C.	1	6.3	5.3	-1.1	5.9	0.6	5.6	1.3
	2	-	5.3	-0.4	6.6	-	5.6	2.4
	3	6.7	5.3	-	6.5	0.5	5.6	0.6
	7	7.6	5.3	1.3	6.4	2.0	5.5	1.1
B. cohaerens #840 Tex.	1	6.8	5.8	4.7	6.9	0.3	6.6	1.8
	2	7.4	5.7	7.1	7.6	-	-	3.1
	3	7.7	5.6	-	7.7	1.2	6.3	3.8
	7	8.4	5.9	13.8	8.5	1.0	6.1	16.0
B. danicus U. S. D. A.	1	6.7	6.1	1.8	6.1	1.3	6.2	-
	2	7.0	5.9	4.3	7.3	-	5.6	2.3
	3	7.4	5.3	-	7.4	2.4	5.7	0.8
	7	8.0	4.9	8.8	8.5	2.2	6.2	3.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. danicus #A. T. C.	1	7.0	5.5	0.2	6.3	0.7	6.3	1.7
	2	7.2	5.8	5.3	6.7	-	5.5	3.1
	3	7.5	5.4	-	6.7	0.2	5.5	5.8
	7	8.3	5.4	11.1	8.2	0.2	6.2	3.2
B. firmus U. S. D. A.	1	6.7	6.3	0.7	6.1	0.5	6.2	0.8
	2	7.1	6.1	0.6	6.9	-	6.5	1.7
	3	7.3	6.1	-	6.5	1.7	6.5	0.1
	7	7.7	6.1	2.2	7.1	1.5	7.3	1.6
B. flexus V. P. I.	1	6.7	6.5	0.5	6.9	0.9	6.3	1.5
	2	7.4	6.5	1.3	6.8	-	-	3.1
	3	7.5	6.5	-	7.1	0.5	6.6	-0.1
	7	7.9	6.3	2.7	7.8	1.6	7.6	0.5

TABLE III (Continued)

Organism	Incubation Period (days	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. freundenreichii A. T. C.	1	6.9	6.5	-2.4	6.4	0.7	6.7	-0.8
	2	7.1	6.8	-2.7	7.0	-	7.1	0.4
	3	6.9	7.0	-	7.1	0.7	6.9	0.2
	7	8.0	-	1.1	7.7	0.4	7.1	1.2
B. fusiformis A. T. C.	1	7.1	7.2	-0.7	6.6	1.5	6.8	0.3
	2	7.5	7.6	1.3	7.4	-	-	1.6
	3	7.6	7.8	-	7.4	1.5	7.7	0.9
	7	8.1	8.0	3.7	8.0	1.5	7.9	2.5
B. fusiformis Tex.	1	7.2	7.2	-4.4	6.5	0.6	6.9	0.8
	2	7.6	7.5	2.0	7.0	-	-	1.5
	3	7.9	7.7	-	7.1	1.2	7.7	0.2
	7	7.9	8.1	1.7	8.1	1.2	8.2	1.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. fusiformis #339 Tex.	1	7.2	6.9	-0.4	6.5	0.3	6.9	0.2
	2	7.5	7.2	-2.2	6.9	-	7.2	1.7
	3	7.6	7.8	-	7.1	0.7	7.3	-0.2
	7	7.9	7.8	3.4	7.9	1.6	7.7	0.3
B. fusiformis Wright Wis.	1	7.0	6.9	0.6	6.7	0.7	6.8	1.4
	2	7.5	7.5	1.8	6.7	-	7.5	2.4
	3	7.8	7.8	1.9	6.9	1.1	7.6	2.0
	7	8.0	8.0	1.6	8.0	2.0	7.7	0.6
B. globigii A. T. C.	1	6.4	5.4	1.6	6.2	0.0	5.5	1.6
	2	7.0	5.5	1.0	6.5	-	5.5	1.7
	3	7.1	5.4	1.2	6.5	1.2	5.6	0.6
	7	7.3	5.4	1.3	7.0	0.7	5.4	0.8

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. globigii #356 Wis.	1	7.0	5.6	2.7				
	2	7.4	5.7	4.6				
	3	7.6	5.7	5.2				
	7	8.1	5.9	8.2				
B. graveolens U. S. D. A.	1	7.0	5.5	1.5	6.7	0.0	5.9	2.1
	2	7.3	5.5	19.8	7.5	-	5.7	2.5
	3	7.7	5.7	-	7.4	0.6	5.7	2.1
	7	-	5.3	11.7	8.2	1.8	5.7	6.1
B. graveolens #615 Tex.	1	6.8	5.8	2.1	6.4	1.0	5.8	1.1
	2	7.6	5.4	6.3	7.0	-	5.6	2.7
	3	7.9	5.3	-	7.1	0.8	5.6	3.3
	7	8.2	5.4	7.5	8.0	0.8	5.8	4.3

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. graveolens Wright Wis.	1	7.5	5.1	5.2	7.0	-0.9	7.1	1.4
	2	8.0	5.0	8.1	8.0	-	7.8	1.7
	3	8.3	5.0	-	8.4	1.3	7.8	1.1
	7	8.6	5.0	12.2	8.7	1.9	7.4	2.4
B. graveolens Neb.	1	7.3	5.7	7.1	6.9	0.1	6.5	4.7
	2	7.8	6.8	13.2	8.2	-	6.8	7.4
	3	8.1	6.4	-	8.0	0.8	6.7	11.4
	7	8.5	8.3	38.1	8.5	1.5	8.0	20.3
B. lacticola Tex.	1	7.0	5.1	2.5	7.0	-0.3	6.9	0.5
	2	7.7	5.1	4.0	7.7	-	7.7	2.3
	3	8.0	5.1	-	8.1	0.3	7.8	3.3
	7	6.9	5.1	8.8	8.6	1.1	8.4	3.2

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. lacticola #876 Tex.	1	7.0	5.1	4.5	7.2	0.0	7.5	1.3
	2	7.7	5.2	6.5	7.8	-	7.8	2.8
	3	7.9	5.2	-	7.8	0.6	8.1	0.2
	7	8.4	5.1	7.0	8.6	0.9	8.2	3.5
B. lactis #618 Tex.	1	6.2	5.2	6.5	7.2	0.5	7.2	2.3
	2	7.5	5.2	6.5	7.6	-	7.7	2.8
	3	8.3	5.3	-	7.9	0.2	7.8	1.5
	7	8.6	5.1	8.8	8.7	1.2	8.2	2.9
B. laterosporus V. P. I.	1	6.7	5.7	3.4	6.2	0.3	6.3	1.5
	2	7.8	5.1	2.5	7.0	-	6.8	3.3
	3	7.9	5.3	-	7.4	0.9	7.1	4.5
	7	8.3	5.3	24.7	8.4	0.2	7.7	8.6

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. lautus V. P. I.	1	7.3	6.9	6.1	7.3	1.0	6.1	4.1
	2	8.1	5.9	19.0	7.9	-	6.3	7.4
	3	8.3	5.9	-	8.2	1.9	6.3	7.7
	7	8.5	6.2	24.0	8.5	1.6	6.0	12.4
B. macerans U. S. D. A.	1	6.4	5.2	2.6	6.1	1.6	5.2	2.3
	2	6.8	5.0	2.5	6.0	-	5.2	2.5
	3	6.7	5.0	2.4	5.9	2.6	5.1	2.9
	7	7.1	5.0	2.3	5.8	5.6	5.0	2.6
B. megatherium U. S. D. A.	1	6.7	6.1	0.8	6.0	1.0	5.2	-0.1
	2	7.0	5.5	3.0	6.8	-	5.4	2.3
	3	7.1	5.5	3.6	6.8	0.4	5.4	1.9
	7	7.8	5.4	5.3	7.7	1.3	5.2	4.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. megatherium #1 A. T. C.	1	6.7	5.7	1.2	6.1	1.8	6.3	1.4
	2	6.8	5.9	2.8	6.8	-	-	1.5
	3	6.6	5.6	1.5	6.9	0.7	5.6	2.7
	7	7.8	5.4	4.2	7.7	1.8	5.5	5.4
B. megatherium #2 A. T. C.	1	6.7	-	0.5	6.9	0.7	6.1	0.5
	2	6.7	5.5	3.6	6.7	-	-	2.0
	3	7.0	5.5	3.5	6.8	0.4	5.6	2.4
	7	7.8	5.4	5.9	7.7	1.7	5.6	4.4
B. megatherium Tex.	1	6.4	5.4	2.4	6.3	1.0	5.8	0.2
	2	6.8	5.2	4.0	6.8	-	-	1.9
	3	7.0	5.1	3.9	6.8	1.2	5.4	0.3
	7	7.8	5.1	2.8	7.6	0.1	5.3	4.8

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. megatherium #240 Tex.	1	6.7	6.0	1.9	6.1	0.6	6.1	1.9
	2	6.5	5.4	2.9	6.7	-	-	2.9
	3	6.9	6.6	3.4	6.9	0.0	5.5	5.0
	7	7.6	5.3	4.0	7.6	1.2	5.5	2.9
B. megatherium Wis.	1	7.4	5.2	1.9	6.9	2.9	7.1	3.2
	2	7.6	5.2	1.7	7.6	-	-	4.1
	3	7.8	5.1	3.4	7.5	2.1	7.8	3.6
	7	8.1	5.1	3.6	8.0	1.4	8.0	2.0
B. megatherium A. T. C. Wis.	1	7.3	5.1	2.8	7.0	2.5	7.1	1.9
	2	7.5	5.1	3.9	7.5	-	-	3.8
	3	7.3	5.1	6.4	7.7	1.6	7.7	4.5
	7	8.3	5.1	6.2	8.0	0.7	7.9	2.7

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. megatherium Neb.	1	6.7	5.1	1.3	6.2	0.1	6.5	0.8
	2	6.9	5.2	1.4	7.2	-	-	1.7
	3	7.1	5.1	1.4	7.3	0.9	7.2	2.1
	7	7.7	5.1	2.8	8.6	1.8	8.0	2.2
B. mesentericus A. T. C.	1	6.7	5.4	1.5			6.5	0.8
	2	6.9	5.0	1.9			6.0	1.2
	3	7.2	5.0	2.0			6.1	2.2
	7	7.7	5.3	5.9			6.0	2.7
B. mesentericus U. S. D. A.	1	7.1	5.3	1.8	6.3	0.9	6.2	1.4
	2	7.7	5.5	2.9	7.8	-	6.6	3.8
	3	7.8	5.6	4.0	8.1	-0.2	6.3	6.1
	7	8.3	7.3	9.8	8.5	0.2	7.4	2.8

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mesentericus Tex.	1	7.8	5.7	8.9	7.4	1.0	5.6	6.3
	2	-	6.0	14.2	8.2	-	6.0	16.7
	3	8.4	6.2	23.5	8.4	0.6	6.4	21.9
	7	8.5	7.6	22.7	8.6	2.4	7.9	25.1
B. mesentericus A. T. C. Wis.	1	7.0	5.4	2.9	6.4	1.8	6.8	0.0
	2	7.5	5.5	3.5	7.8	-	7.0	2.8
	3	7.7	5.7	3.8	7.8	0.4	7.0	6.5
	7	8.2	6.3	13.6	8.6	1.3	7.5	6.5
B. mesentericus Frost Wis.	1	6.8	5.4	2.6	6.4	0.1	6.3	0.4
	2	7.4	5.5	2.9	7.4	-	6.6	2.9
	3	7.8	5.7	3.9	7.4	1.3	6.6	8.2
	7	8.1	6.0	19.4	8.3	1.7	7.2	20.5

TABLE III (Continued)

Organism	Incubation Period(days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mesentericus Neb.	1	7.4	5.0	3.6	6.4	1.4	6.5	-0.1
	2	8.0	5.0	2.0	8.0	-	8.0	1.6
	3	8.2	5.0	2.0	8.1	1.8	8.2	3.0
	7	8.6	5.0	1.9	8.6	2.0	8.4	4.9
B. mycoides #1 V. P. I.	1	6.4	5.2	3.1	6.8	-	6.6	3.7
	2	7.3	5.3	4.4	7.4	1.3	7.2	1.9
	3	7.4	5.0	4.7	7.5	0.9	7.5	3.3
	7	8.0	5.2	11.4	8.0	1.3	7.0	4.1
B. mycoides #101 V. P. I.	1				7.0	-		
	2				7.1	1.2		
	3				7.3	0.9		
	7				7.9	0.3		

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. mycoides Beaded Tex.	1	7.4	5.1	4.1	7.0	-		
	2	7.5	5.1	4.1	7.3	1.3		
	3	7.6	5.0	3.8	7.5	0.5		
	7	7.8	5.1	3.7	7.8	0.6		
B. mycoides A. T. C. #80 Tex.	1	7.5	6.5	3.2				
	2	7.6	5.0	4.7				
	3	7.7	5.0	3.4				
	7	7.9	4.9	4.5				
B. mycoides A 2 Tex.	1	7.5	5.0	1.5	6.6	1.7		
	2	7.5	5.0	4.3	7.2	1.8		
	3	7.5	5.0	2.7	7.3	0.9		
	7	7.9	5.0	2.6	7.9	1.4		

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mycoides A 5 Tex.	1	7.5	5.1	1.7	7.0	-		
	2	7.6	5.0	3.9	7.4	0.7		
	3	7.5	5.0	2.8	7.5	1.7		
	7	8.1	5.0	4.6	8.1	1.2		
B. mycoides #23 Tex.	1	7.3	5.0	1.7				
	2	7.6	5.0	2.3				
	3	7.7	5.0	3.2				
	7	8.1	5.0	4.9				
B. mycoides #65 Tex.	1	7.4	5.2	1.2	6.7	2.3	6.3	3.8
	2	7.7	5.3	0.7	7.2	-	6.9	1.8
	3	7.5	5.2	1.8	6.5	0.8	7.4	3.2
	7	7.8	5.2	0.9	7.8	1.7	7.5	1.9

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mycoides #405 Tex.	1	7.3	5.2	-	7.0	-	7.2	1.7
	2	7.5	5.2	1.9	7.6	1.9	7.1	1.5
	3	7.5	5.1	4.3	7.6	1.6	7.2	1.6
	7	8.2	5.0	2.6	8.1	1.7	8.0	2.3
B. mycoides #410 Tex.	1	7.4	5.2	3.6	7.2	-	7.3	2.7
	2	7.6	5.3	4.1	7.5	0.7	7.4	2.4
	3	7.4	5.2	3.0	7.5	1.6	7.4	3.3
	7	7.7	5.2	7.0	7.9	1.3	8.0	1.9
B. mycoides #417 Tex.	1	7.4	5.1	2.3			7.1	5.0
	2	7.7	5.0	2.8			7.5	3.4
	3	7.8	5.1	4.4			7.6	2.3
	7	8.5	5.1	4.5			8.2	0.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mycoides #420 Tex.	1	7.4	5.2	2.5	7.2	-	7.1	1.9
	2	7.6	5.2	4.9	7.2	1.0	7.7	4.6
	3	7.5	5.1	3.6	7.4	1.5	7.7	3.5
	7	8.0	5.2	3.9	8.0	0.8	8.0	2.4
B. mycoides #421 Tex.	1	7.3	5.1	1.5	7.1	-	6.9	2.0
	2	7.7	5.3	3.2	7.5	0.2	7.5	2.8
	3	7.9	5.1	3.8	7.5	1.9	7.7	5.1
	7	8.0	5.1	1.5	7.9	1.0	8.3	1.4
B. mycoides #422 Tex.	1	7.4	5.1	1.1	6.6	-	6.5	4.2
	2	7.6	5.3	2.2	7.5	1.2	7.4	4.1
	3	7.4	5.4	2.2	7.4	0.8	7.4	6.7
	7	8.0	5.1	2.1	8.0	1.5	7.9	2.9

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. mycoides #425 Tex.	1	7.4	5.1	1.9	7.1	-	7.1	1.3
	2	7.6	5.3	2.5	7.2	1.2	7.6	2.8
	3	7.5	5.1	3.2	7.4	1.7	7.5	2.7
	7	7.7	5.2	2.2	8.0	0.6	7.8	1.9
B. mycoides #426 Tex.	1	7.3	5.0	2.5	6.7	-	7.1	4.1
	2	7.6	5.3	1.5	7.3	0.9	7.4	2.9
	3	7.5	5.2	2.2	7.4	0.7	7.2	4.9
	7	7.8	5.1	1.5	7.8	-0.1	7.6	0.9
B. mycoides #427 Tex.	1	7.5	5.3	1.5	7.2	-	7.1	2.0
	2	7.6	5.3	1.6	7.4	1.0	7.4	2.1
	3	7.6	5.1	2.6	7.5	1.4	7.8	1.7
	7	8.1	5.0	1.5	7.9	0.5	8.1	1.3

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mycoides #430 Tex.	1	7.5	5.3	1.6	7.0	-	6.7	1.8
	2	7.7	5.3	2.1	7.2	1.4	7.2	1.8
	3	7.6	5.2	1.6	7.4	1.2	7.5	3.8
	7	7.9	5.2	2.1	7.9	0.5	7.7	0.4
B. mycoides Wright Wis.	1	7.7	5.2	2.0				
	2	7.6	5.2	2.8				
	3	7.5	5.1	3.7				
	7	7.9	5.1	2.2				
B. mycoides Neb.	1	7.5	5.3	1.9	7.1	-	7.3	3.5
	2	7.5	5.3	2.3	7.5	1.5	7.5	4.2
	3	7.4	5.1	4.1	7.4	1.9	7.3	1.9
	7	8.0	5.1	4.6	7.1	1.4	7.9	0.8

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. mycoides Left-Hand Sarles Wis.	1	6.9	5.0	1.5	7.0	-	7.0	3.5
	2	7.7	5.0	4.3	7.0	1.2	7.5	2.3
	3	7.6	5.0	3.9	7.5	1.5	7.8	3.5
	7	8.6	5.8	3.4	8.2	1.7	8.2	3.0
B. niger A. T. C.	1	7.2	5.9	1.3	6.8	-	6.1	0.2
	2	7.6	6.0	4.1	7.3	1.2	7.2	3.2
	3	7.9	5.7	9.3	7.2	1.0	7.2	3.9
	7	8.4	5.8	19.6	8.4	1.2	7.8	7.8
B. niger U. S. D. A.	1	6.9	5.9	0.6	6.7	-	6.2	0.8
	2	7.3	5.7	4.1	7.4	1.2	6.7	1.4
	3	7.3	5.6	9.1	7.7	0.3	-	2.7
	7	8.2	5.7	23.0	8.4	1.1	7.7	4.4

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. niger #228 Wis.	1	7.0	5.9	1.1	6.3	-	6.3	1.2
	2	7.9	6.0	3.6	7.4	0.6	7.4	2.3
	3	7.8	5.8	7.8	7.7	0.5	7.4	4.0
	7	8.4	5.7	18.0	8.3	0.1	7.9	12.0
B. niger 'Old-Wis'	1	7.3	5.8	1.8	6.9	-	6.7	0.9
	2	7.7	6.1	4.1	7.3	0.7	-	0.7
	3	7.8	6.2	7.2	7.5	1.3	7.1	1.9
	7	8.1	6.2	21.9	8.2	1.8	7.5	5.7
B. niger 'S' Wis.	1	7.2	6.0	0.8	6.3	-	6.3	0.2
	2	7.8	6.1	3.6	7.1	1.0	7.3	1.7
	3	7.9	6.0	10.6	7.7	0.9	7.3	3.7
	7	8.4	6.0	19.9	8.3	0.3	7.9	10.9

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. panis A. T. C.	1	6.9	5.5	0.8	5.8	-	6.4	1.4
	2	7.0	5.5	2.2	6.6	1.4	6.2	1.7
	3	6.8	5.3	3.8	6.6	1.1	5.9	2.3
	7	7.2	5.5	7.0	7.4	0.6	5.9	1.9
B. panis U. S. D. A.	1	6.9	6.6	-0.5	6.2	-	6.5	0.5
	2	7.0	6.6	-0.3	6.8	0.8	6.5	0.5
	3	7.0	6.7	-0.2	6.8	1.2	6.5	0.0
	7	7.4	6.9	2.4	7.0	1.1	6.6	-0.1
B. paraalvei Tex.	1	6.1	5.3	0.3	5.7	-	6.1	0.8
	2	7.0	5.3	1.6	6.5	1.8	6.1	1.5
	3	6.8	5.1	1.3	6.7	0.5	6.9	0.6
	7	8.1	5.1	2.3	7.8	0.5	7.4	0.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. polymyxa V. P. I.	1	6.9	5.4	3.6	6.7	-	5.1	2.5
	2	7.2	5.4	14.4	6.9	1.3	4.4	4.2
	3	7.3	5.9	21.7	7.1	0.9	5.0	4.0
	7	7.7	6.7	21.9	7.2	0.5	5.6	24.5
B. polymyxa Tex.	1	6.5	5.0	4.1	6.8	-	5.0	4.2
	2	6.7	5.7	12.7	6.9	0.6	5.4	6.9
	3	6.8	5.9	24.5	6.9	1.3	5.2	16.2
	7	6.9	6.1	22.6	7.1	1.1	5.1	24.5
B. prausnitzii Neb.	1	7.0	5.1	1.5			7.2	2.6
	2	7.5	5.3	1.4			7.5	1.7
	3	7.6	5.2	1.6			7.9	2.9
	7	8.0	5.1	1.5			8.0	1.1

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. robur #946 Tex.	1	6.4	5.1	2.2	6.1	-	6.3	1.0
	2	6.5	5.0	3.0	6.6	0.7	6.9	1.7
	3	6.9	5.0	2.5	7.4	1.2	7.2	2.3
	7	7.6	5.1	9.3	8.0	1.2	7.5	1.1
B. rotans A. T. C.	1	7.2	6.8	0.2	6.7	-	6.9	-1.1
	2	7.3	7.1	0.4	7.1	1.1	-	-0.4
	3	7.5	7.3	0.5	7.1	0.8	-	-
	7	7.8	7.6	1.9	7.8	0.7	7.6	-0.3
B. rotans Tex.	1	6.9	6.6	-0.1	6.5	-	6.8	2.2
	2	6.8	6.6	0.1	6.8	1.1	-	-0.7
	3	7.0	6.6	0.4	6.9	0.6	-	-
	7	7.0	6.8	1.3	7.4	1.3	6.8	-0.6

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. rotans (probably Wis.)	1	7.2	6.6	-0.6	6.6	-	7.0	4.7
	2	7.2	7.4	-1.5	7.1	1.0	-	-0.1
	3	7.5	7.7	0.6	7.3	1.1	-	-
	7	8.0	8.1	2.8	7.8	1.7	7.9	2.2
B. ruminatus #952 Tex.	1	7.2	5.3	1.6	6.9	-	6.0	0.5
	2	7.3	5.1	2.6	6.7	0.9	5.6	0.3
	3	7.7	5.0	5.2	7.0	1.1	5.3	3.4
	7	7.8	5.3	5.9	7.7	1.0	5.3	6.2
B. serositidis Neb.	1	7.3	6.9	-0.3	6.2	-	7.1	0.2
	2	7.3	7.7	-0.2	7.4	0.7	7.8	0.5
	3	7.5	8.0	0.2	7.8	1.3	8.1	1.6
	7	8.2	8.3	3.3	8.1	2.0	-	2.4

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. silvaticus #957 Tex.	1	6.8	6.0	1.3	5.8	-	6.1	1.5
	2	6.9	5.3	2.7	6.8	1.3	5.9	1.7
	3	7.1	5.3	4.7	7.1	0.7	5.4	2.2
	7	7.7	5.3	8.5	7.6	1.3	5.4	5.0
B. simplex Tex.	1	7.1	6.7	0.5	6.3	-	6.8	0.5
	2	7.2	6.7	0.5	7.0	1.1	7.0	-1.1
	3	7.2	6.5	0.6	7.1	0.5	7.1	-0.4
	7	7.6	6.4	5.9	7.4	1.2	7.4	1.8
B. simplex #335 Tex.	1	7.1	6.8	-1.0	6.4	-	6.9	0.9
	2	7.4	6.9	-0.7	7.0	1.3	7.2	0.9
	3	7.3	6.7	18.6	7.0	1.1	7.3	0.1
	7	7.7	6.6	6.2	7.4	1.3	7.4	0.7

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. sphaericus #348 Tex.	1	7.3	7.0	-0.8	6.5	-	7.0	0.9
	2	7.6	6.7	-0.1	7.1	0.5	7.3	0.6
	3	7.7	5.9	1.1	7.4	0.8	7.4	0.0
	7	7.9	5.6	2.1	7.8	1.7	7.7	3.8
B. subtilis #231 A.T.C.	1	6.9	5.7	2.4	6.4	-	6.5	0.3
	2	7.3	5.7	3.8	6.9	1.7	6.7	2.0
	3	7.4	6.4	9.1	7.3	0.8	7.1	3.1
	7	8.1	6.4	15.1	8.3	1.8	7.5	9.3
B. subtilis Tex.	1	6.9	5.8	1.2	6.4	-	6.5	1.2
	2	6.9	5.5	2.7	7.0	1.3	6.8	1.4
	3	7.0	5.6	7.2	7.3	0.7	7.0	2.3
	7	8.4	6.0	14.2	8.4	1.3	7.8	12.6

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. subtilis Koch-Novy Tex.	1	6.7	5.6	2.4	6.5	-	6.5	0.1
	2	6.8	5.7	-	6.7	1.7	6.8	2.2
	3	7.0	5.5	9.7	6.9	1.1	7.0	3.9
	7	8.1	6.4	16.4	8.2	0.6	7.8	5.3
B. subtilis Marburg Tex.	1	6.7	5.7	0.7	6.6	-	6.7	0.5
	2	6.8	5.6	0.9	7.2	0.7	6.7	1.9
	3	7.3	5.8	4.2	7.3	1.3	7.2	1.9
	7	8.3	6.8	11.9	8.0	0.8	7.4	10.5
B. subtilis Gram Neg. Tex.	1	7.2	5.9	2.7	6.8	-	6.6	0.4
	2	7.6	5.6	5.7	7.0	0.7	6.4	3.6
	3	7.7	5.7	9.1	7.2	0.6	7.1	5.8
	7	8.3	5.9	22.0	8.1	0.7	8.0	15.7

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. subtilis #231 Tex.	1	6.7	5.8	2.0	6.7	-	6.7	-0.2
	2	7.0	5.6	6.8	6.8	0.9	6.7	1.6
	3	7.7	5.8	10.7	7.3	0.8	7.2	1.8
	7	8.3	6.6	15.6	8.2	1.3	7.6	11.4
B. subtilis Marburg Wis.	1	7.1	6.0	0.7	6.8	-	6.6	-0.8
	2	7.5	5.7	4.4	7.1	0.5	7.1	2.2
	3	7.9	5.8	9.8	7.3	0.6	7.5	4.8
	7	8.4	6.1	19.3	8.2	0.7	8.0	15.2
B. subtilis Marburg N.Y.	1	6.7	6.2	1.2	7.0	-	6.6	0.3
	2	7.2	5.4	6.5	7.1	2.5	7.1	2.2
	3	7.6	5.3	11.3	7.4	1.3	7.4	4.0
	7	8.2	5.9	19.3	8.3	0.7	7.0	16.3

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. subtilis Marburg Neb.	1	7.2	5.8	2.0	6.6	-	6.7	0.2
	2	7.1	5.8	3.9	7.2	0.9	6.8	3.6
	3	7.6	5.8	8.5	7.7	0.3	6.8	7.0
	7	8.2	6.0	19.3	8.1	-0.3	7.5	13.6
B. subtilis 'L' Neb.	1	7.7	6.0	2.9	7.3	-	6.8	1.2
	2	-	5.4	7.2	7.4	0.7	5.5	4.2
	3	7.8	5.2	9.3	7.8	1.0	5.6	4.3
	7	8.2	5.1	13.1	8.4	1.4	5.8	6.1
B. subtilis #600 Neb.	1	7.5	5.0	1.6	7.0	-	7.2	0.0
	2	7.5	4.9	2.8	7.1	1.1	7.3	-0.1
	3	7.5	5.0	6.9	7.4	0.3	7.4	-0.6
	7	8.2	5.1	12.2	8.0	6.6	8.0	1.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. subtilis 'Milk' V.P.I.	1	6.7	5.8	1.6	6.4	-	6.5	-0.7
	2	7.0	5.6	4.5	6.7	-0.1	6.8	1.9
	3	7.4	5.8	8.5	7.6	1.4	7.2	1.7
	7	8.2	6.2	16.9	8.1	1.1	7.6	7.2
B. tumefaciens Tex.	1	7.4	5.3	1.6	7.0	-	6.9	-0.5
	2	7.4	5.1	1.6	7.1	1.6	7.5	0.0
	3	7.4	5.1	4.9	7.4	0.5	7.7	0.7
	7	8.4	5.1	11.6	8.3	1.2	8.1	0.5
B. tumescens Tex.	1	6.9	5.8	1.9	5.8	-	5.9	0.6
	2	7.0	5.3	2.6	6.7	0.6	5.6	1.4
	3	7.2	5.3	4.6	6.9	1.2	5.4	2.9
	7	7.9	5.5	7.1	7.5	1.3	5.4	3.8

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
B. tumescens #995 Tex.	1	6.5	5.4	-0.8	6.2	-	6.2	3.0
	2	7.1	5.2	-0.7	6.8	1.1	5.8	2.7
	3	7.0	5.2	0.3	7.2	1.2	6.6	2.5
	7	8.0	5.4	2.4	8.3	1.7	5.5	9.8
B. vulgatus A. T. C.	1	6.9	5.9	2.4	6.7	-	6.3	2.8
	2	7.0	5.9	4.6	7.2	1.2	6.9	4.4
	3	7.9	6.4	10.7	7.7	1.4	6.8	5.9
	7	8.3	7.3	14.6	8.4	1.2	7.3	6.7
B. vulgatus U. S. D. A.	1	6.9	6.0	2.0	6.5	-	6.3	2.7
	2	7.0	5.7	6.8	7.1	0.9	6.8	3.8
	3	7.5	6.2	8.6	7.7	1.1	7.0	5.7
	7	8.3	6.5	14.3	8.4	1.5	6.3	7.5

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose pH	Glucose mg Utilized	d-Arabinose pH	d-Arabinose mg Utilized	l-Arabinose pH	l-Arabinose mg Utilized
B. vulgatus Neb.	1	6.8	5.9	1.8	6.5	-	6.3	3.7
	2	7.7	5.8	7.5	7.3	0.0	6.9	3.1
	3	7.7	5.9	9.1	7.9	1.4	7.3	3.5
	7	8.4	6.6	16.5	8.3	1.7	7.2	4.8
B. vulgatus Tex.	1	6.5	5.8	-	6.6	-	6.3	0.4
	2	6.9	5.6	-	7.3	-0.9	6.7	2.8
	3	7.7	5.7	-	7.5	1.0	6.9	4.2
	7	8.4	6.6	-	8.2	0.0	7.3	5.3
B. globigii (Probably Vogel) Wis.	1	6.8	5.9	1.2	6.6	-	6.5	1.3
	2	7.6	5.7	5.9	7.1	0.8	7.4	2.2
	3	7.8	5.7	8.6	7.9	1.3	7.7	4.6
	7	8.4	6.1	8.3	8.4	1.4	7.9	13.7

TABLE III (Continued)

Organism	Incubation Period (days)	pH Peptone (alone)	Glucose		d-Arabinose		l-Arabinose	
			pH	mg Utilized	pH	mg Utilized	pH	mg Utilized
DeLong Pectin Fermenter Wis.	1	7.2	7.1	-0.4	6.6	-	6.7	0.8
	2	7.3	7.2	2.6	6.8	1.1	7.0	1.1
	3	7.5	7.5	1.6	7.1	1.4	7.3	1.5
	7	8.0	8.0	2.8	8.0	1.2	7.6	2.5

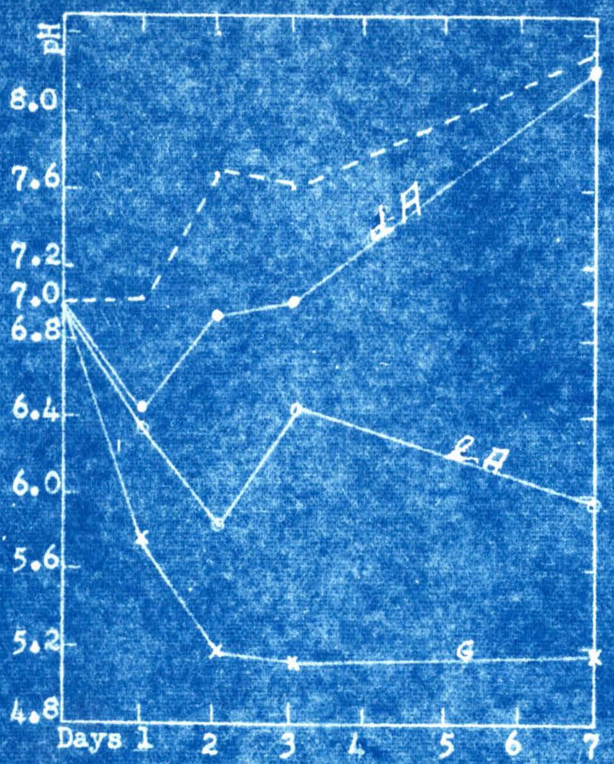
EXPLANATION OF FIGURES 1-130

The graphs in Figures 1-130 are intended to express the data recorded in Table II. The pH values resulting from the activity of a particular *Bacillus* in peptone alone, glucose-peptone, and d- and l- arabinose-peptone media are given in curves in the upper half of each figure. The curves which express the ability of each organism tested to utilize these carbohydrates as food for energy are in the lower half of each figure.

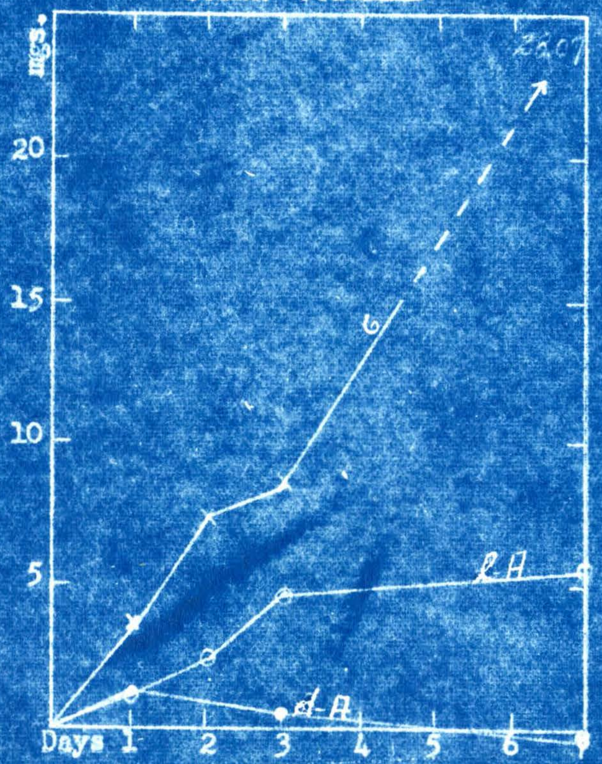
LEGEND:

------	pH change in peptone medium alone.
G	Abbreviation for glucose-peptone medium.
d-A	" " d-arabinose-peptone medium
l-A	" " l-arabinose-peptone medium

Fig. 1

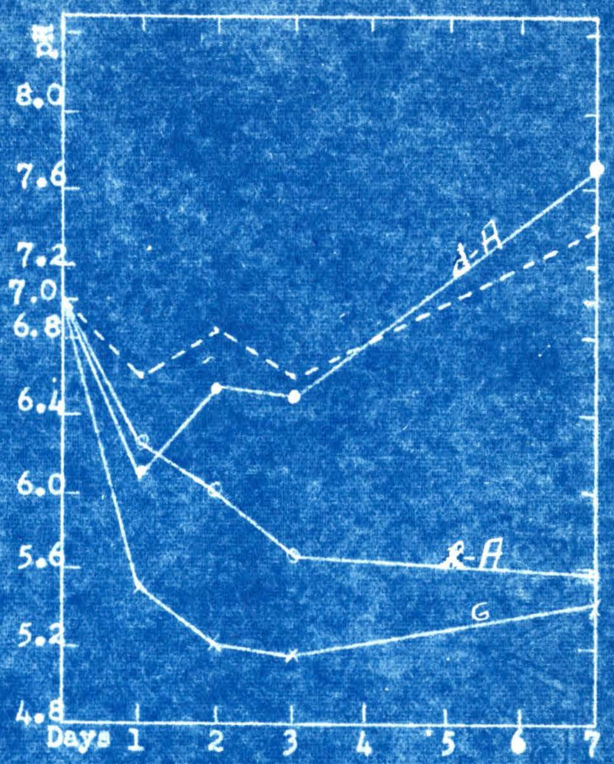


SUGAR UTILIZED



B. <u>adhaerens</u> A.T.C.

Fig. 2



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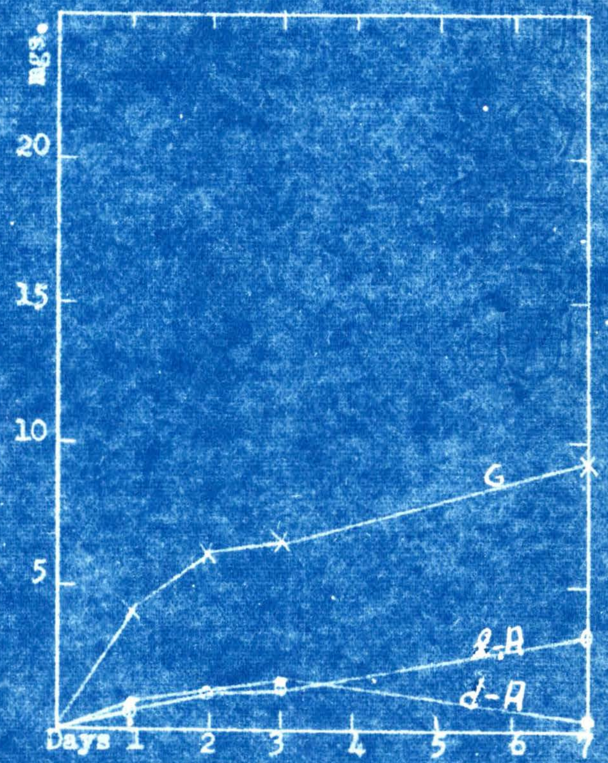
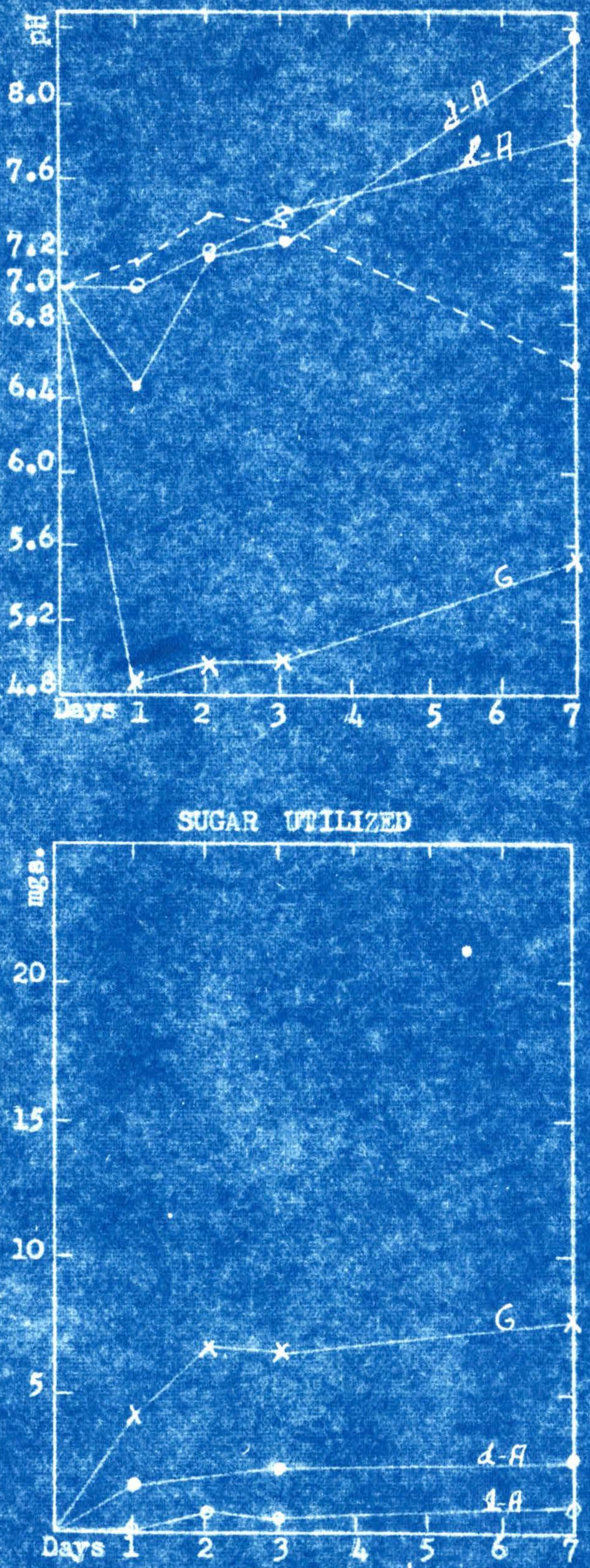


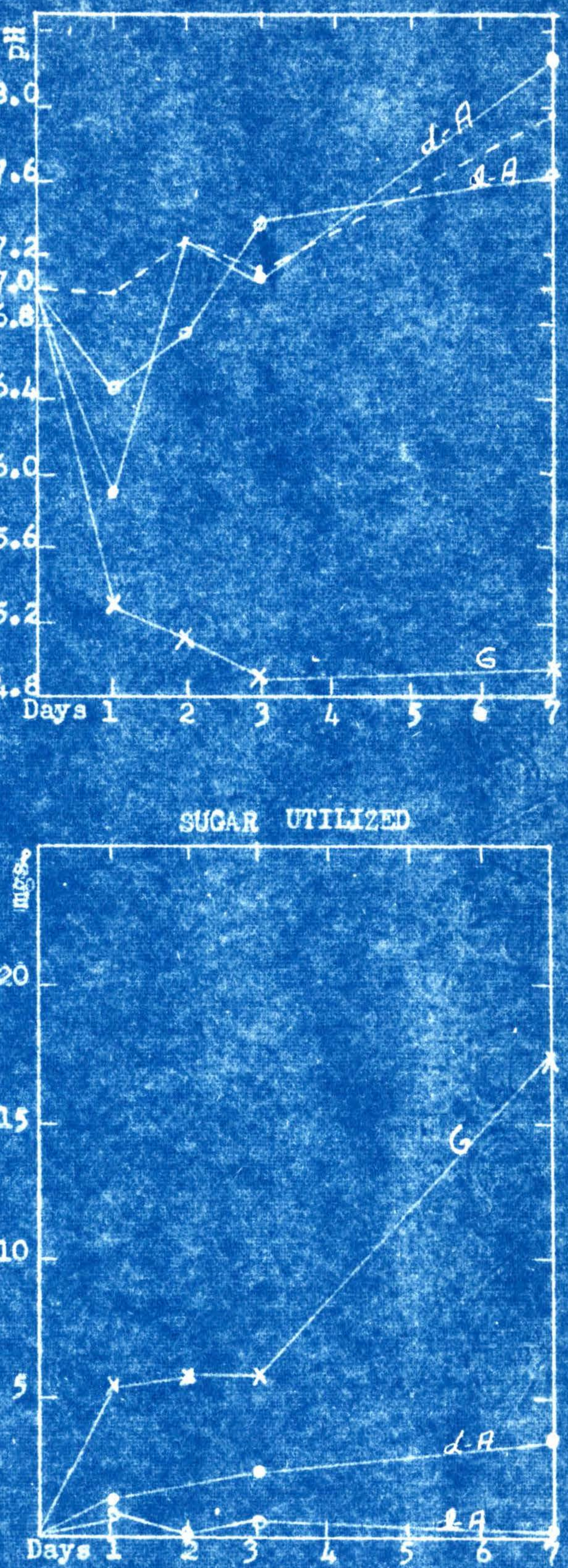
B. <u>agri</u> U.S.D.A.

Fig. 3



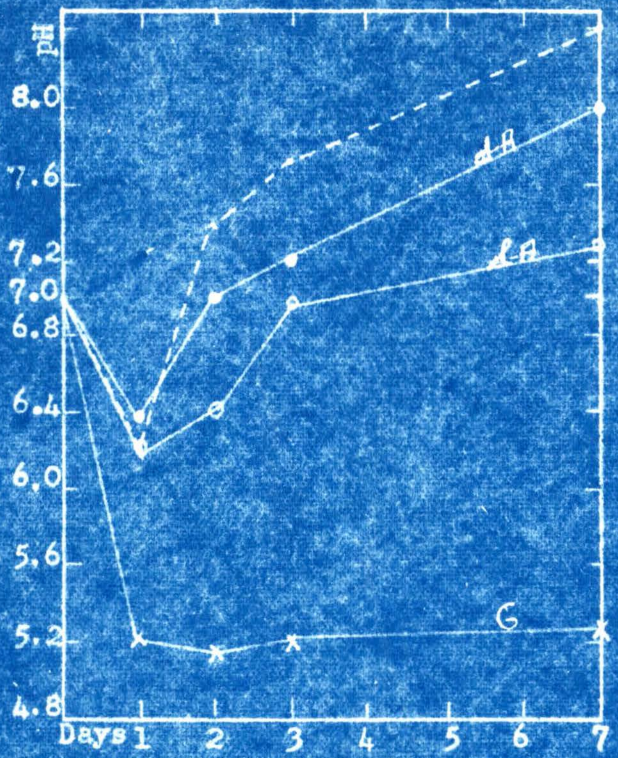
B. albolactis V.P.I.

Fig. 4



B. albolactis Tex.

Fig. 5



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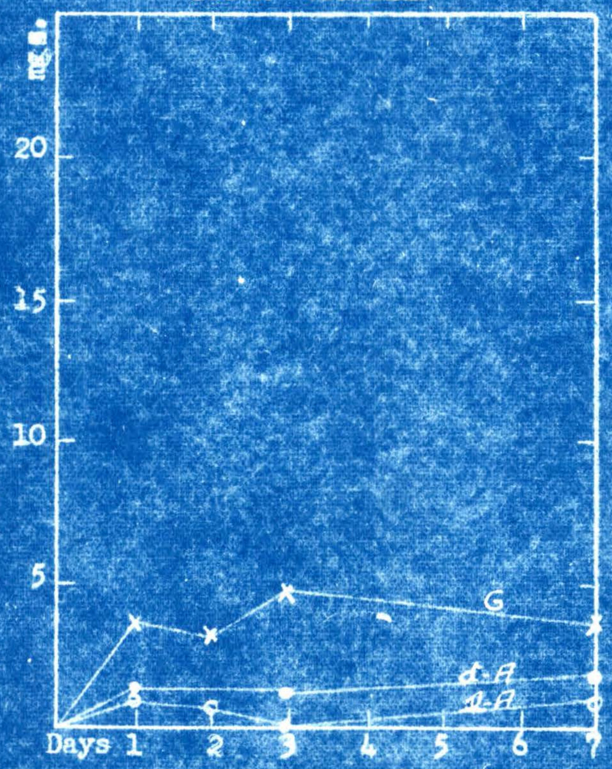
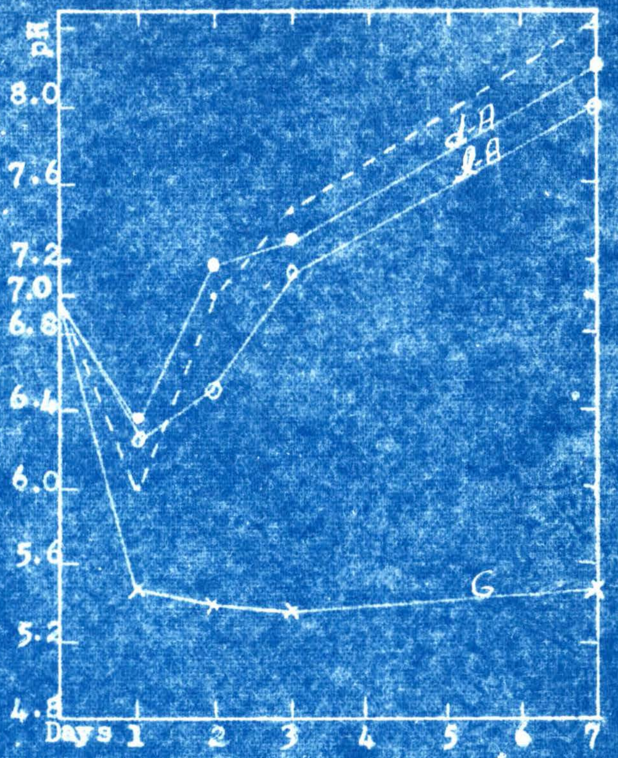


B. alvei A. T. C.

Fig. 6



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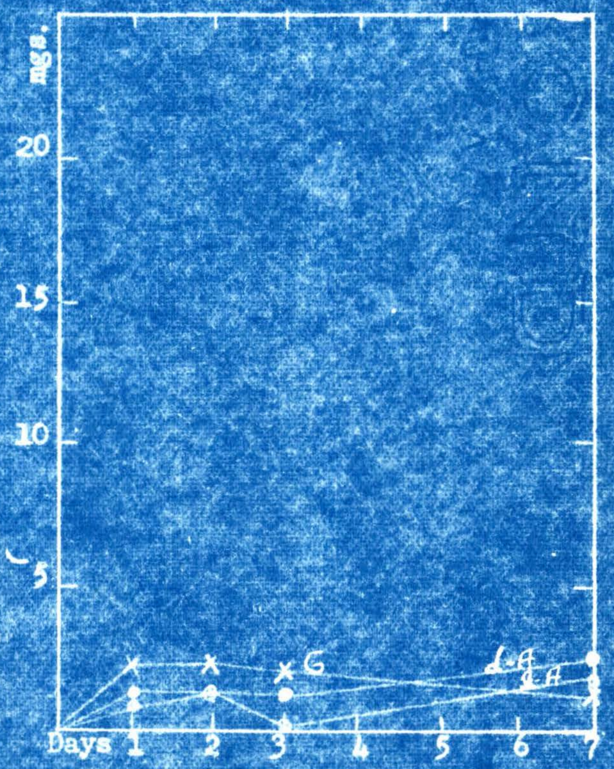
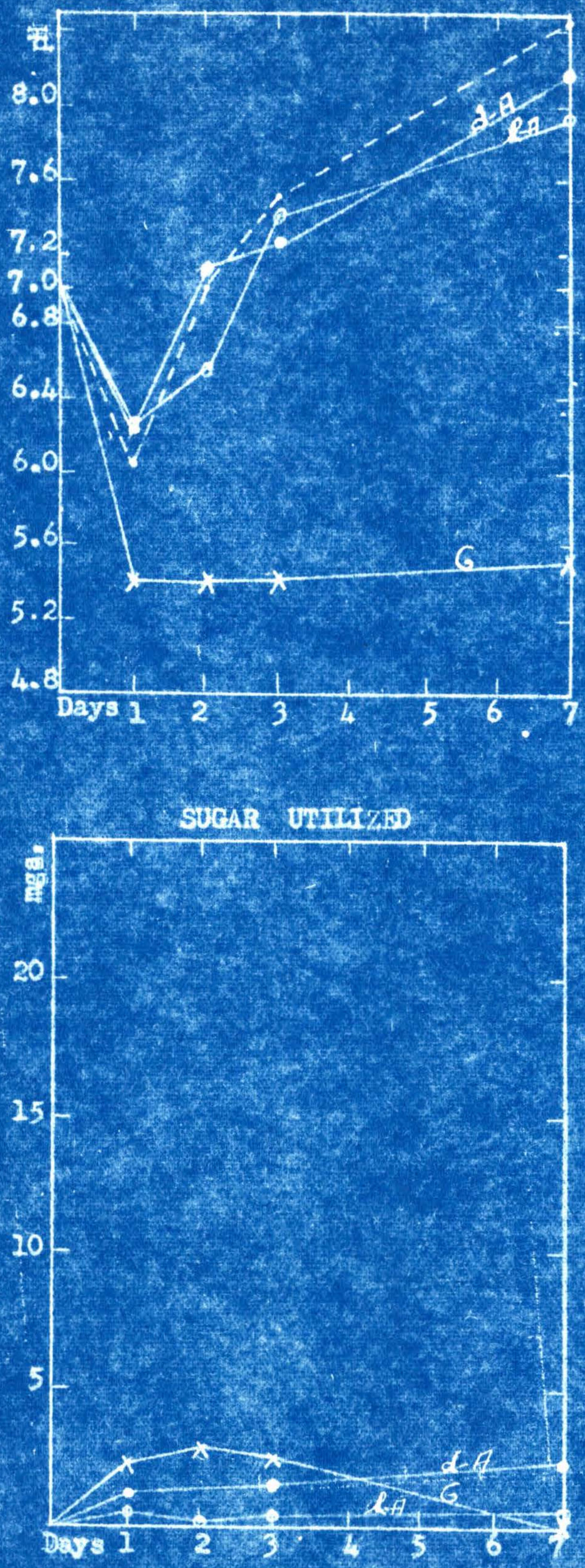


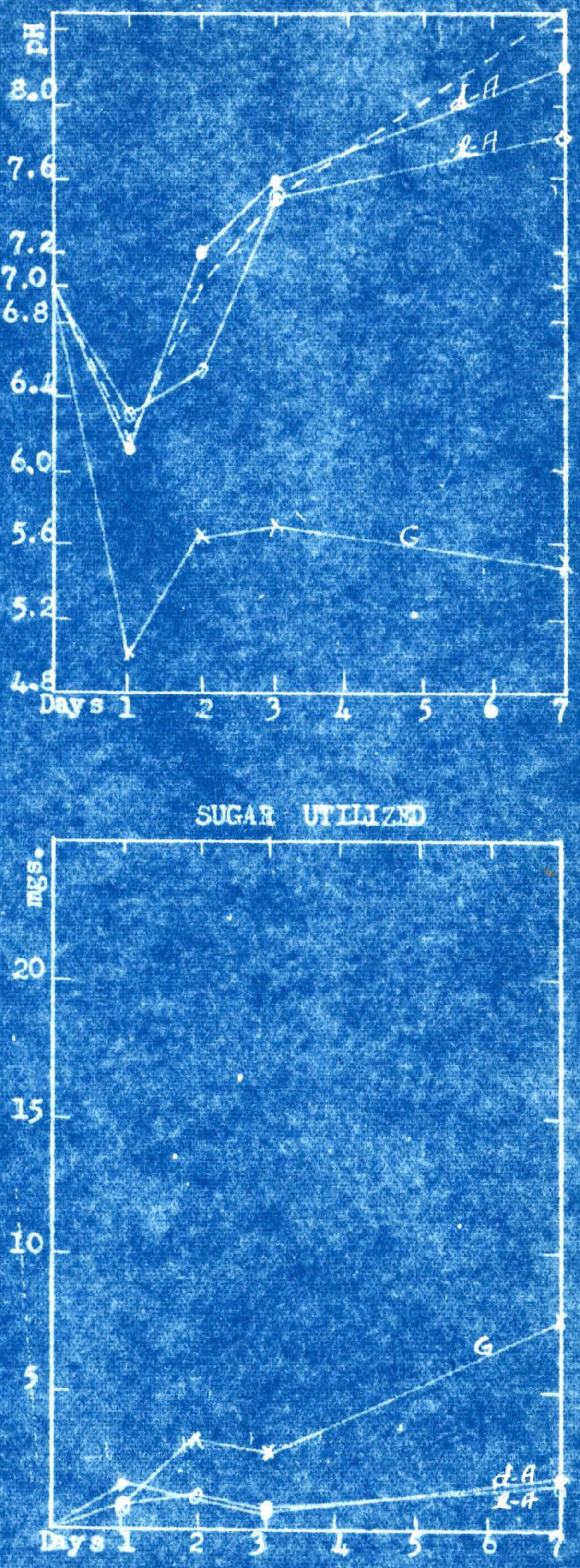
B. alvei Tex.

Fig. 7



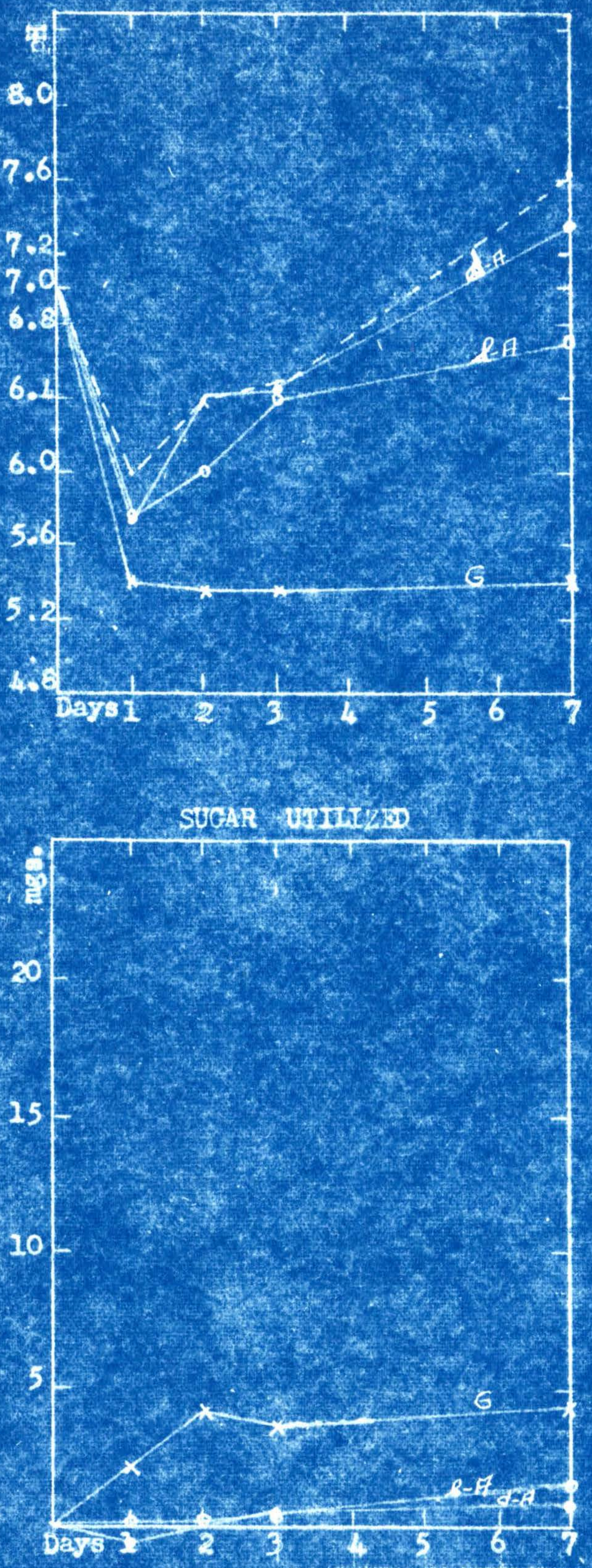
B. alvei #622 Tex.

Fig. 8



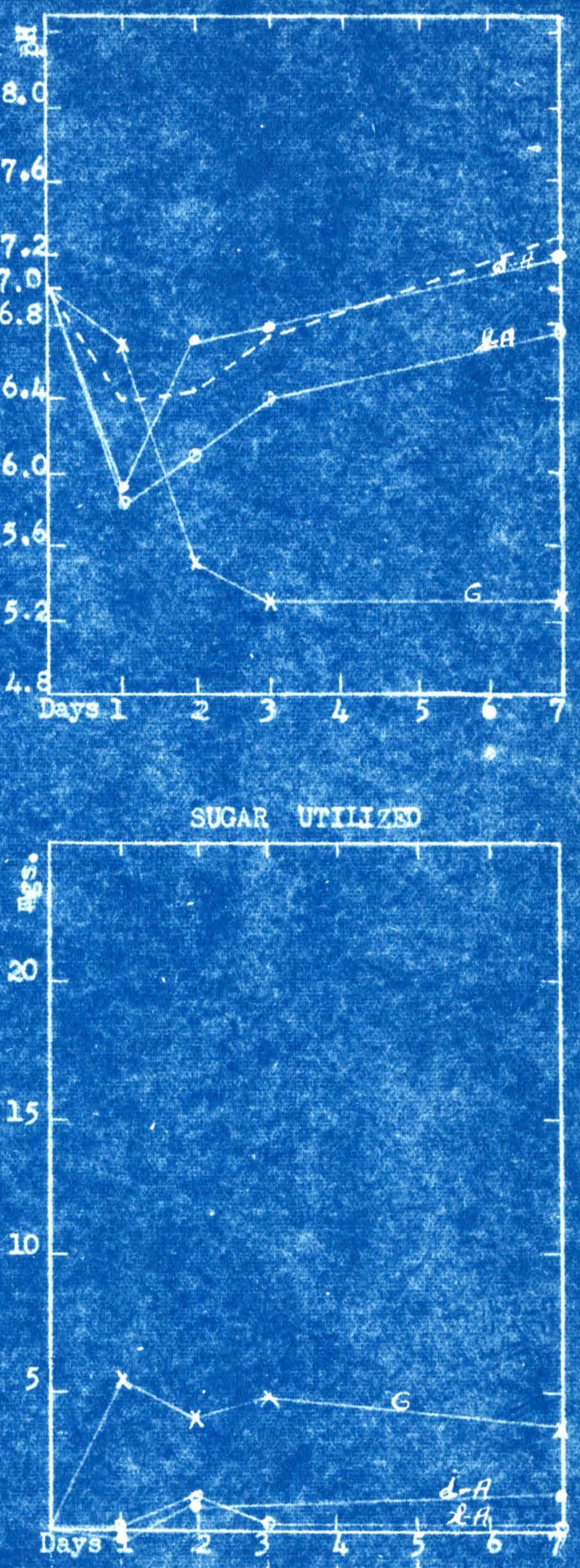
B. alvei #622 Lockheed, Tex.

Fig. 9



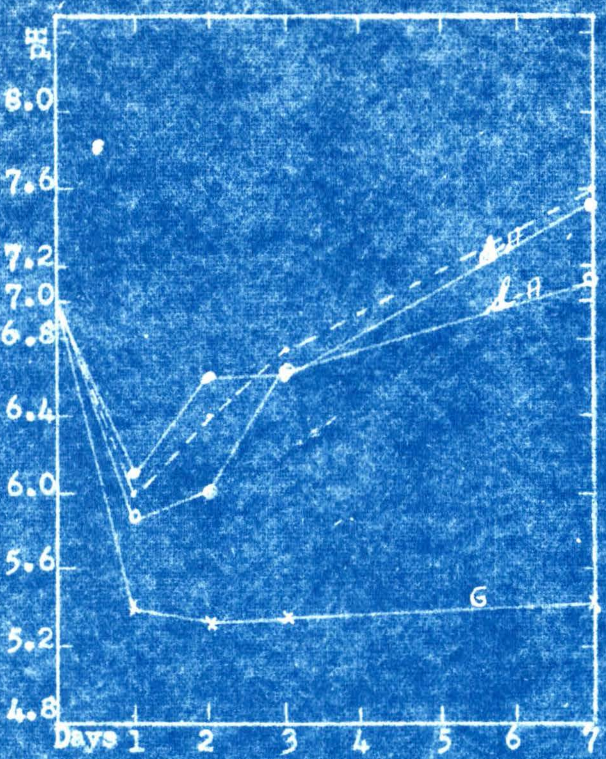
B. anthracis Wis.

Fig. 10

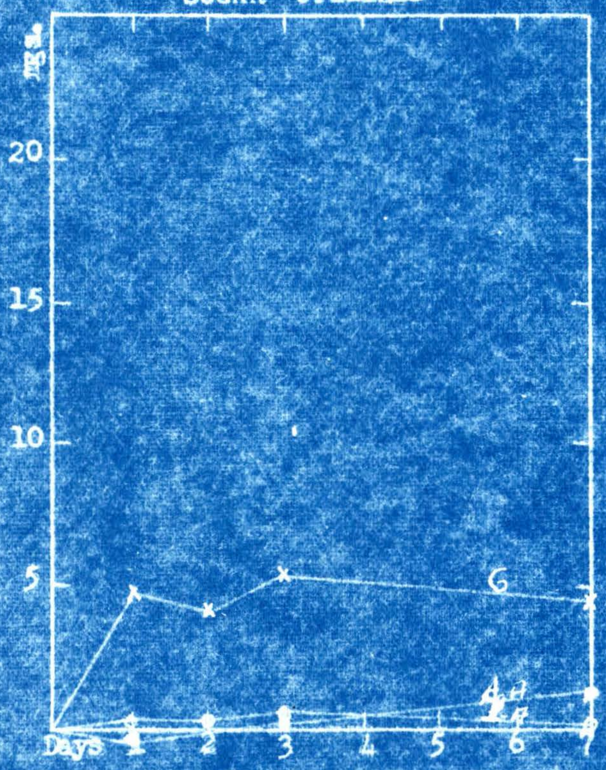


B. anthracis Tex.

Fig. 11

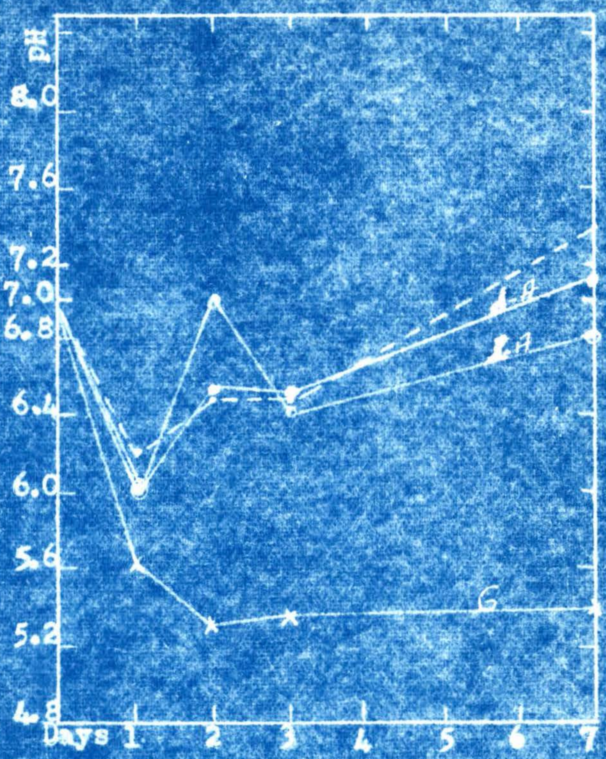


SUGAR UTILIZED



B. anthracis (Wis.) Tex.

Fig. 12



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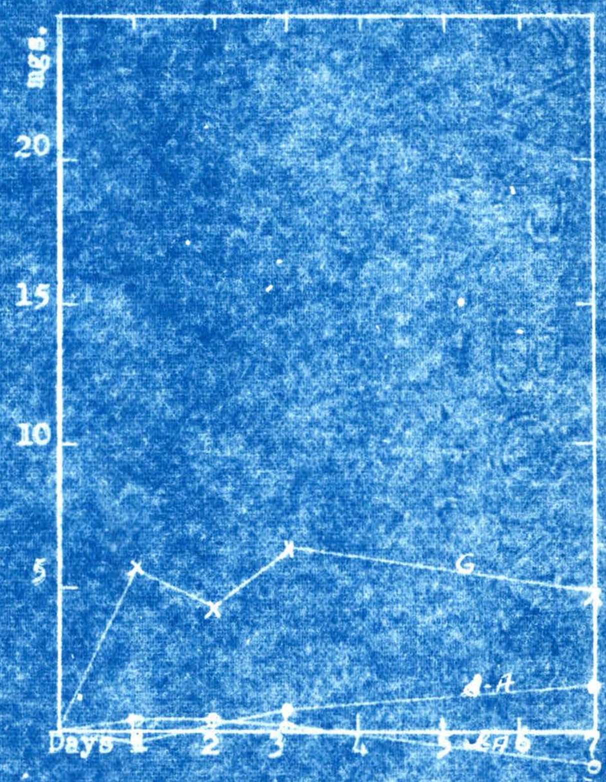
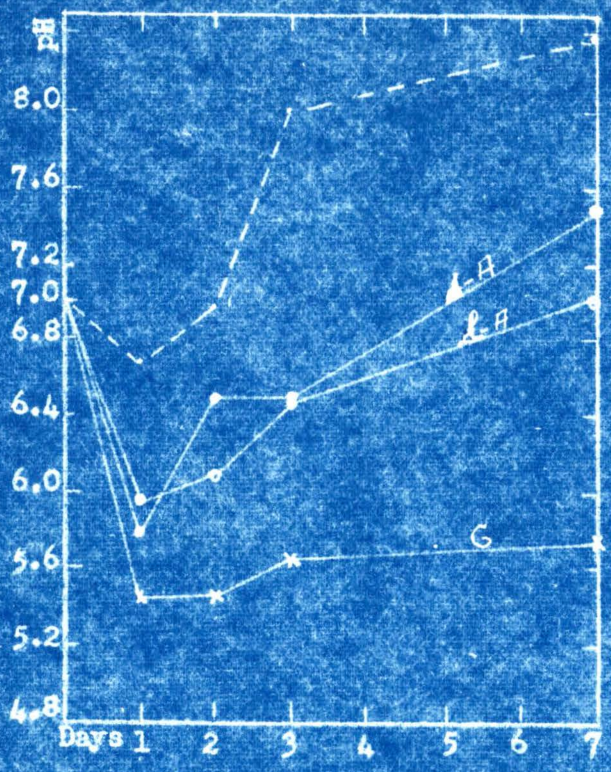
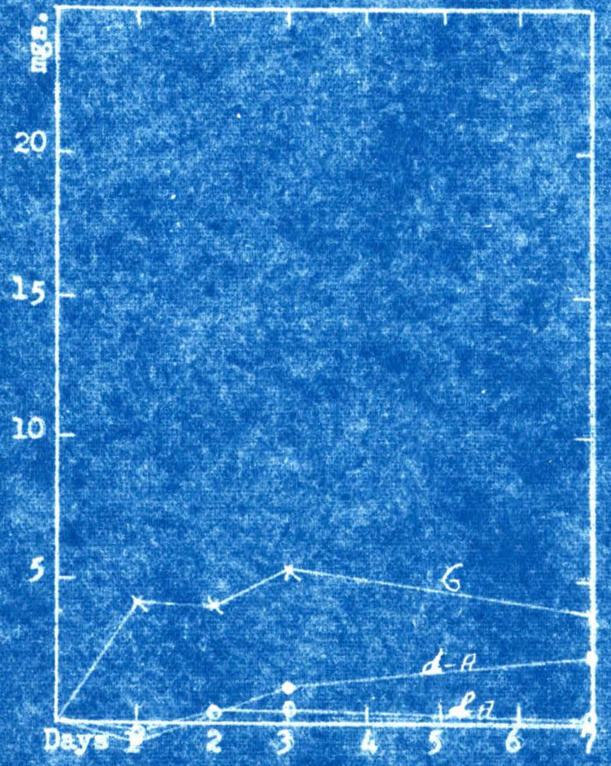


B. anthracis Rall. Tex.

Fig. 13

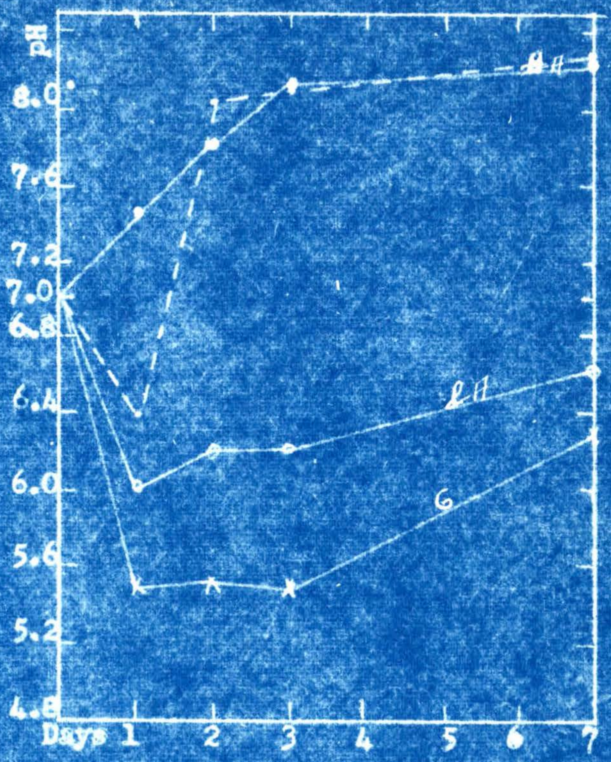


SUGAR UTILIZED

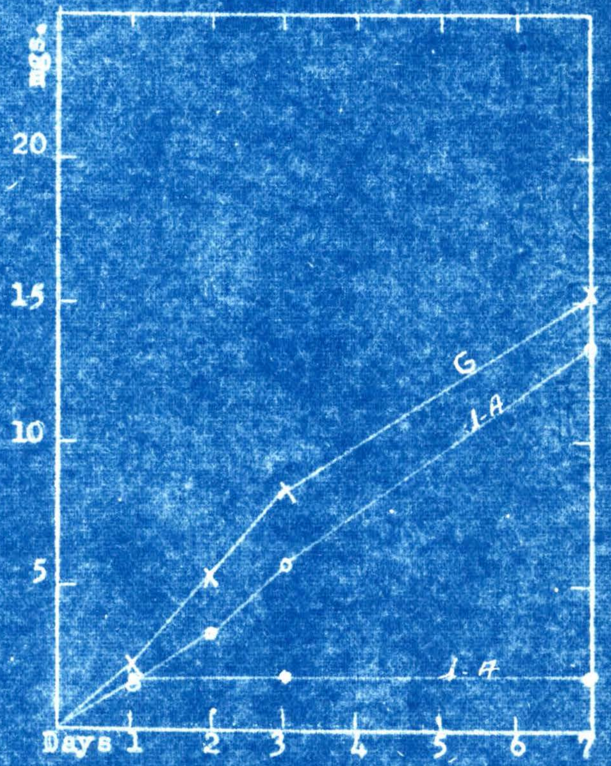


B. anthracis (Smooth) Tex.

Fig. 14



SUGAR UTILIZED



B. aterrimus #353 Wis.

Fig. 15

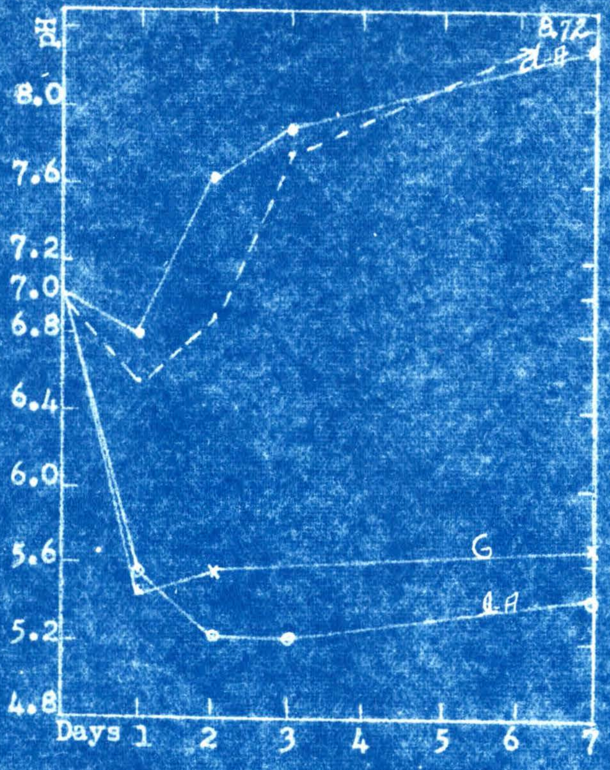
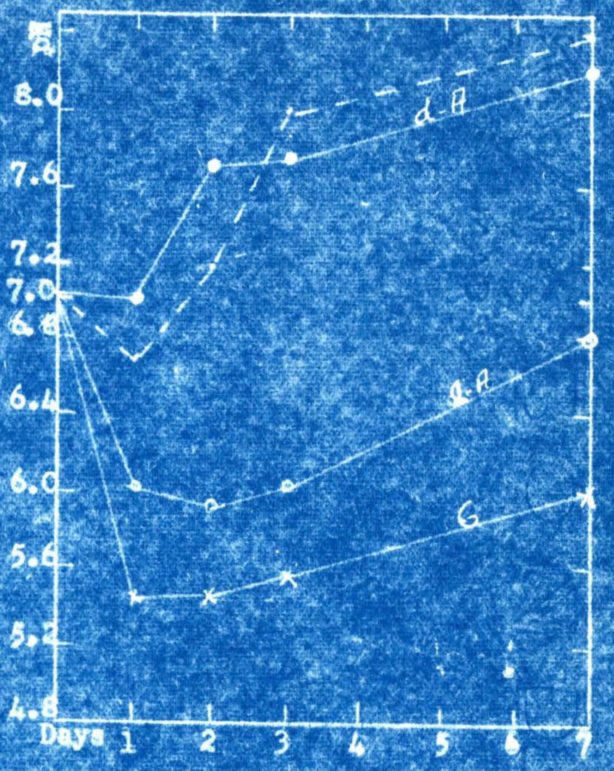


Fig. 16



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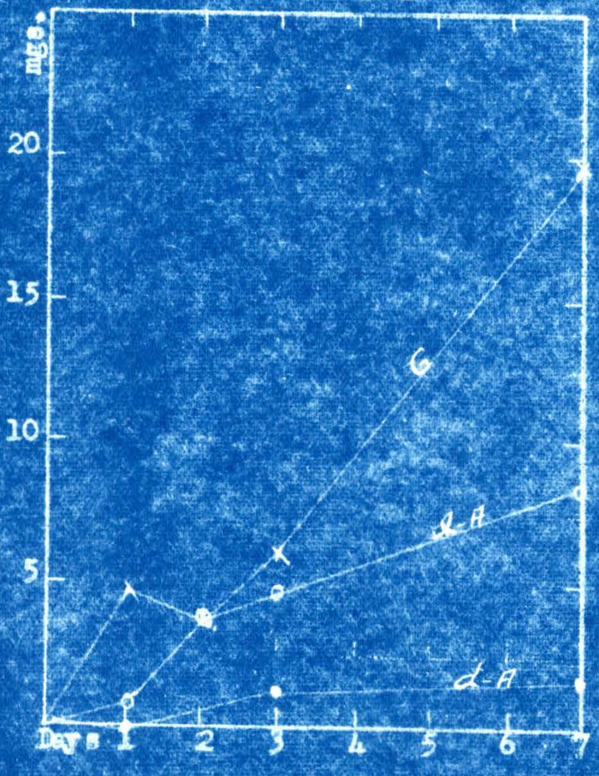
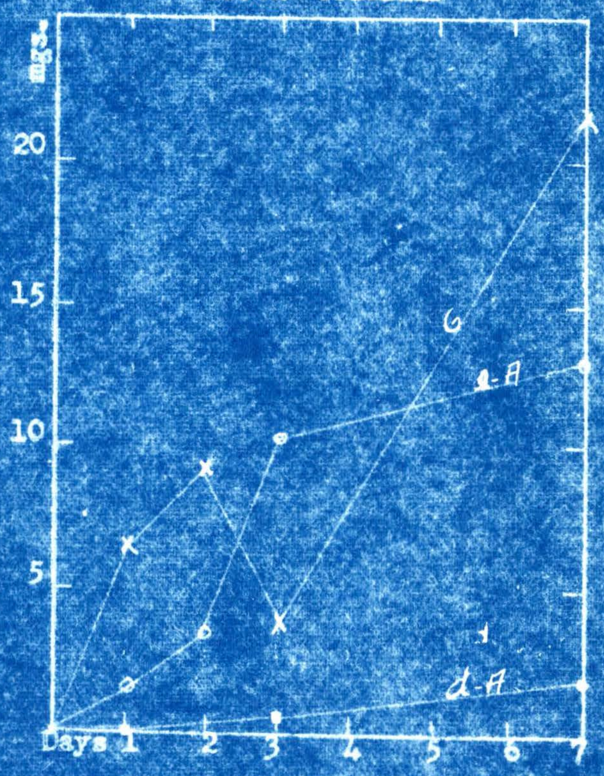


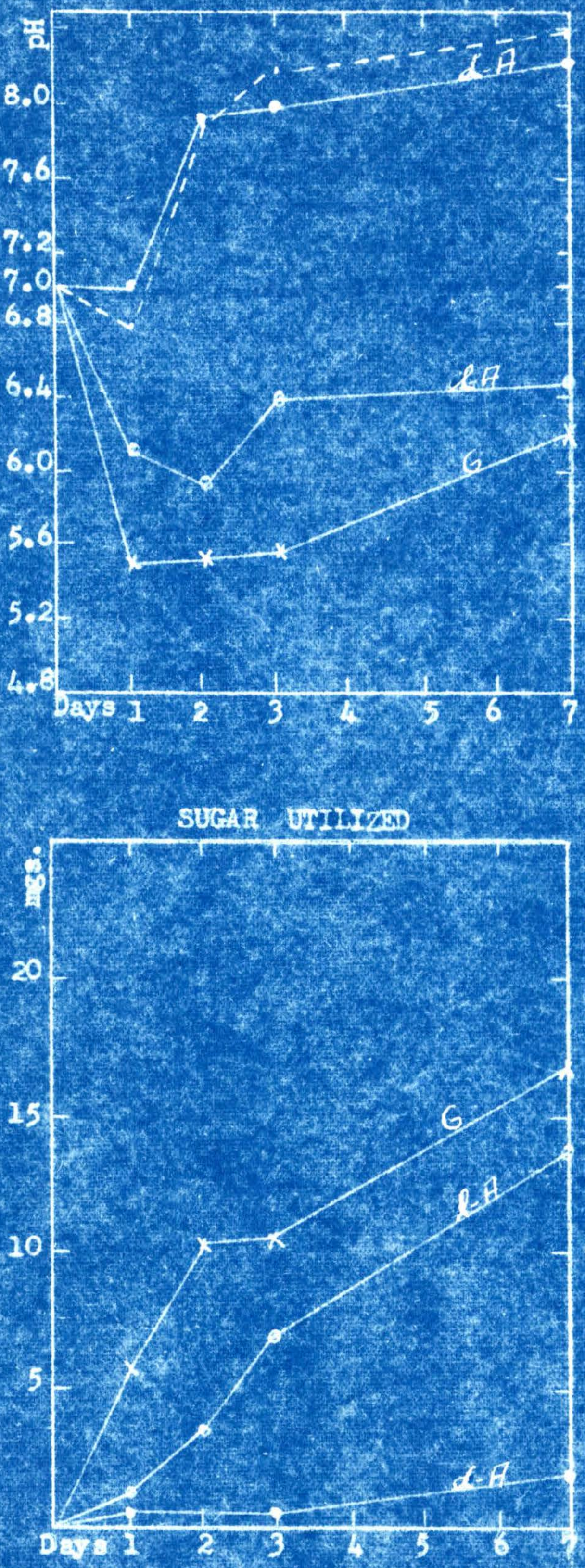
SUGAR UTILIZED



B. <u>aterrimus</u> U.S.D.A.

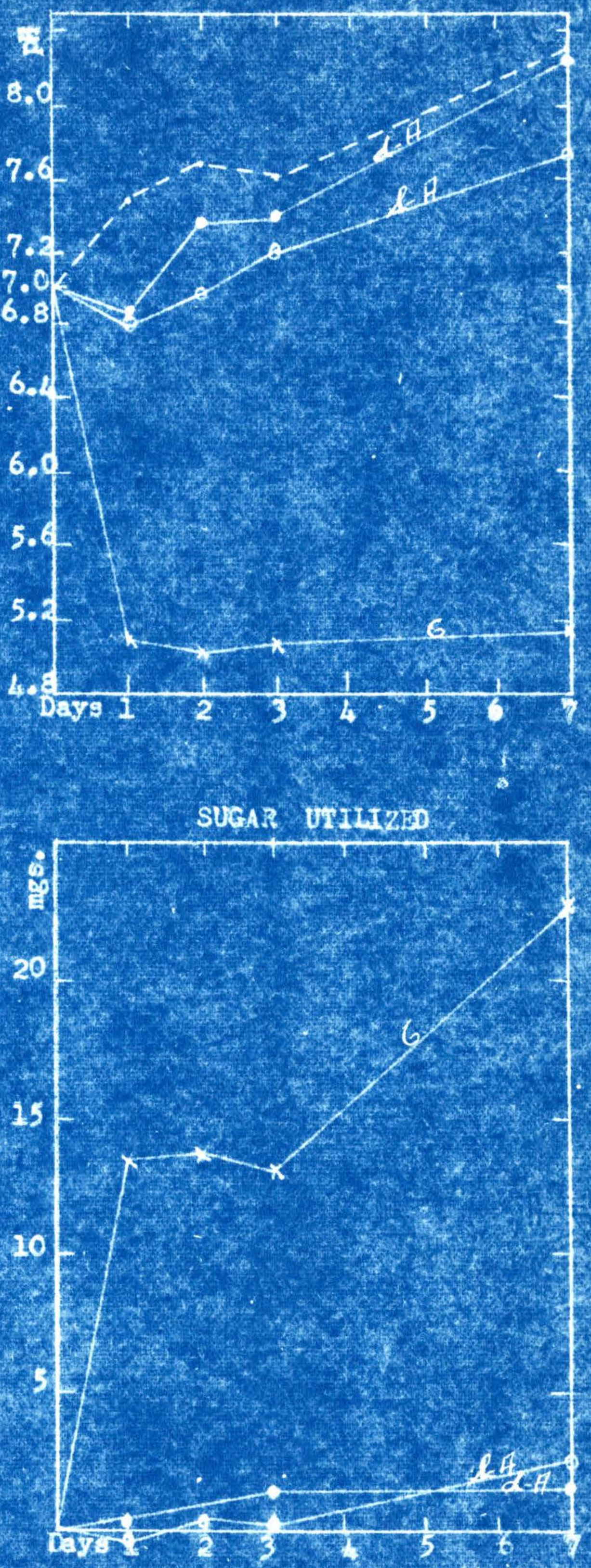
B. <u>atterimus</u> Pesek, Wis.

Fig. 17



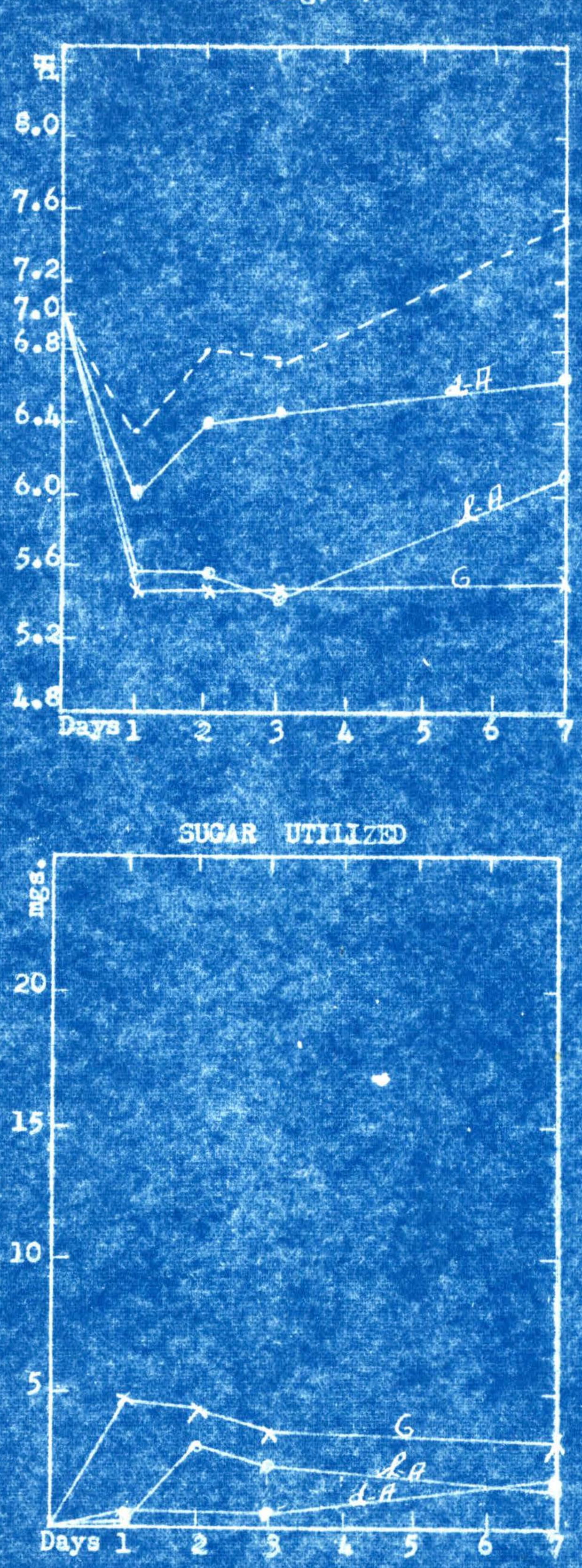
B. aterrimis R.C. Wis.

Fig. 18



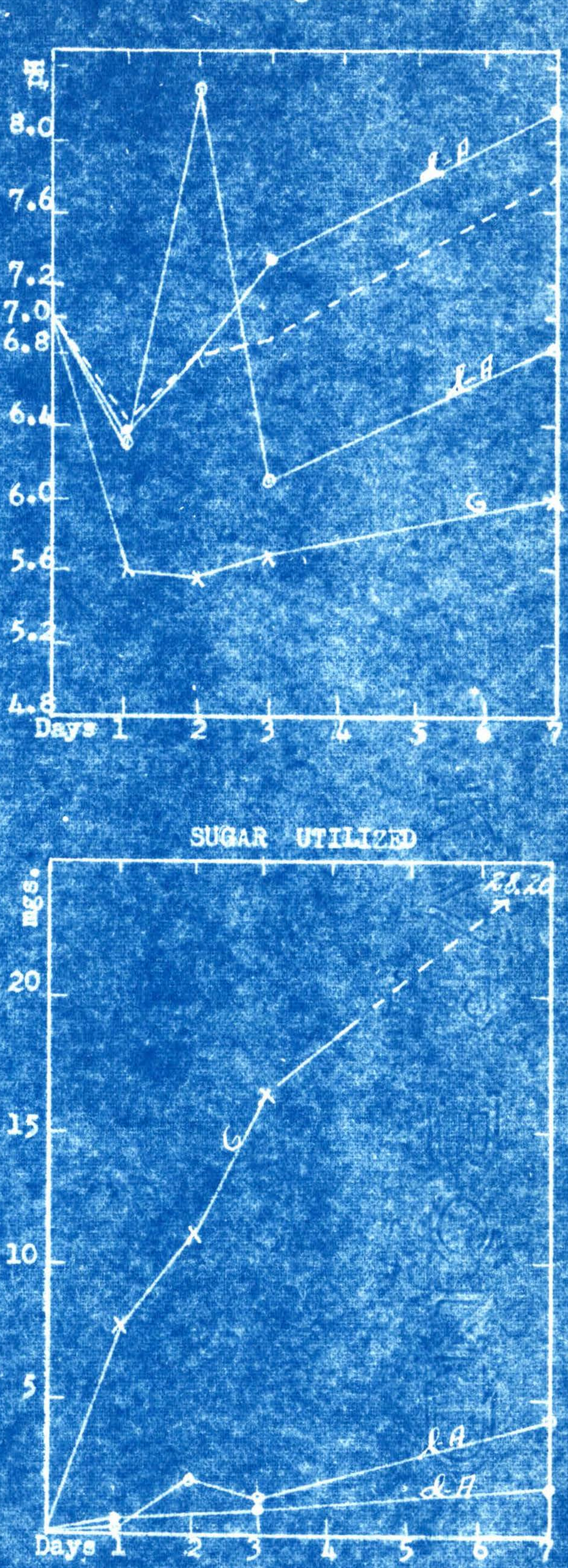
B. cereus #1 A.T.C.

Fig. 19



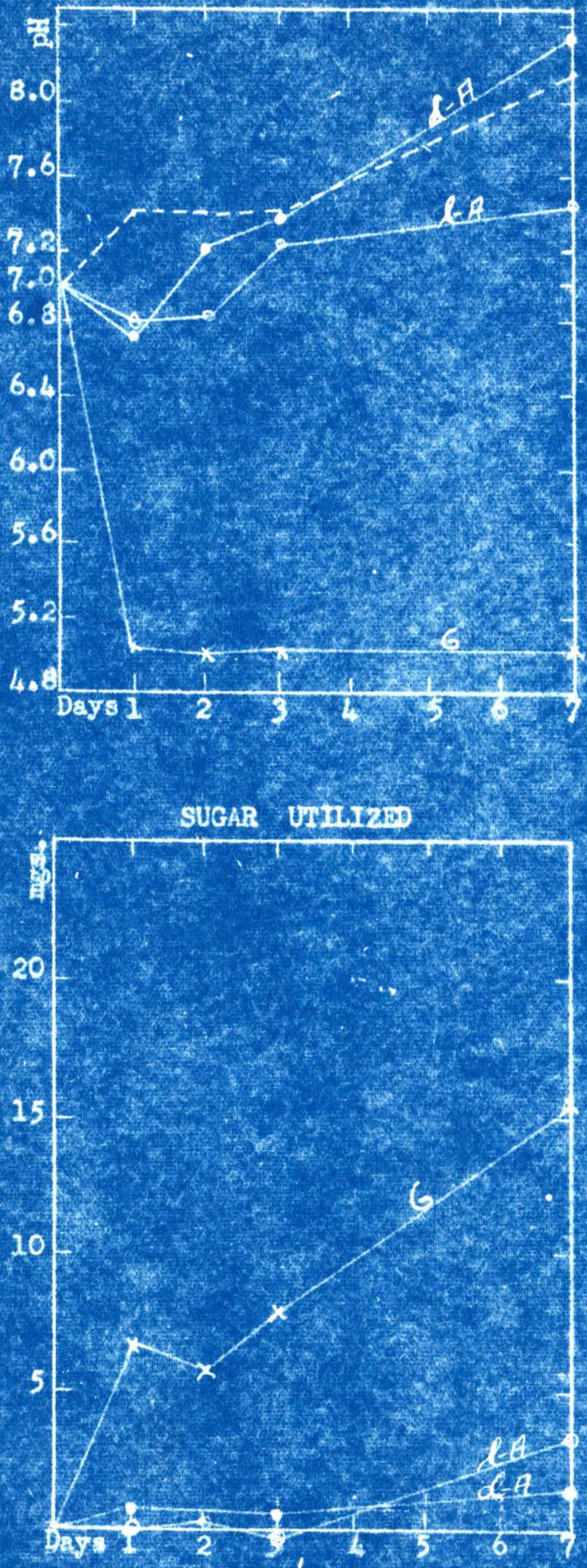
B. cereus #2 A.T.C.

Fig. 20



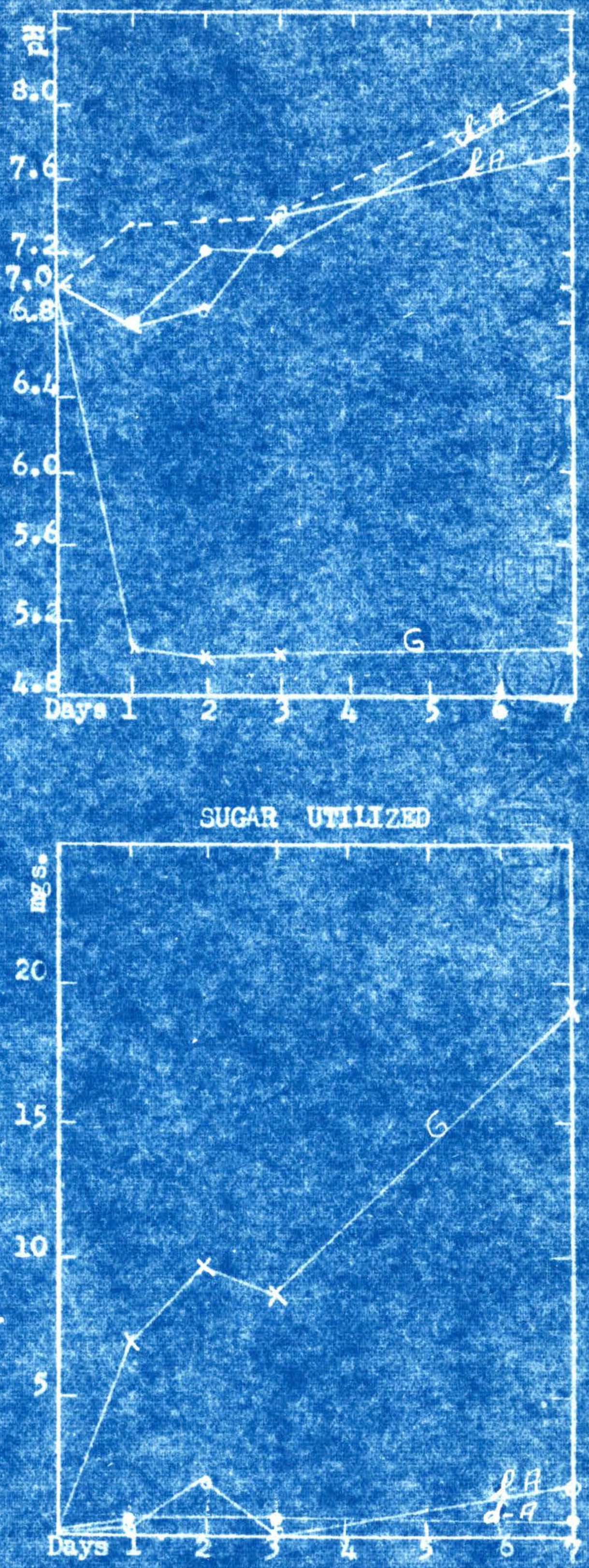
B. cereus Tex.

Fig. 21



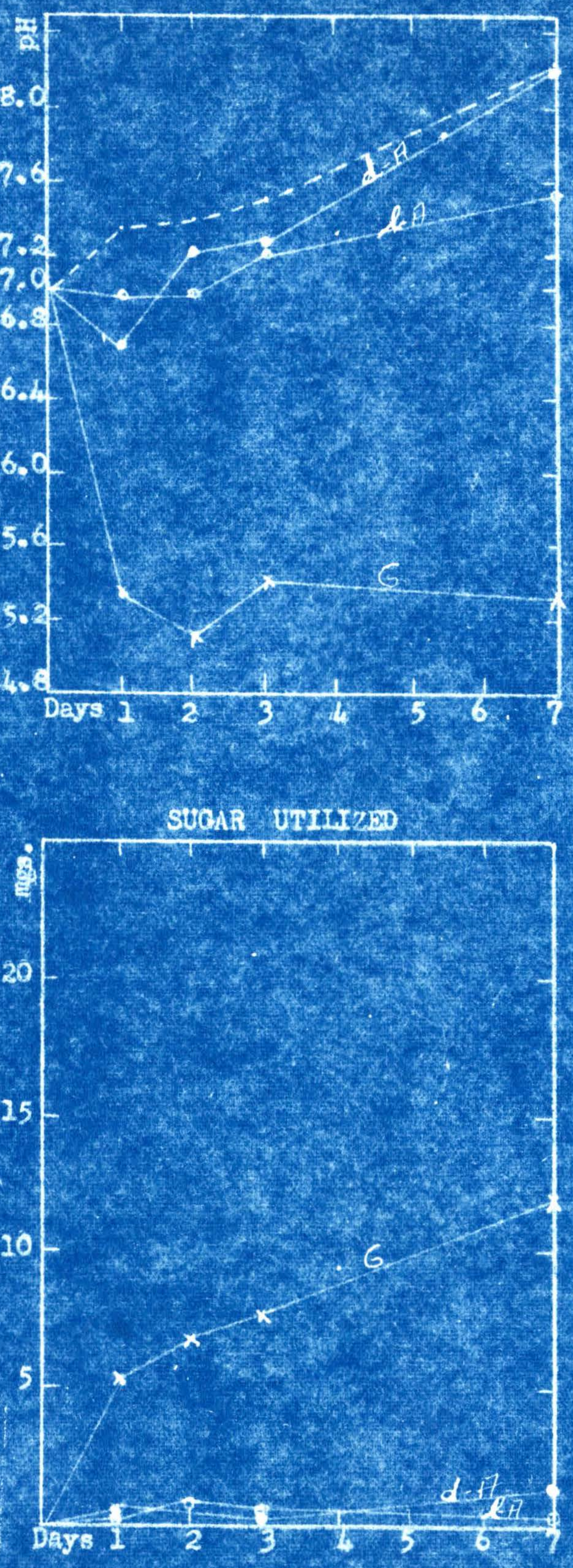
B. cereus (Stock) Tex.

Fig. 22



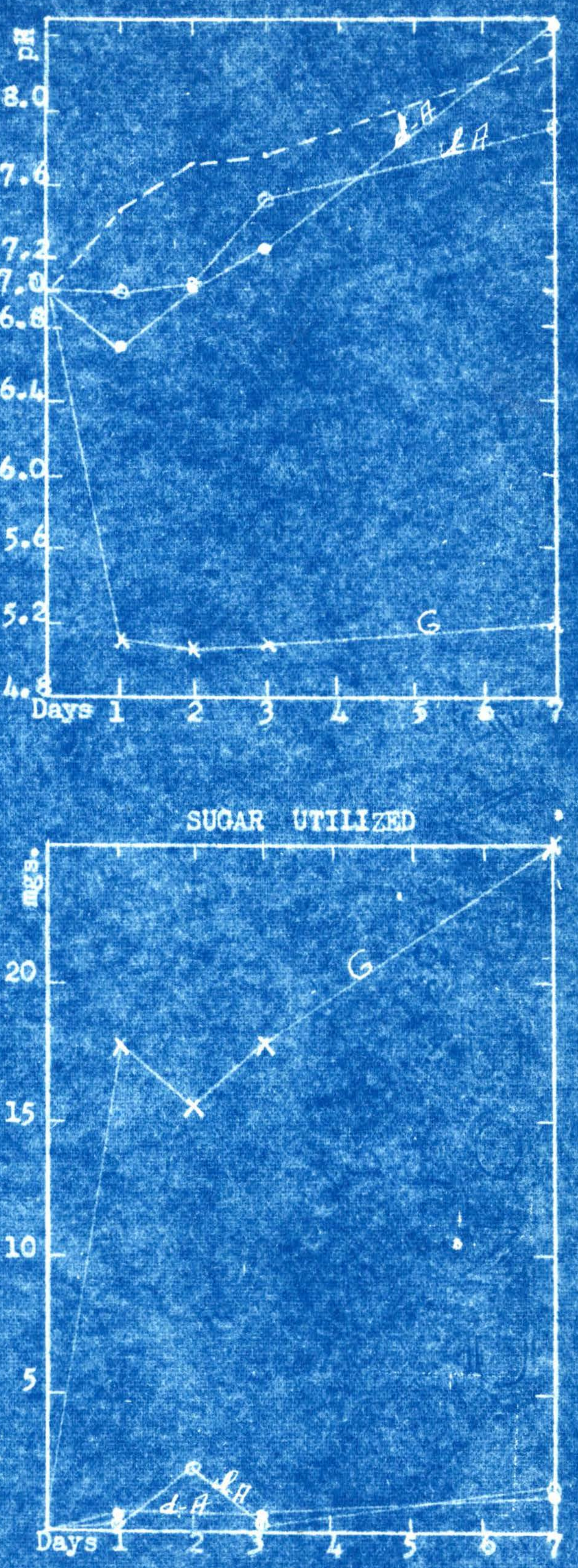
B. cereus (Non-Motile) Tex.

Fig. 23



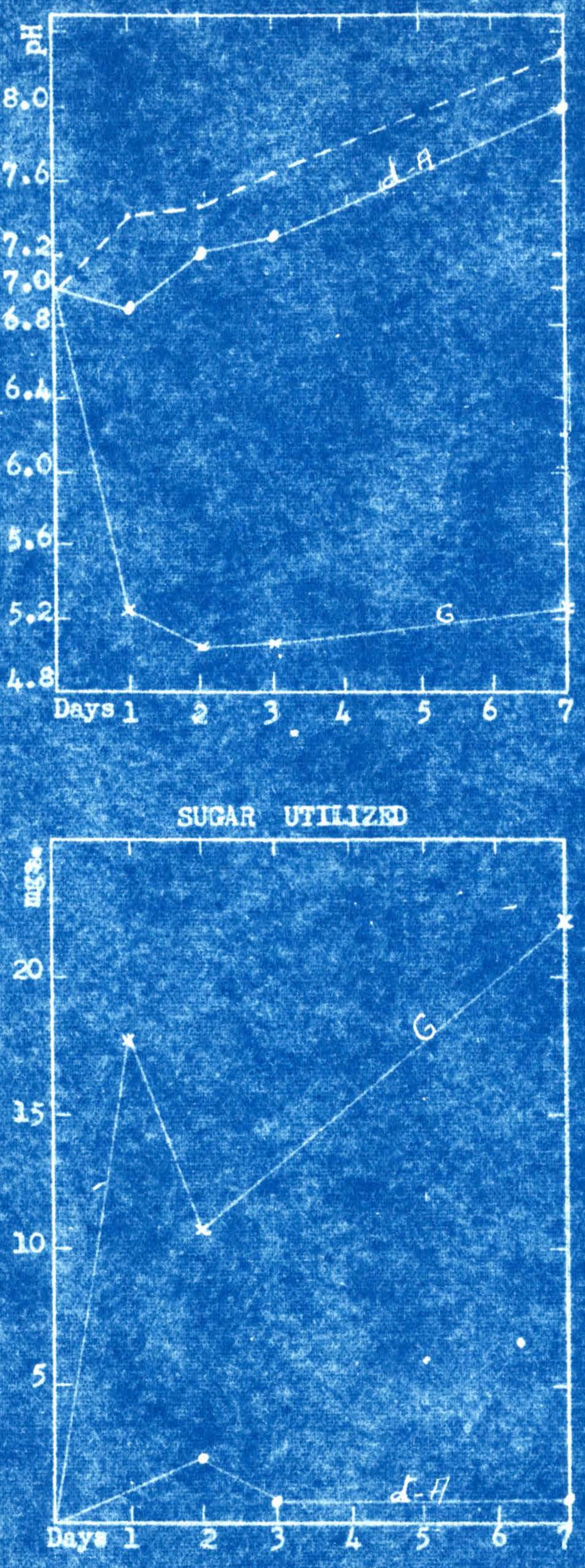
B. cereus #244 Tex.

Fig. 24



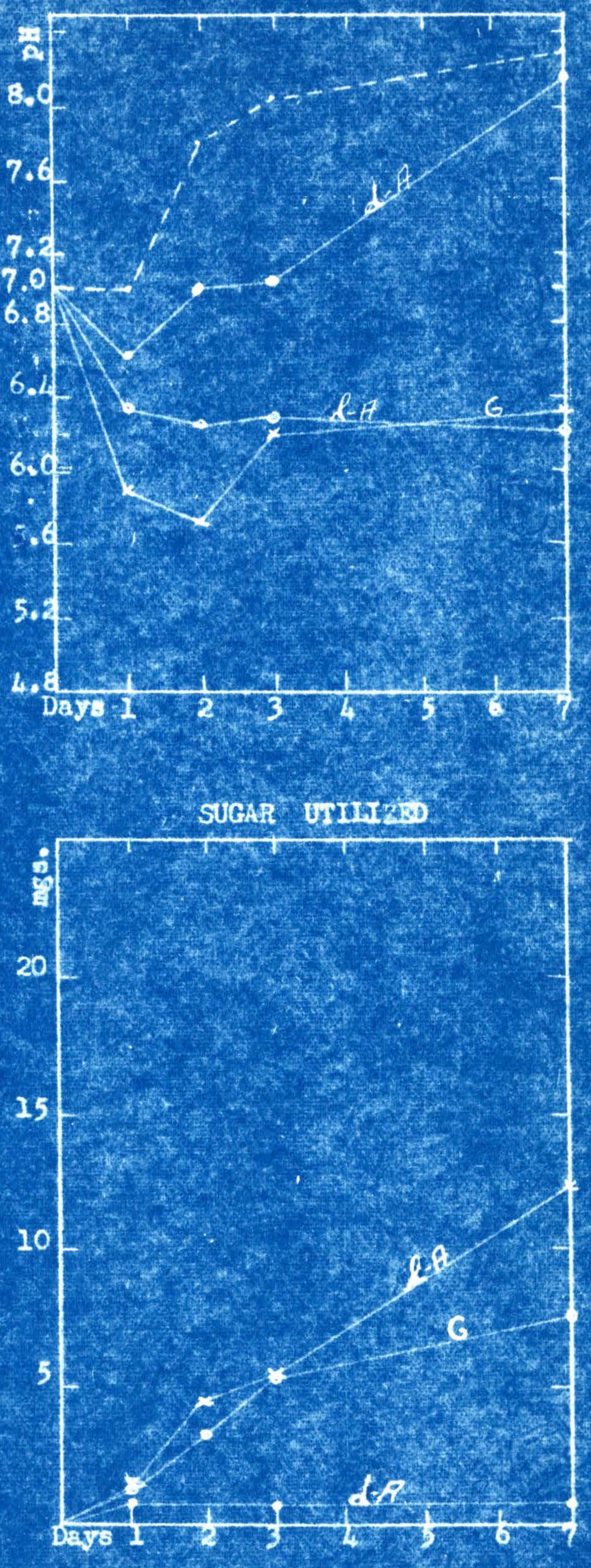
B. cereus Clark Tex.

Fig. 25



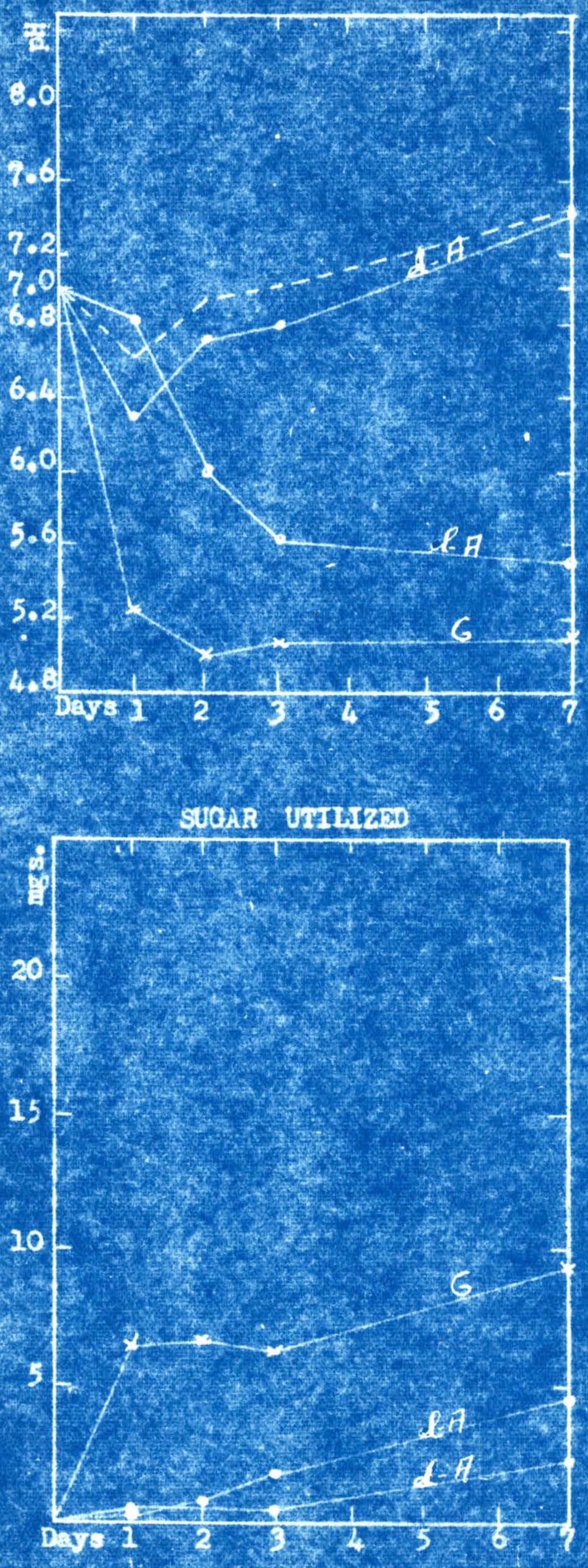
B. cereus Neb.

Fig. 26



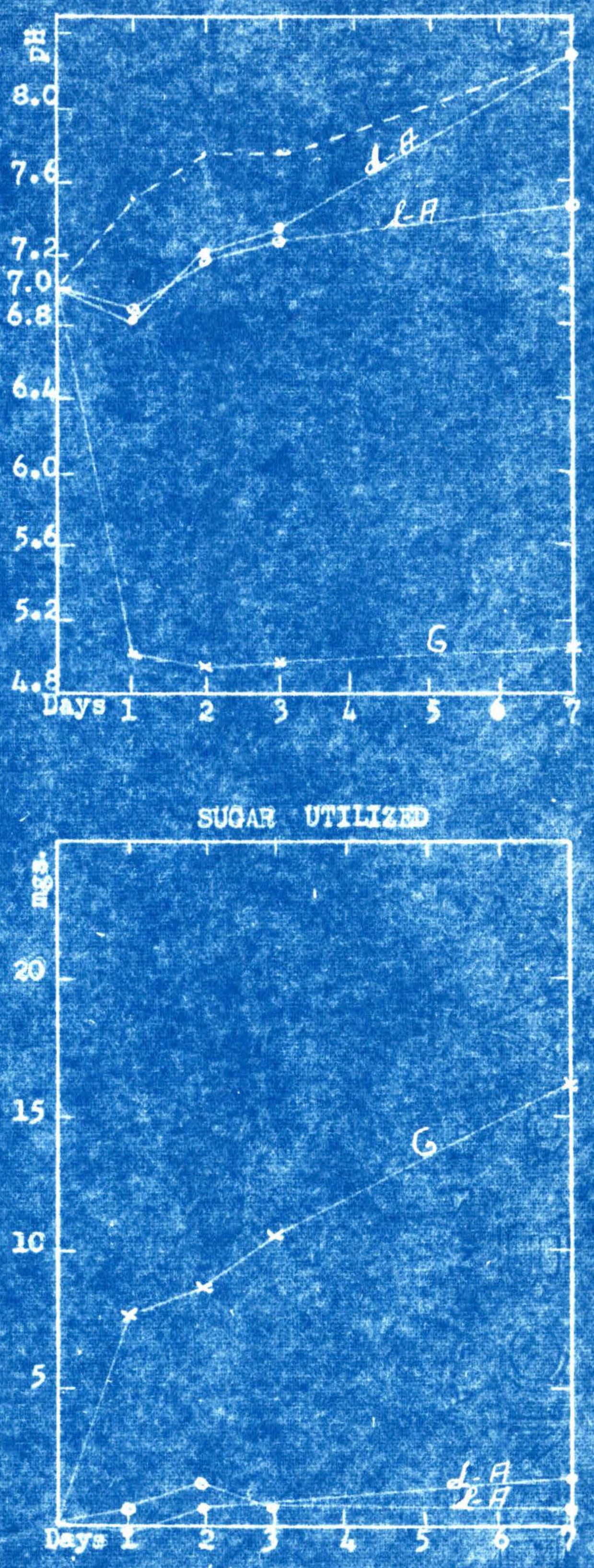
B. cereus Smith-Thom Tex.

Fig. 27



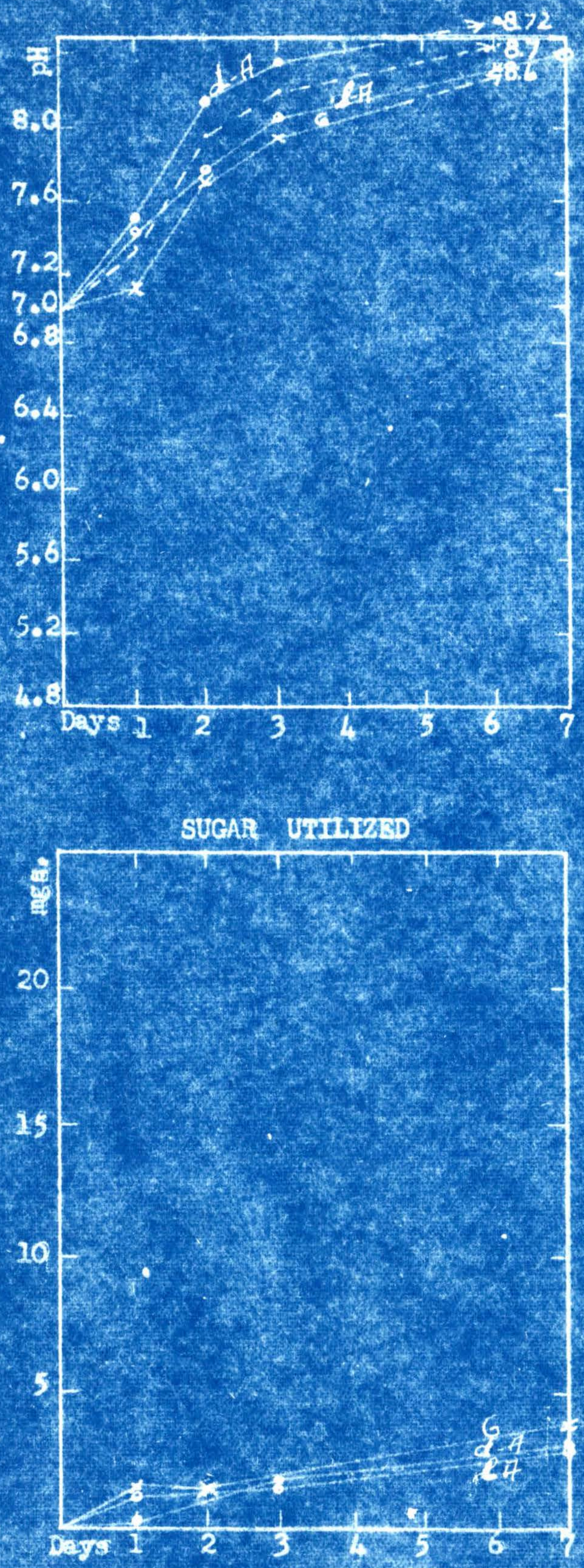
B. cereus Wis.

Fig. 28



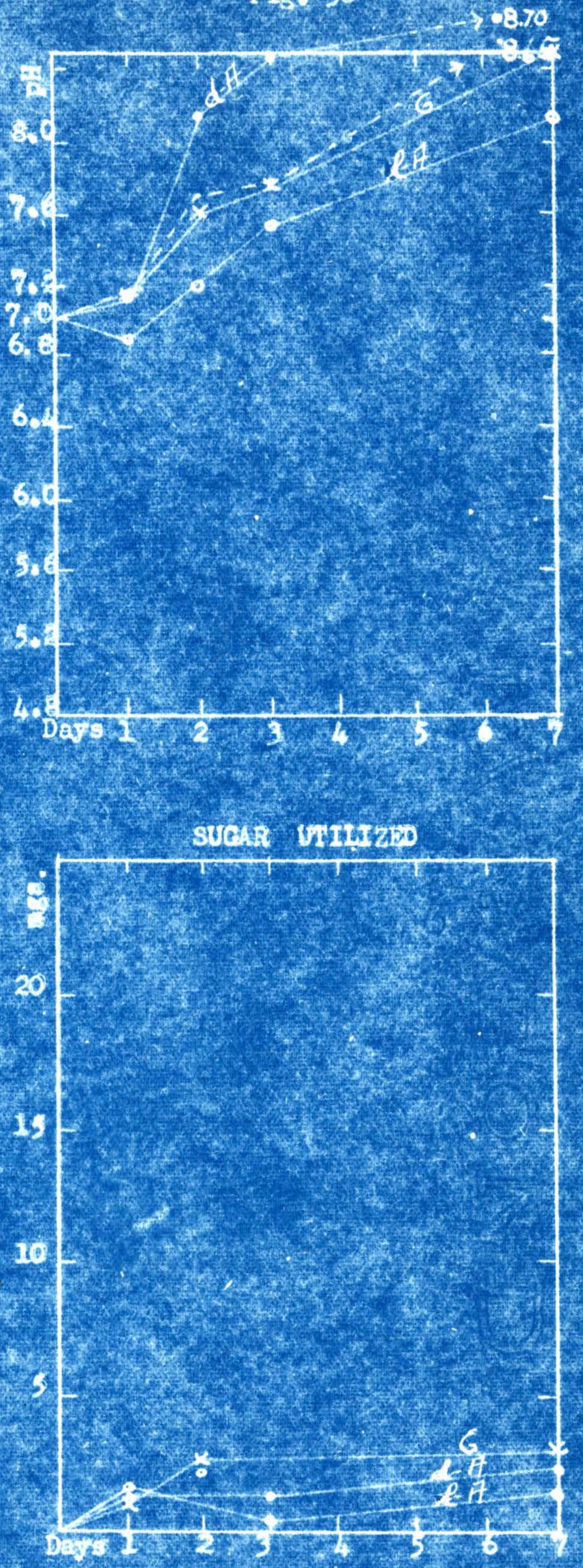
B. cereus A.T.C. Wis.

Fig. 29



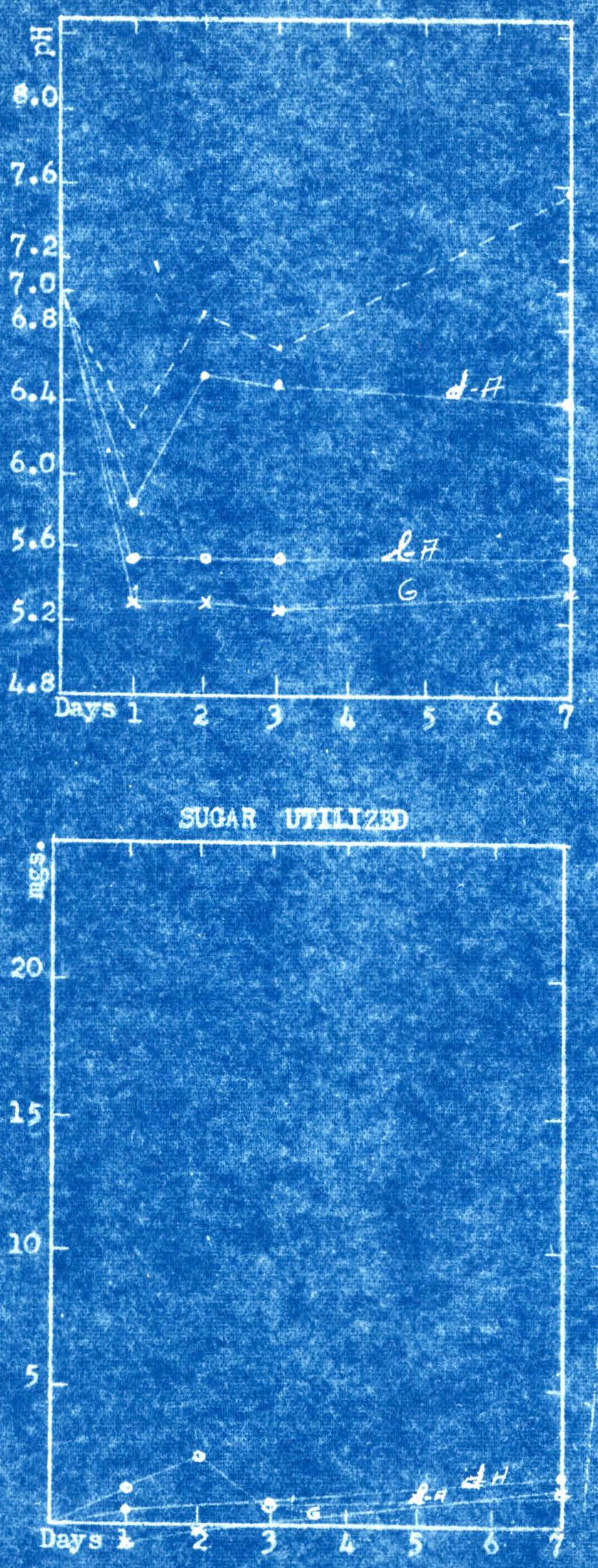
B. brevis #604 A.T.C.

Fig. 30



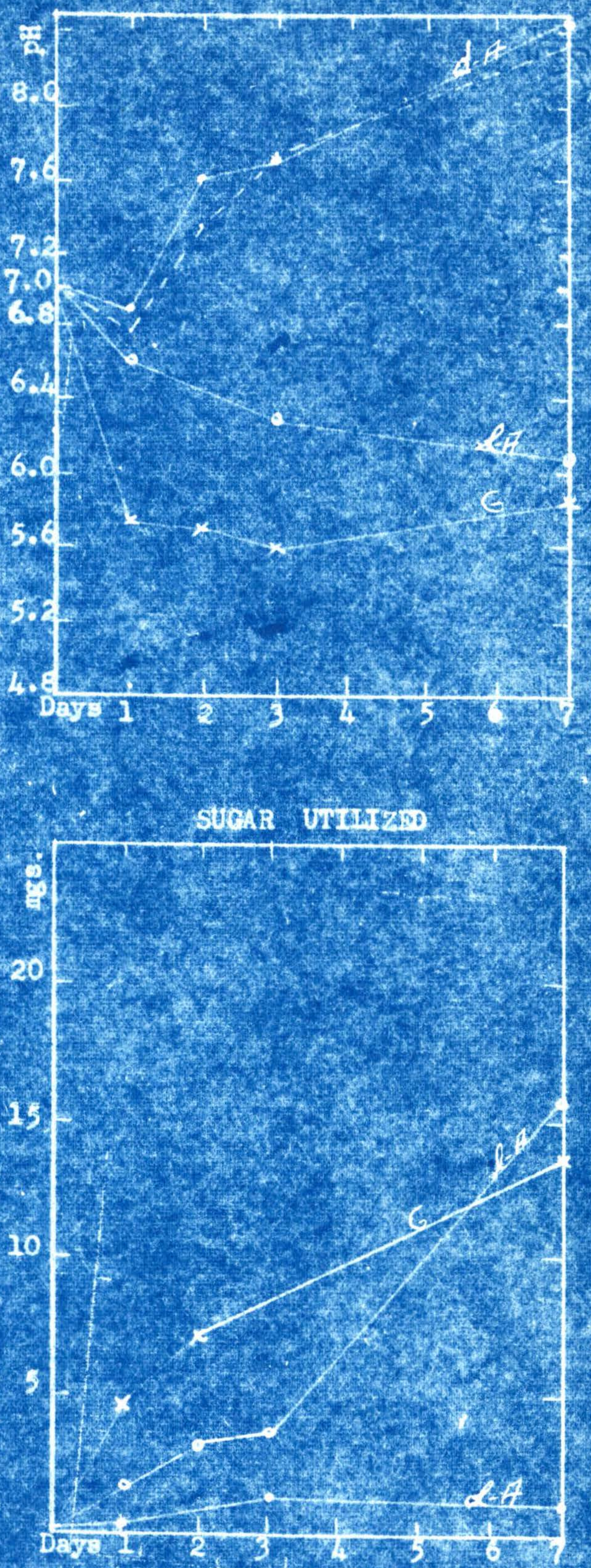
B. brevis U.S.D.A.

Fig. 31



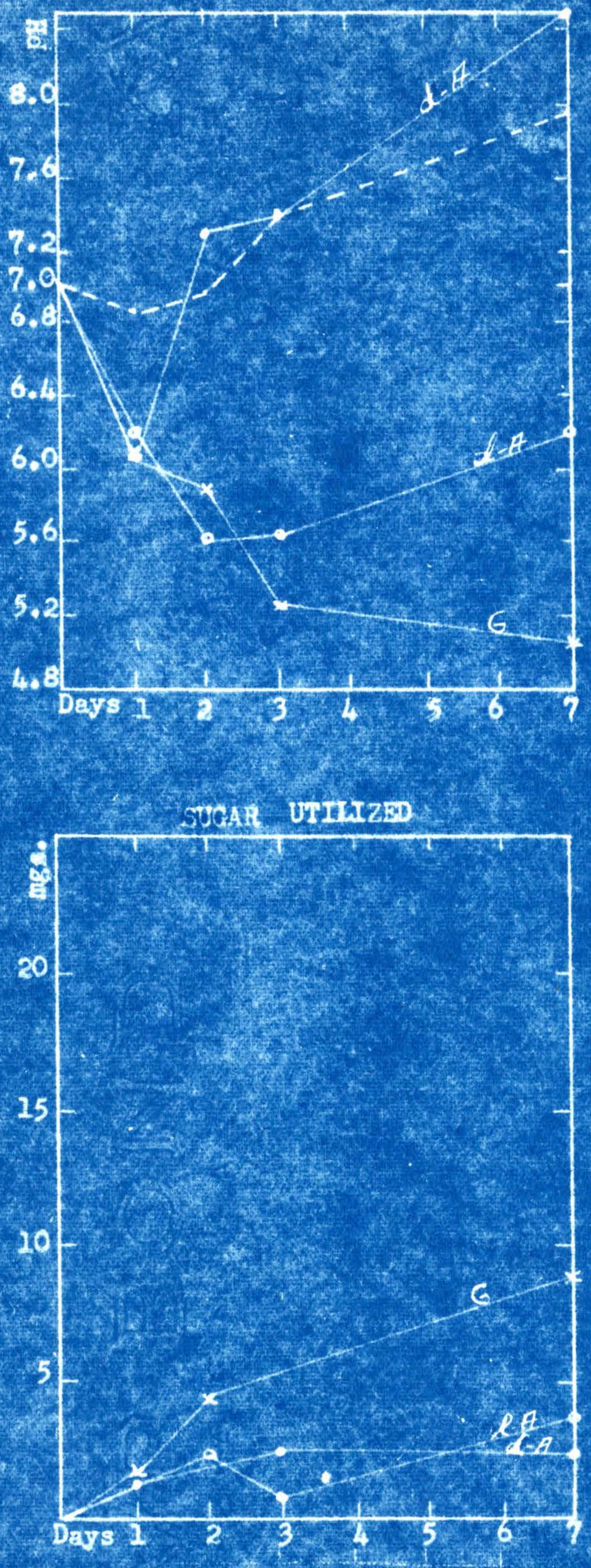
B. circulans A. T.C.

Fig. 32



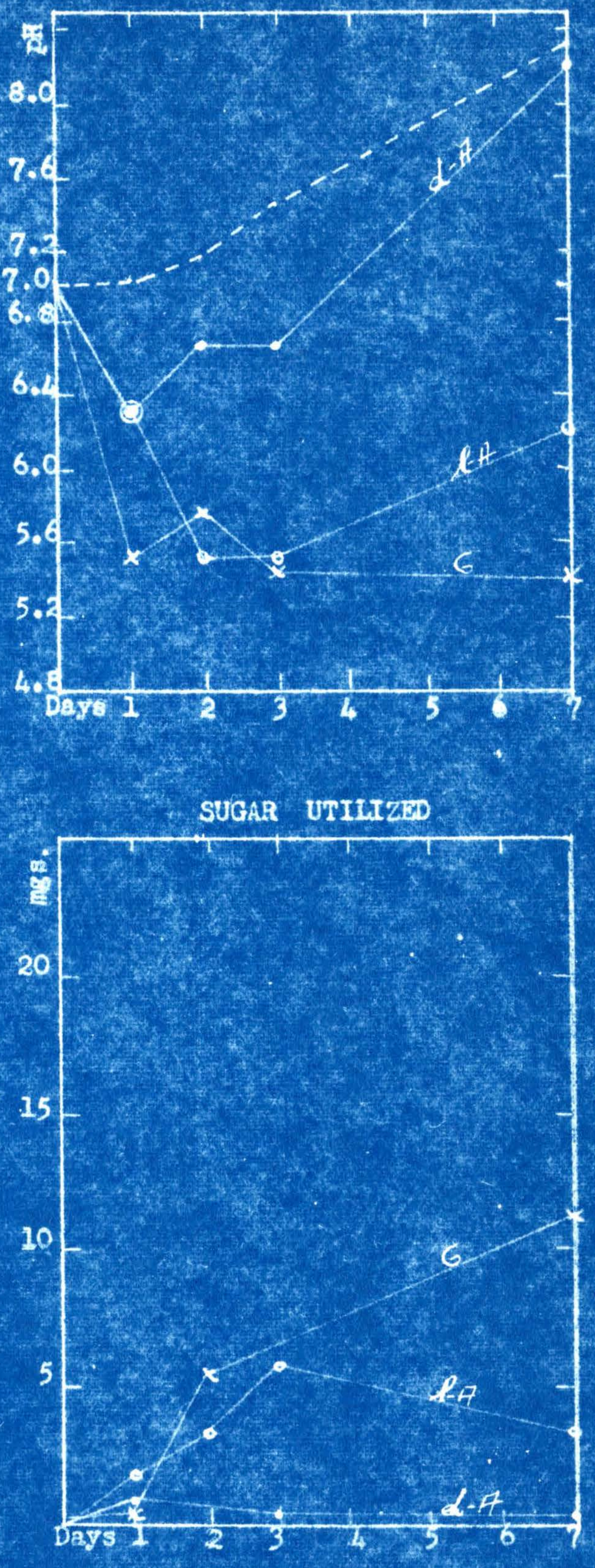
B. cohaerens #840 Tex.

Fig. 33



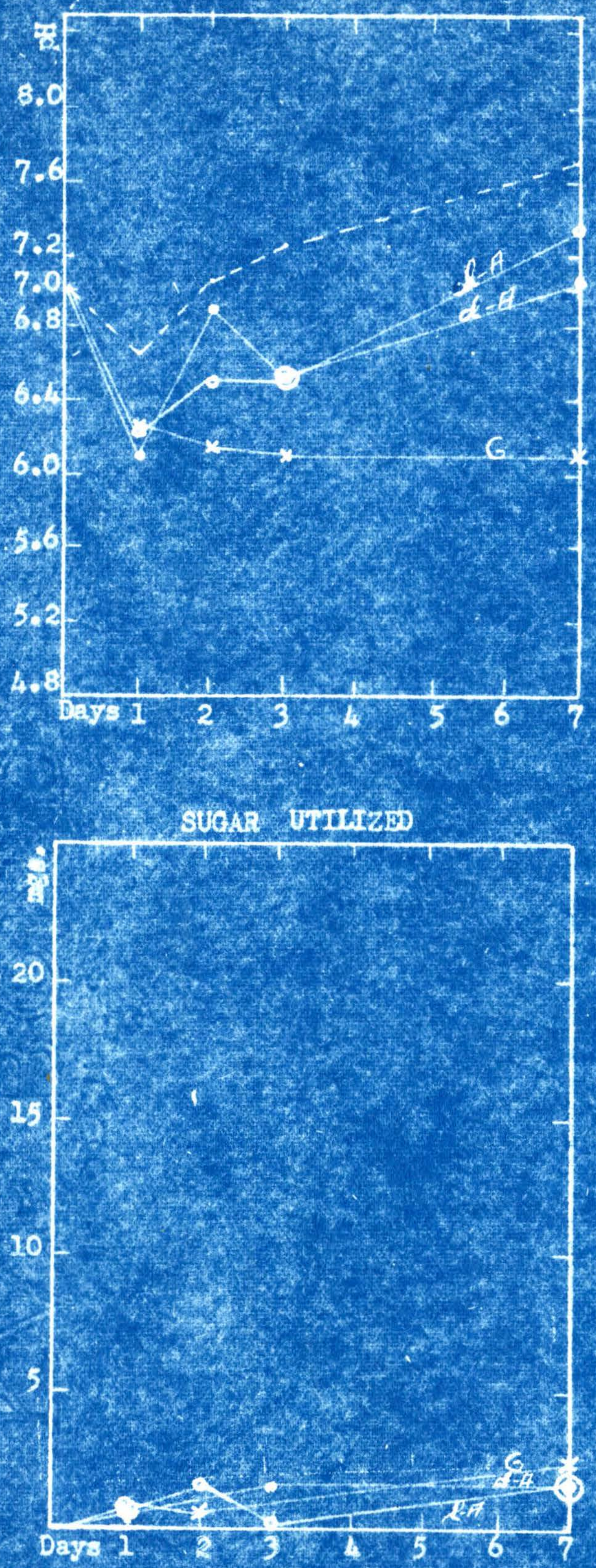
B. danicus U.S.D.A.

Fig. 34



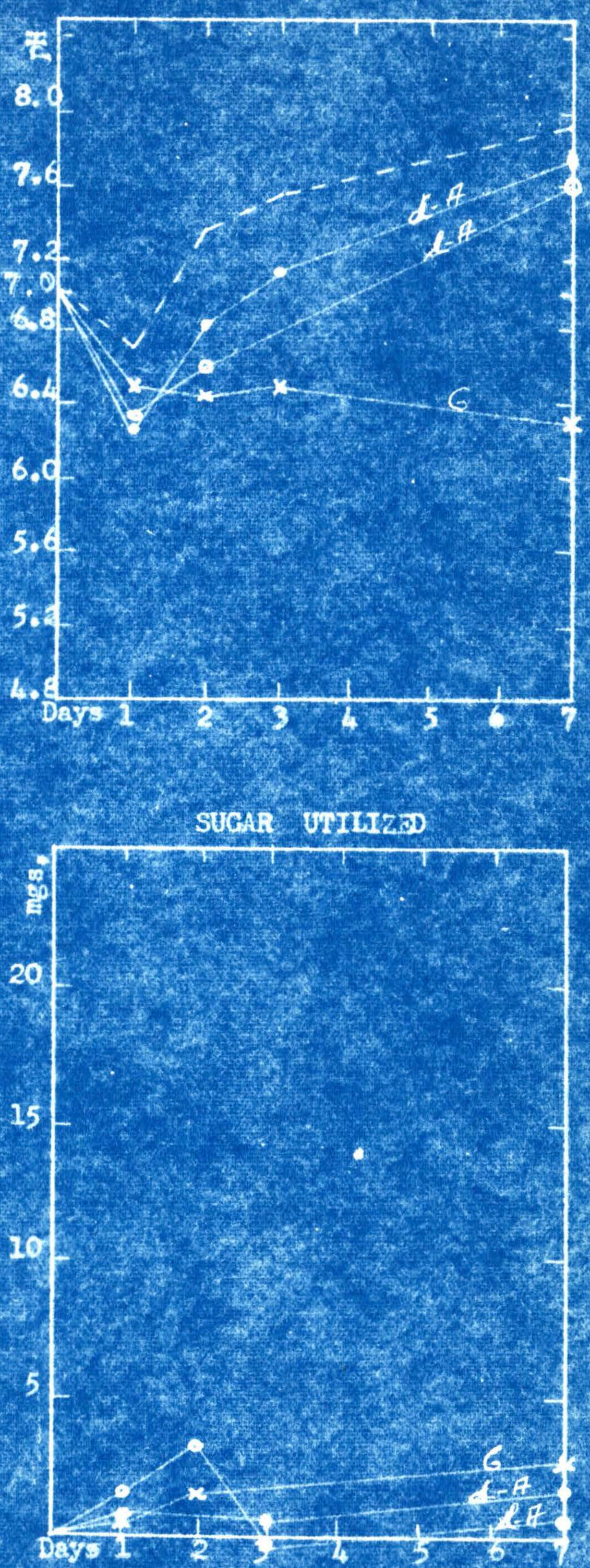
B. danicus #1 A.T.C.

Fig. 35



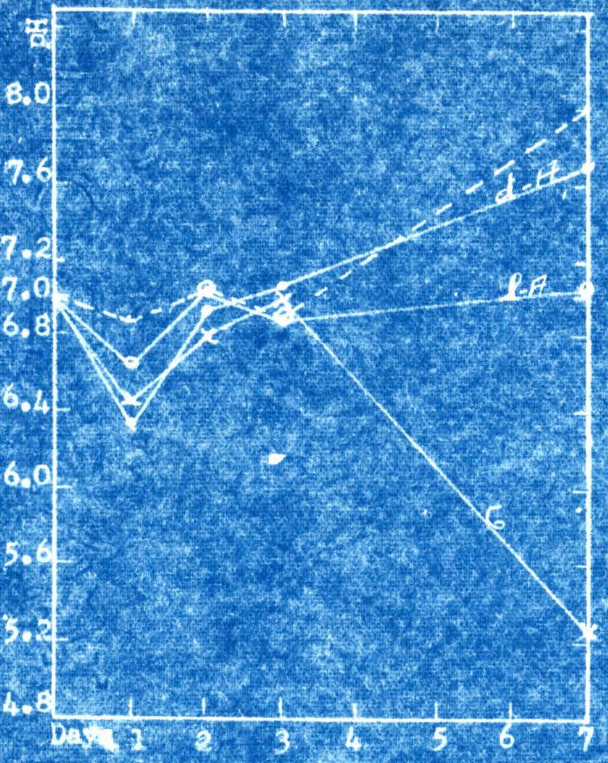
B. firmus U.S.D.A.

Fig. 36

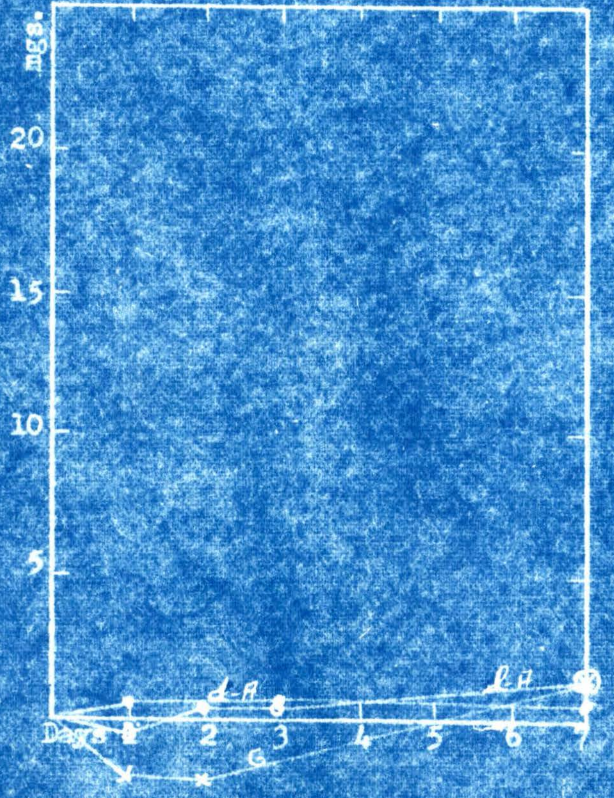


B. flexus V.P.I.

Fig. 37

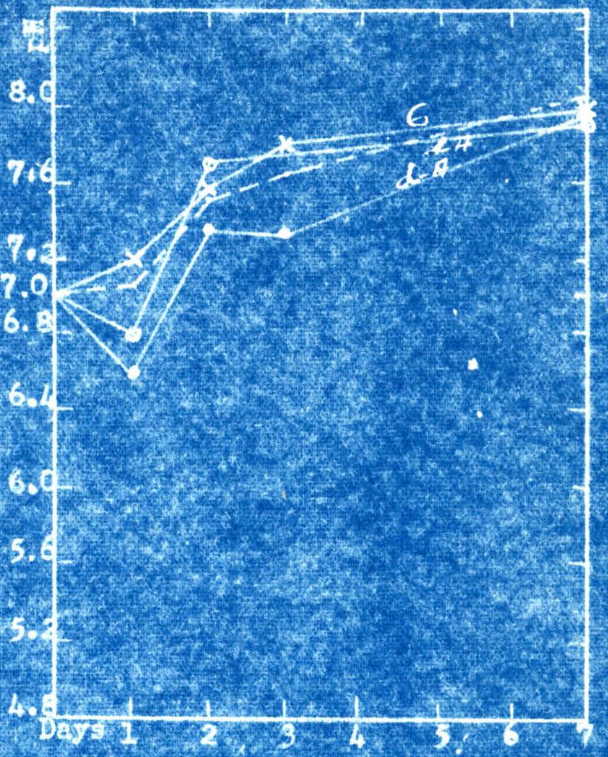


SUGAR UTILIZED



B. freudenreichii A.T.C.

Fig. 38



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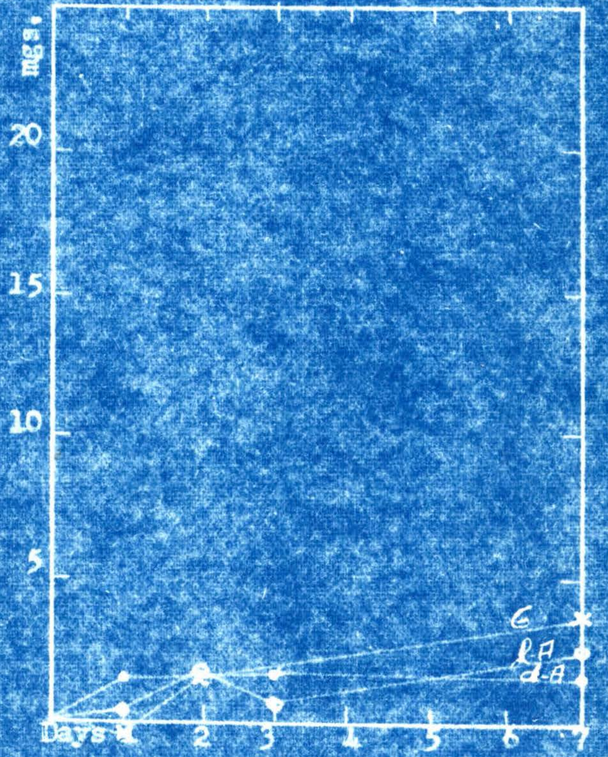
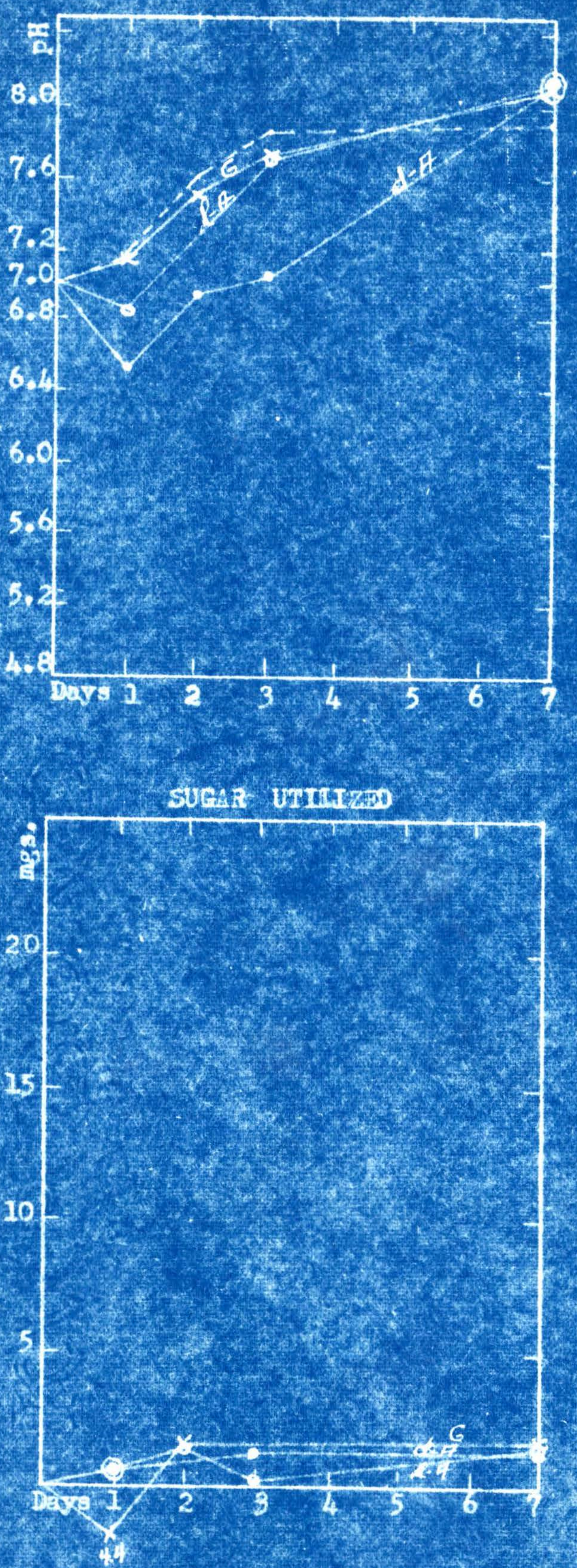


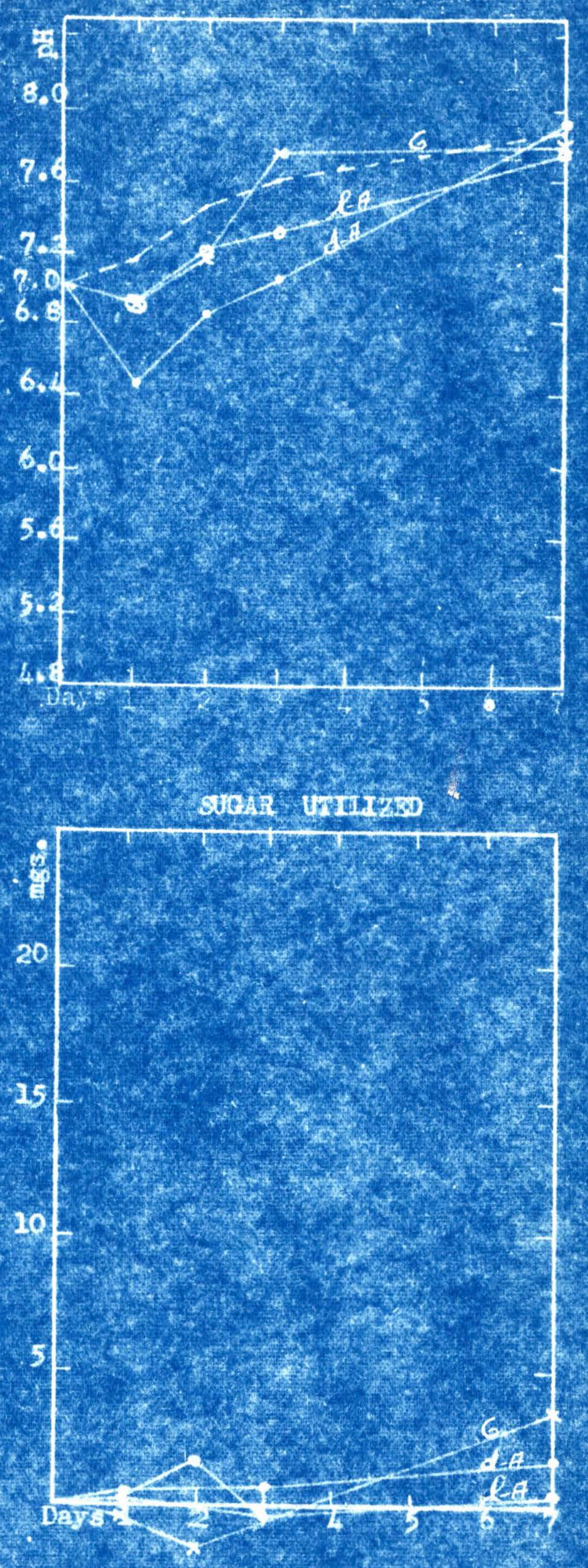
B. fusiformis A.T.C.

Fig. 39



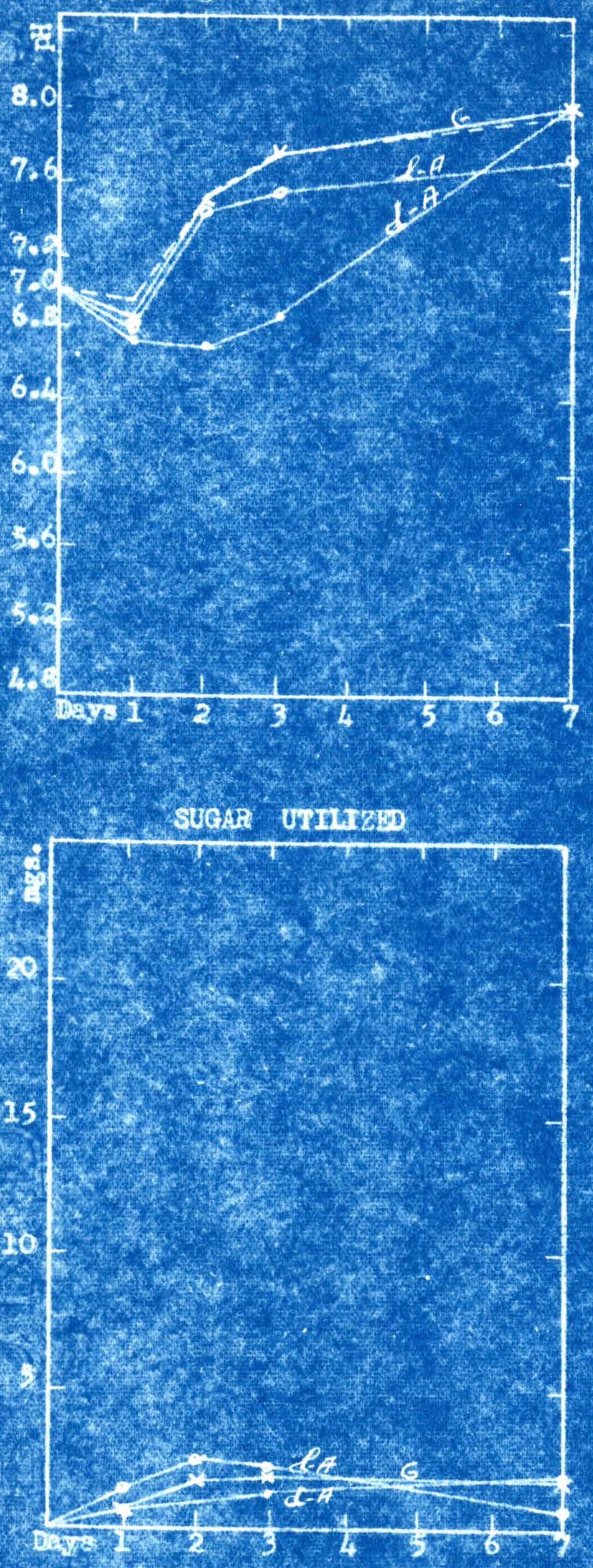
B. fusiformis Tex.

Fig. 40



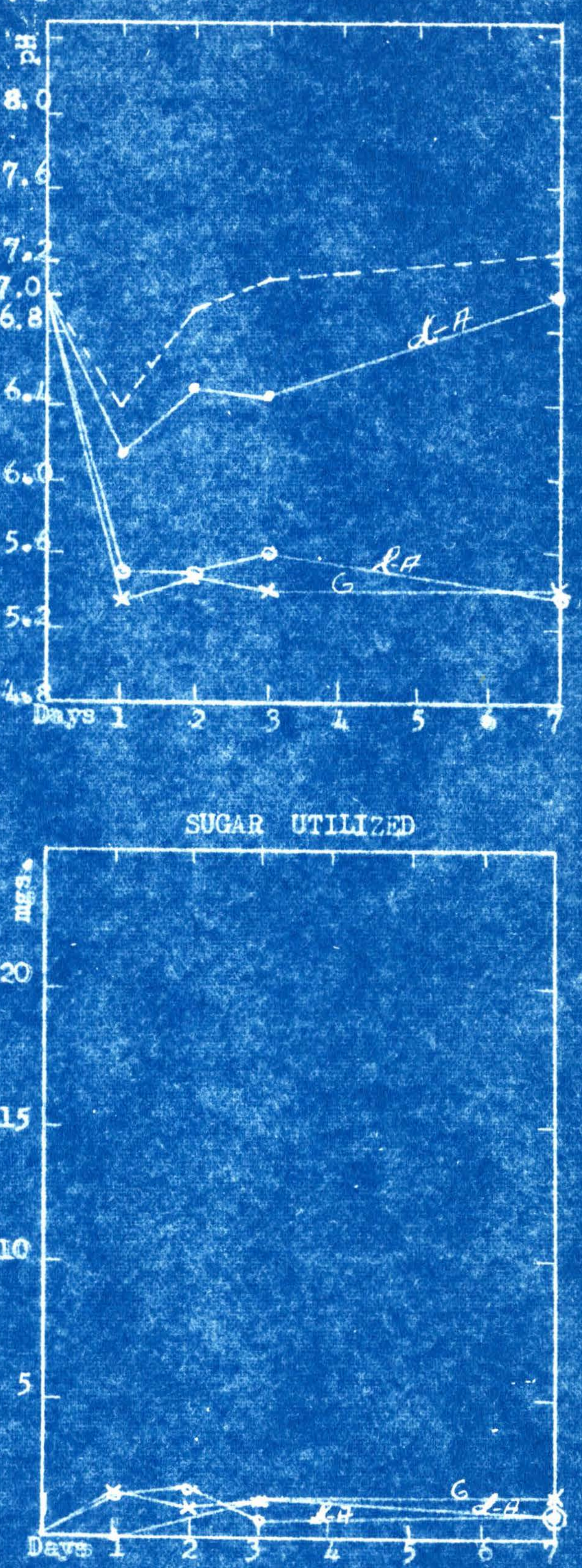
B. fusiformis #339 Tex.

Fig. 41



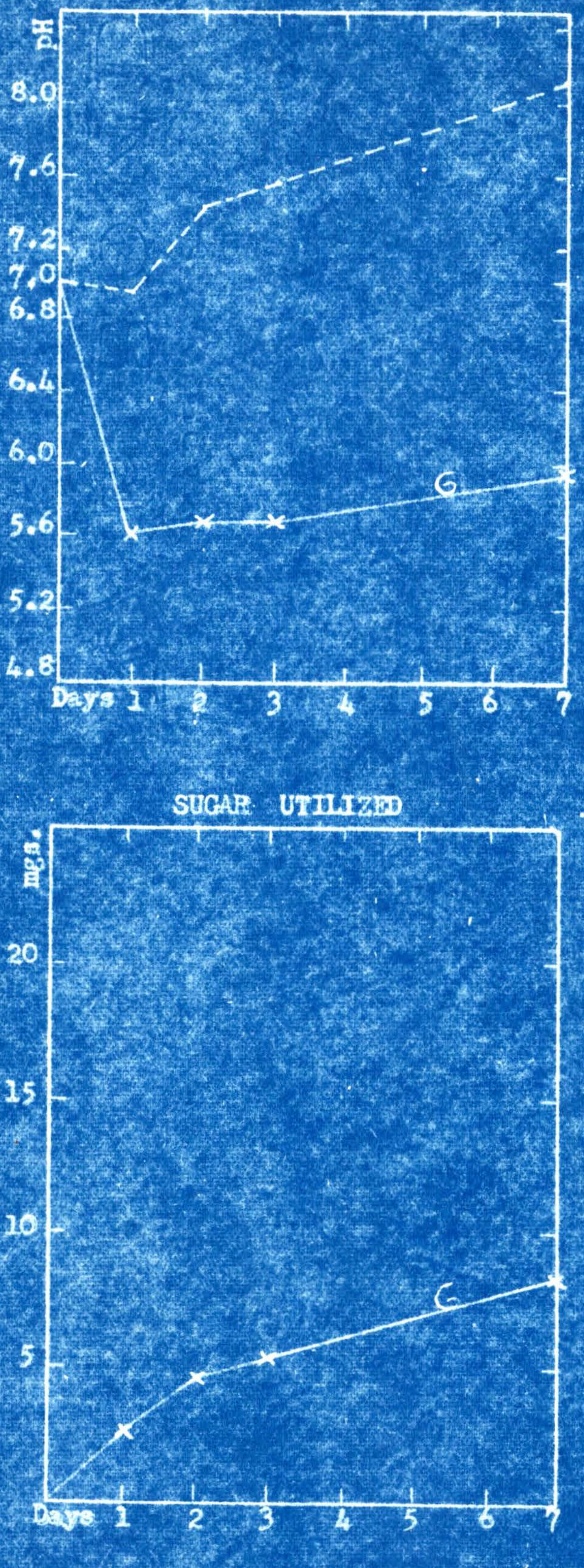
B. fusiformis Wright Wis.

Fig. 42



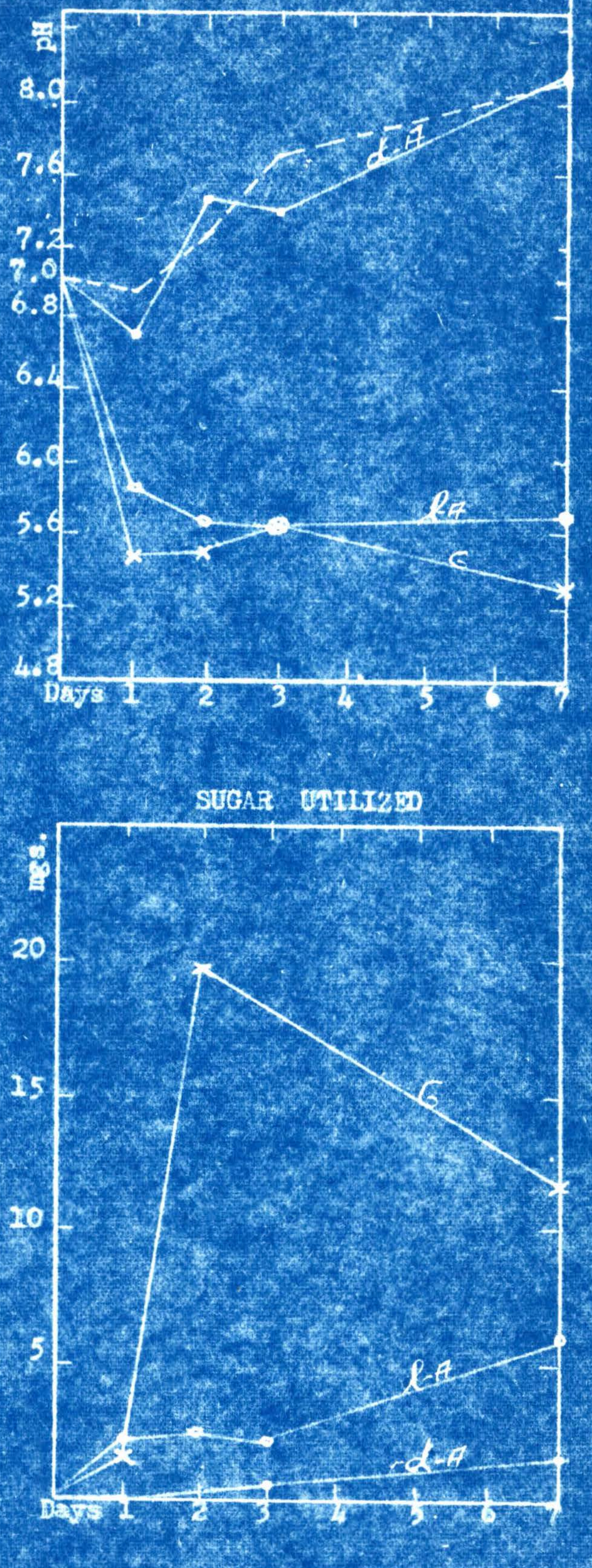
B. globigii A.T.C.

Fig. 43



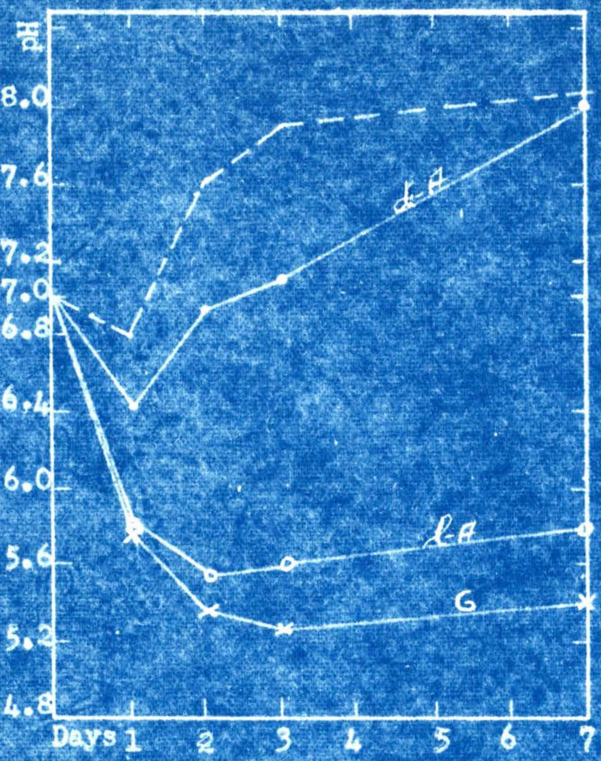
G. globigii #356 Wis.

Fig. 44

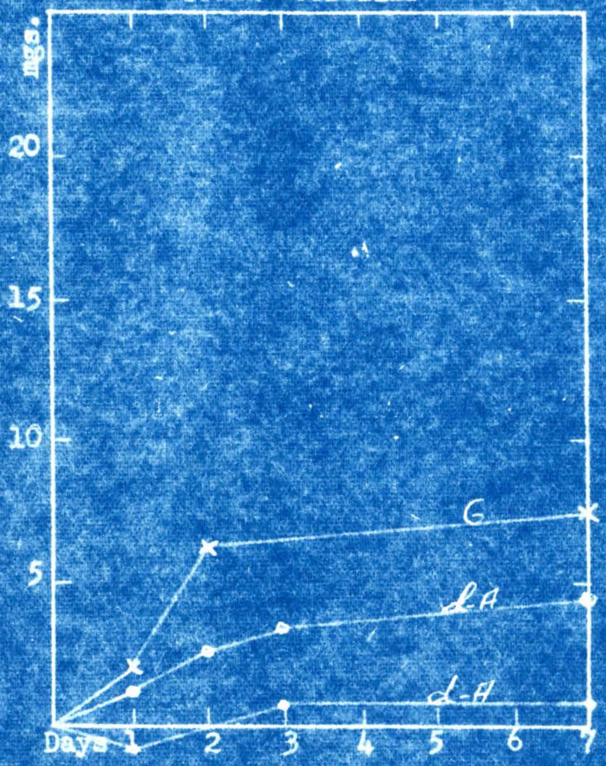


B. graveolens U.S.D.A.

Fig. 45

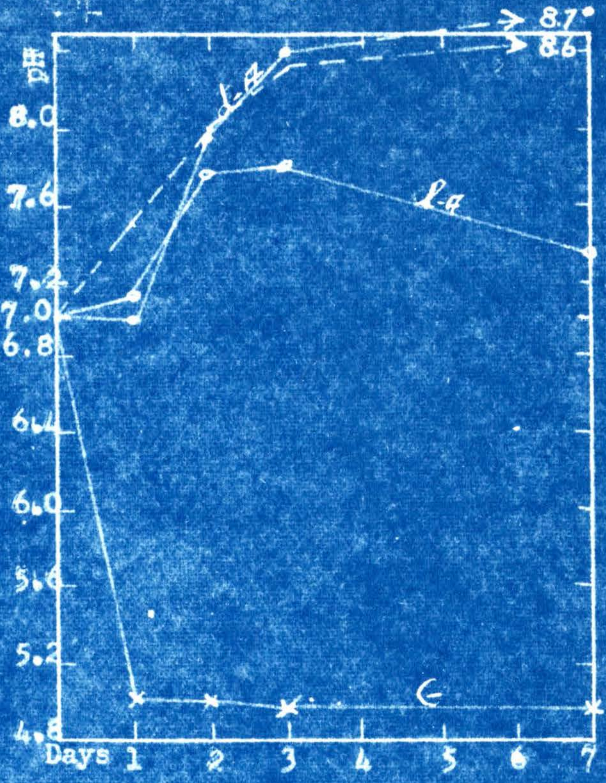


SUGAR UTILIZED

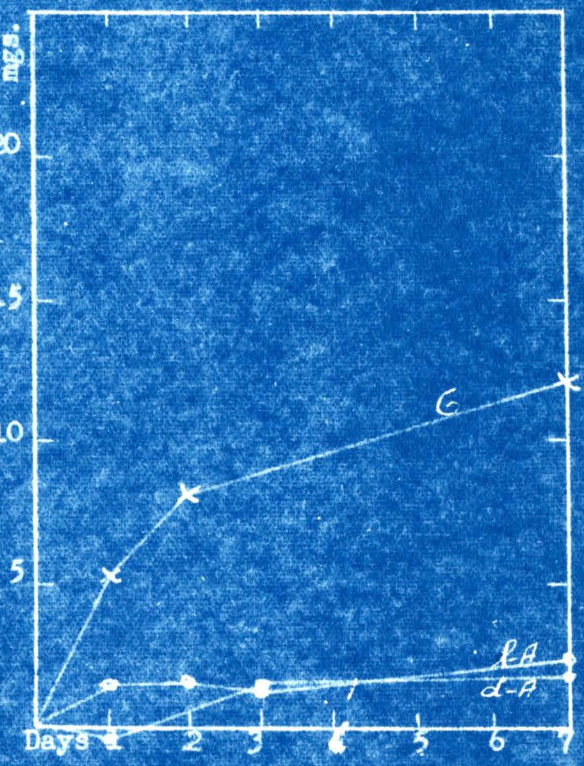


B. graveolens #615 Tex.

Fig. 46

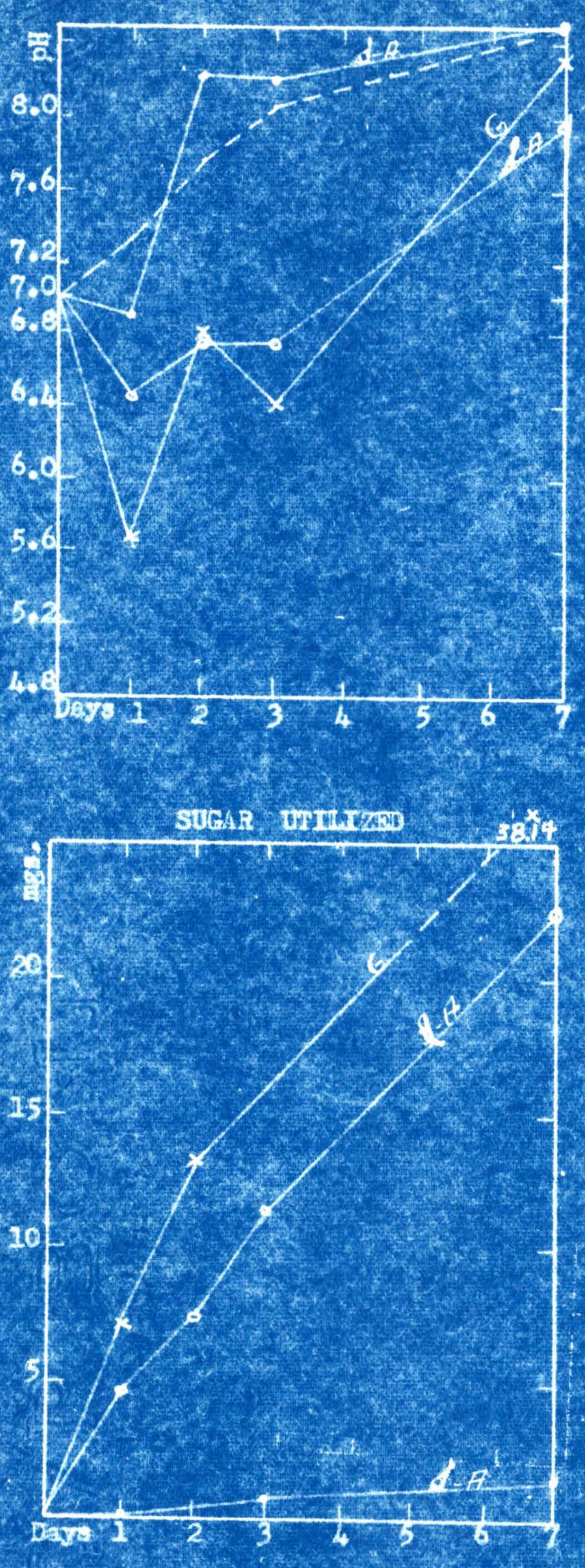


SUGAR UTILIZED



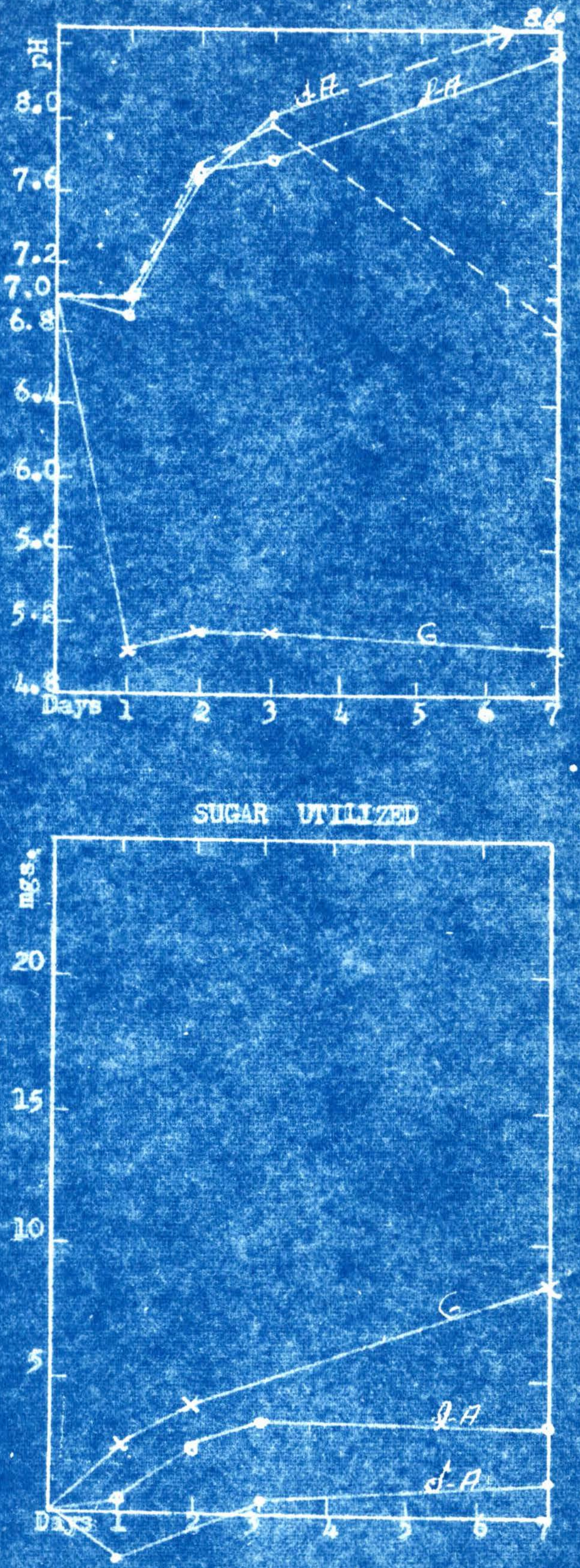
G. graveolens Wright Wis.

Fig. 47



B. graveolens Neb.

Fig. 48



B. lacticola Tex.

Fig. 49

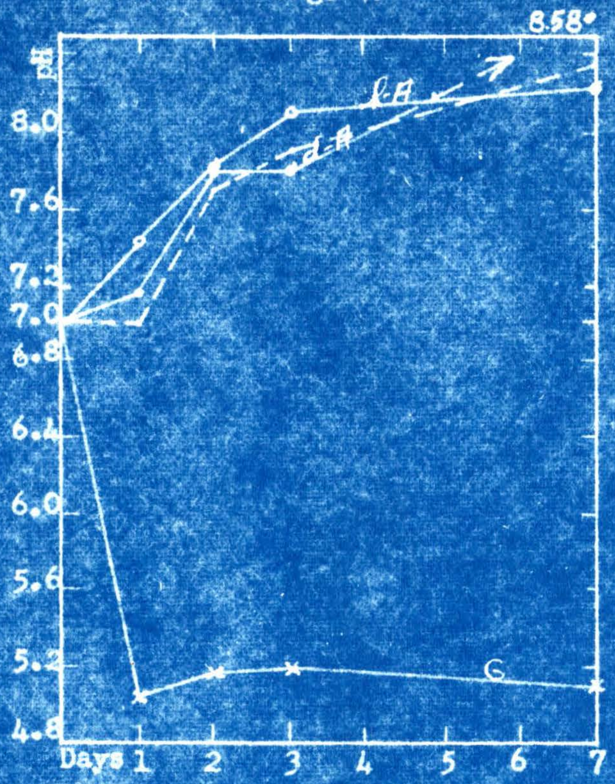
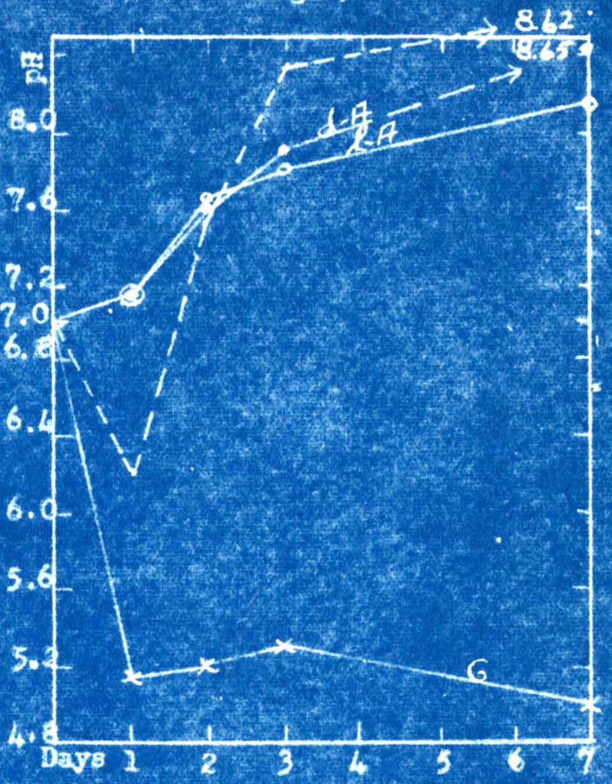
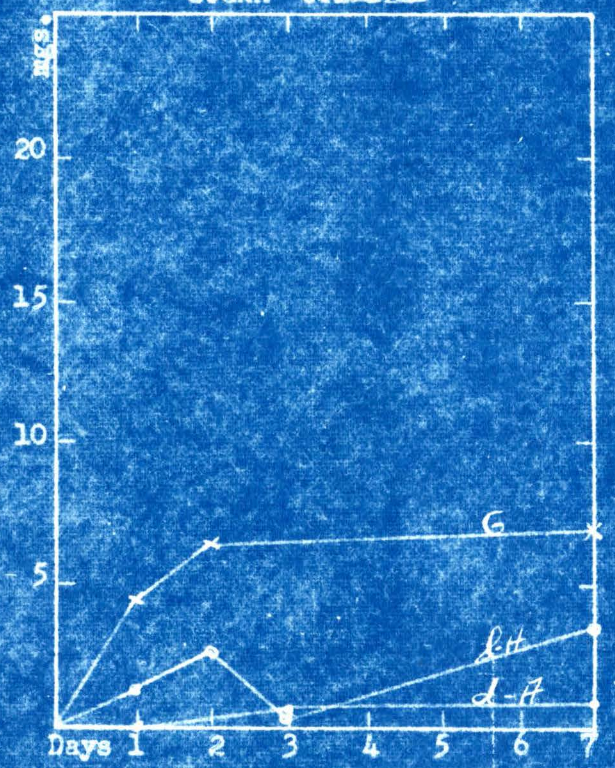


Fig. 50

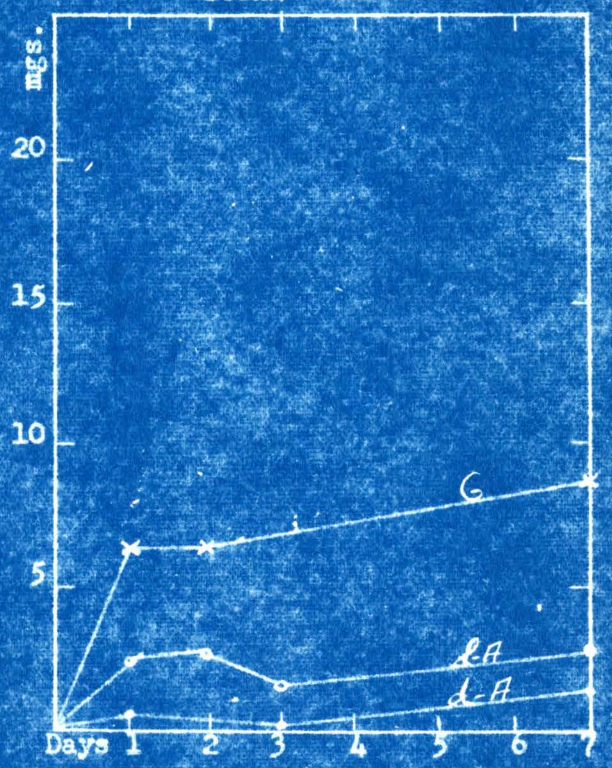


SUGAR UTILIZED



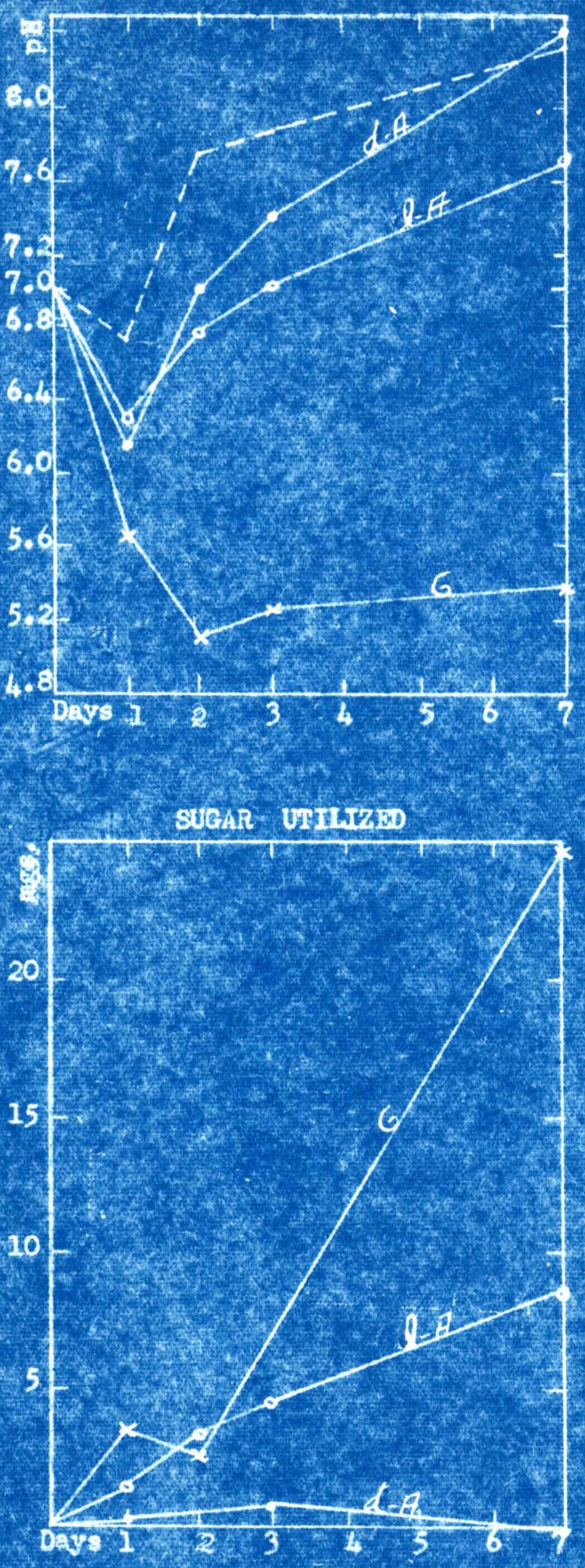
B. lacticola #876 Tex.

SUGAR UTILIZED



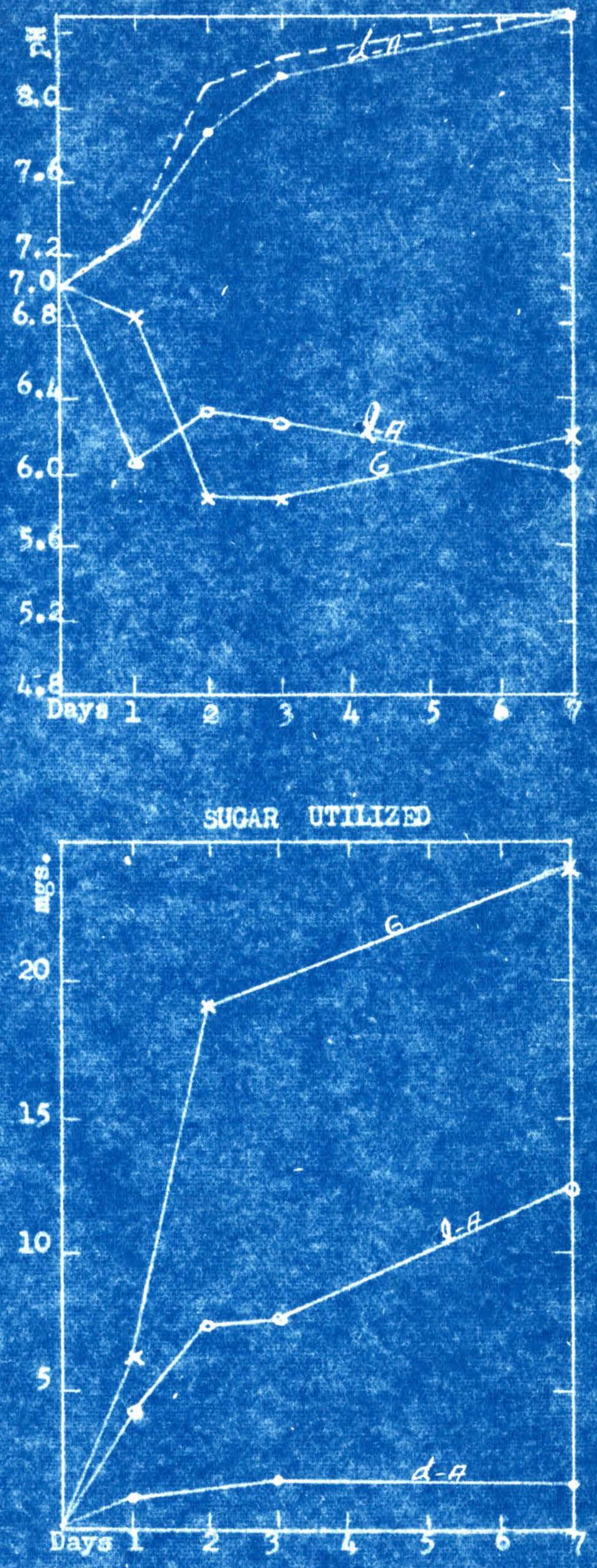
B. lactis #618 Tex.

Fig. 51



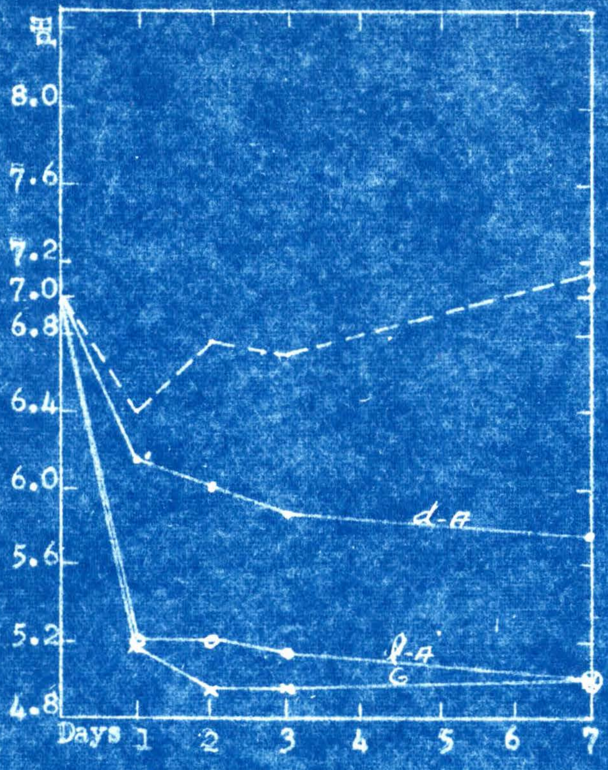
B. laterosporus V.P.I.

Fig. 52



B. lautus V.P.I.

Fig. 53



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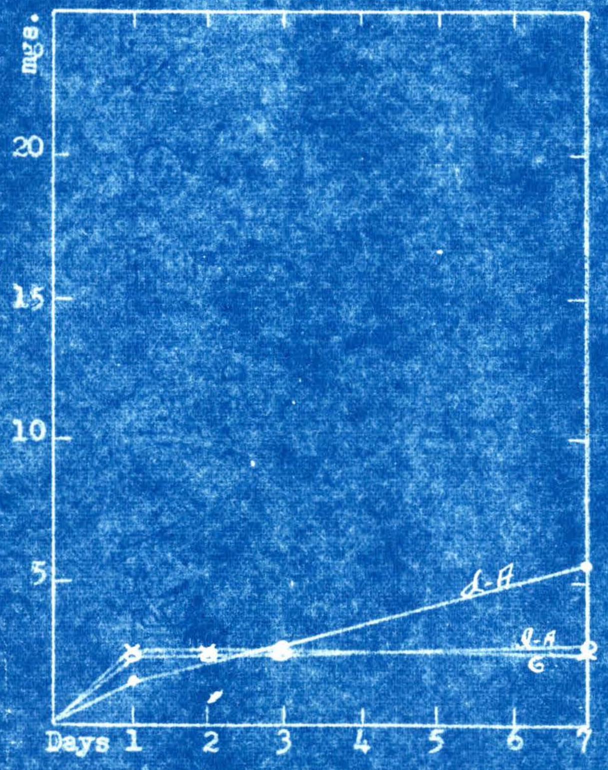
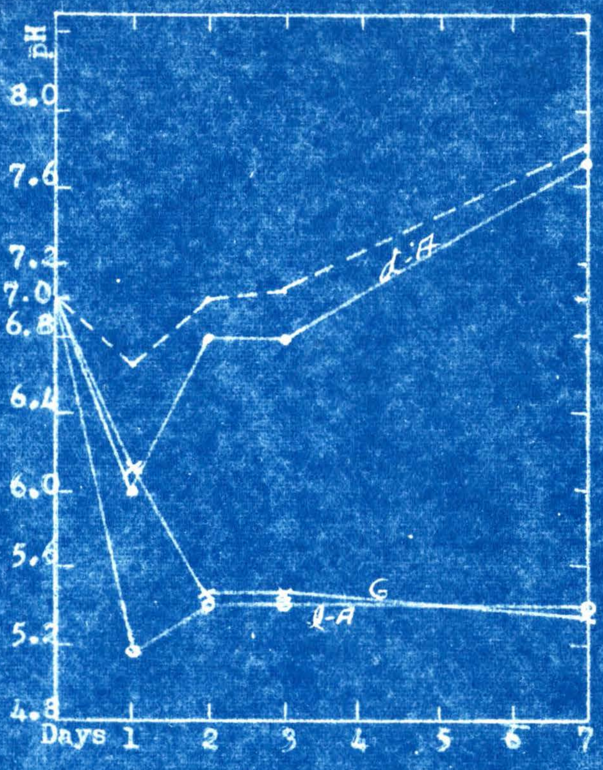
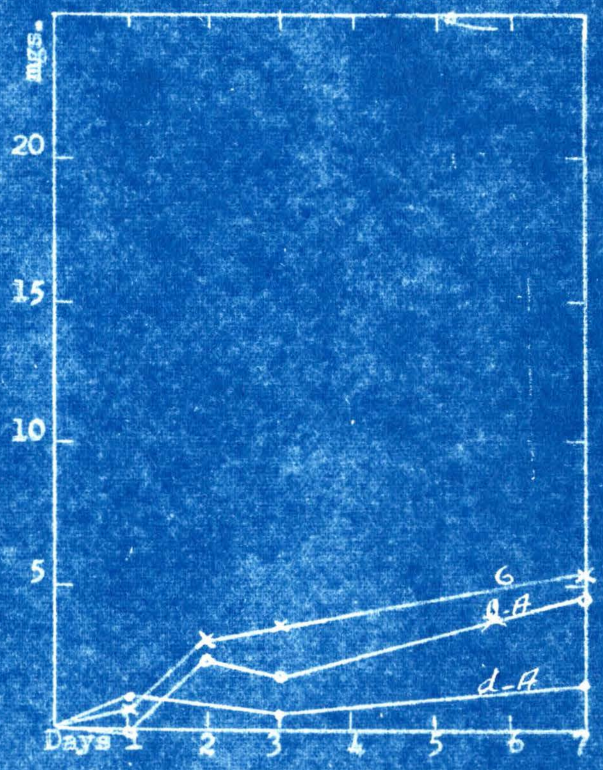


B. macerans U.S.D.A.

Fig. 54

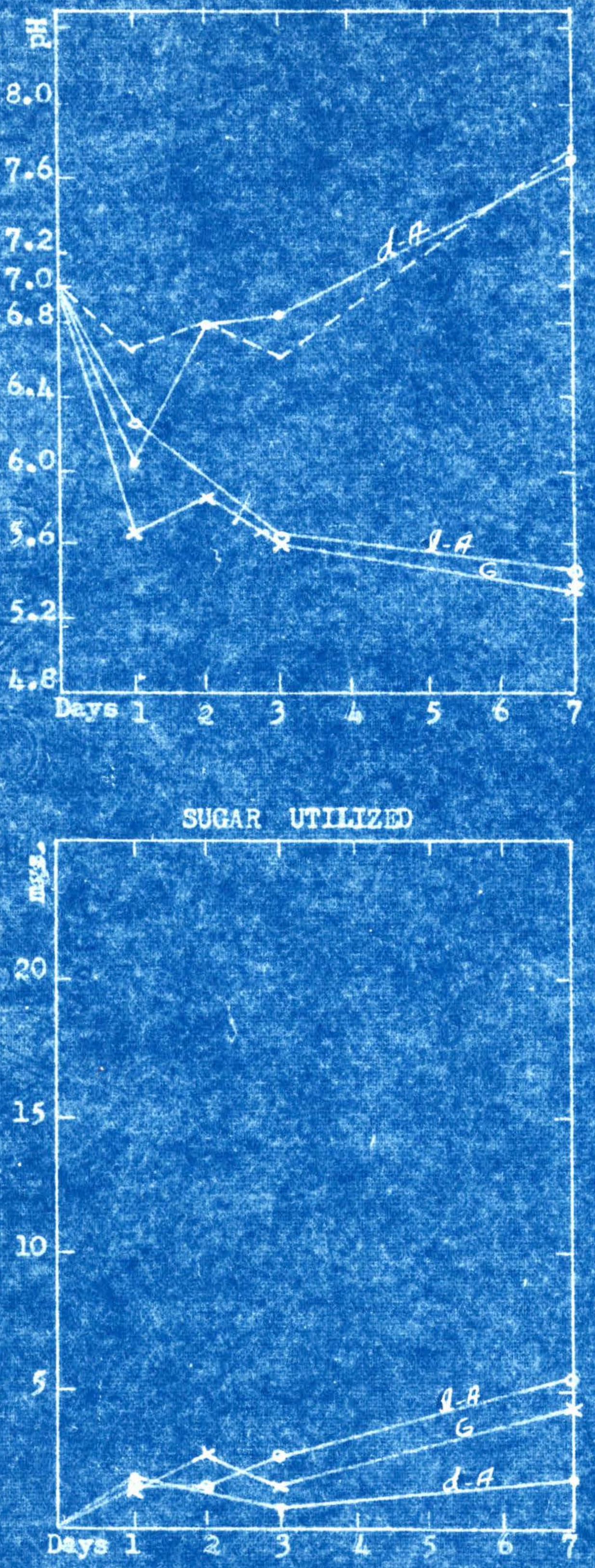


SUGAR UTILIZED



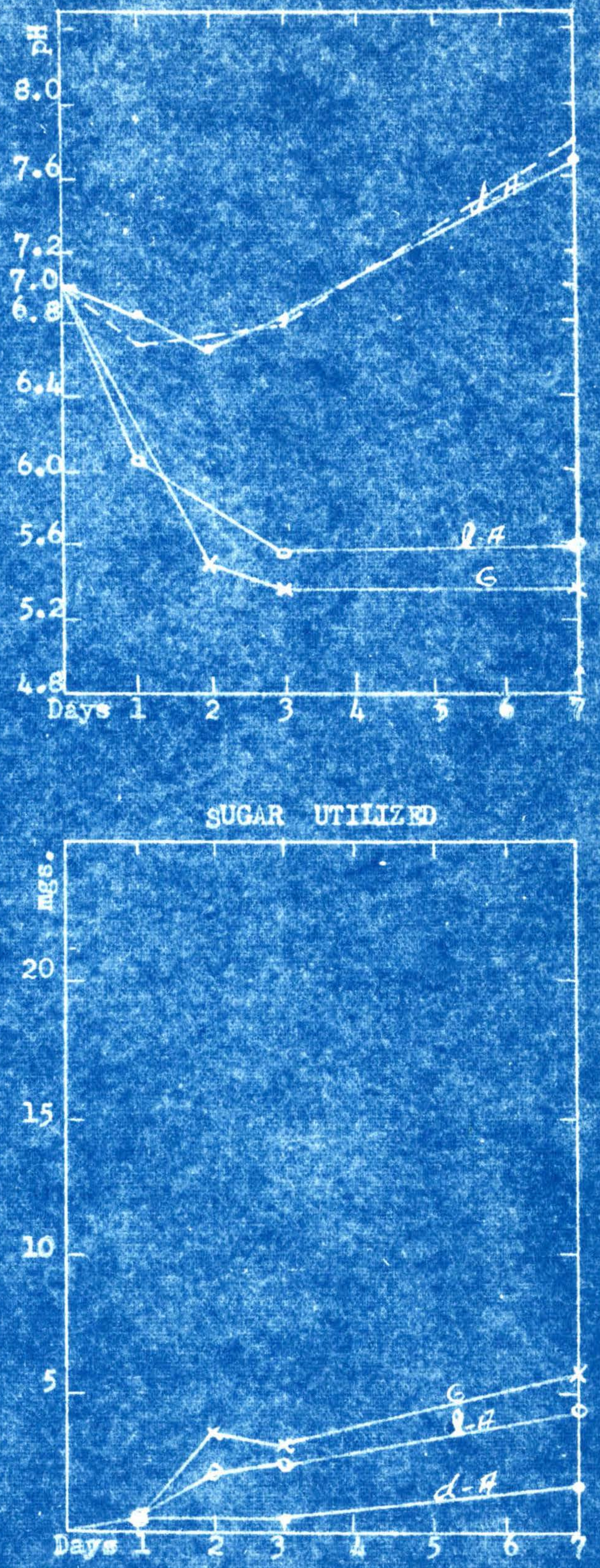
B. megatherium U.S.D.A.

Fig. 55



B. megatherium #1 A.T.C.

Fig. 56



B. megatherium #2 A.T.C.

Fig. 57

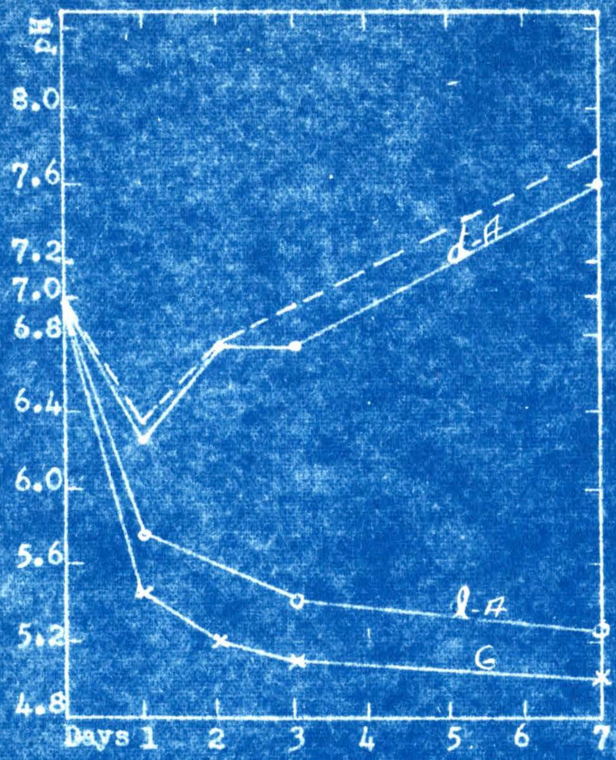
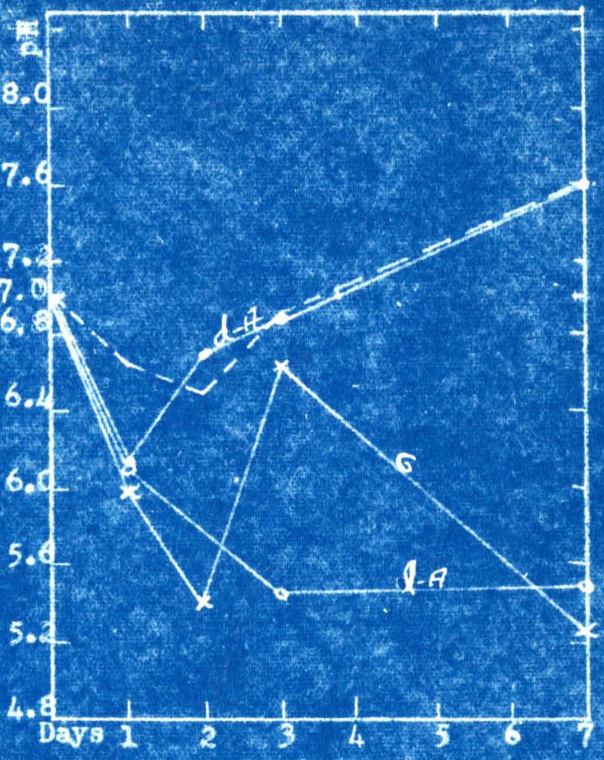


Fig. 58



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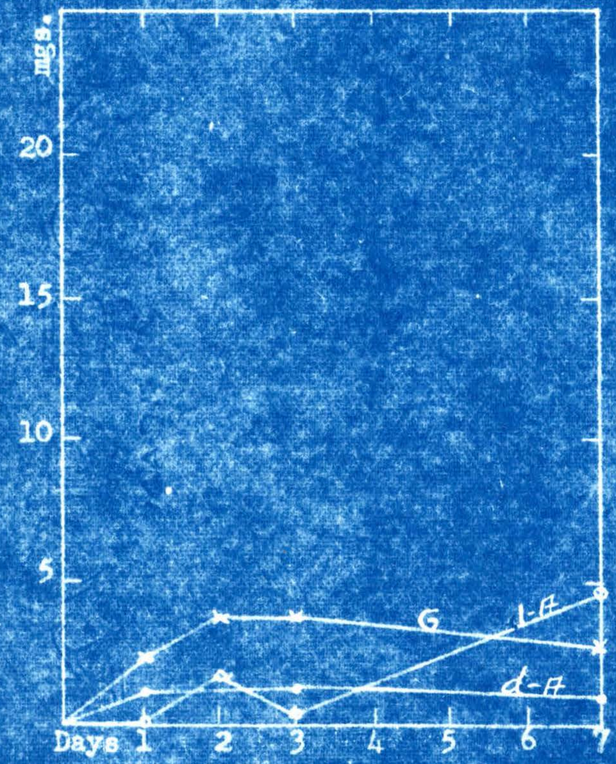

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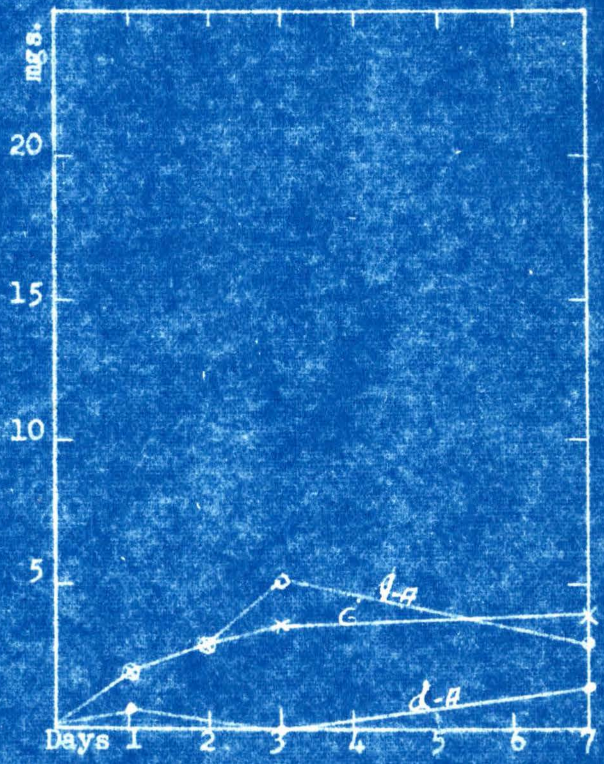


B. *megatherium* Tex.

B. *megatherium* #240 Tex.

Fig. 59

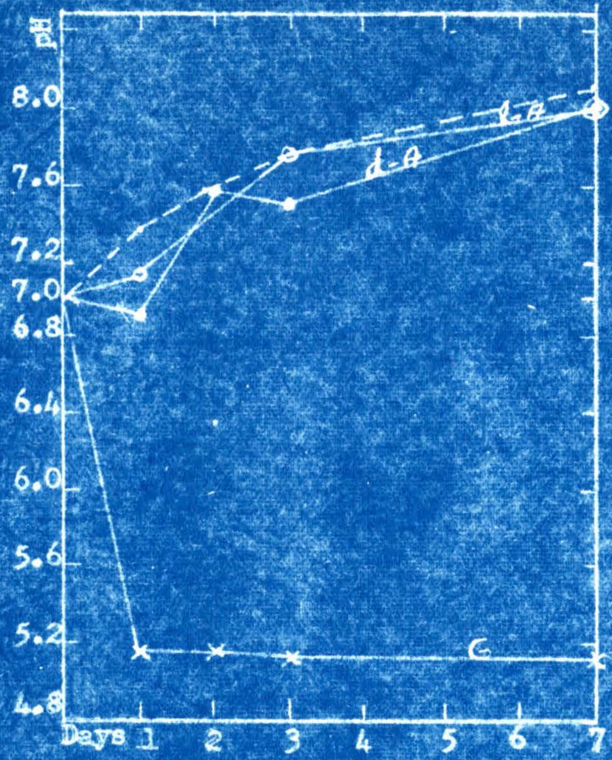
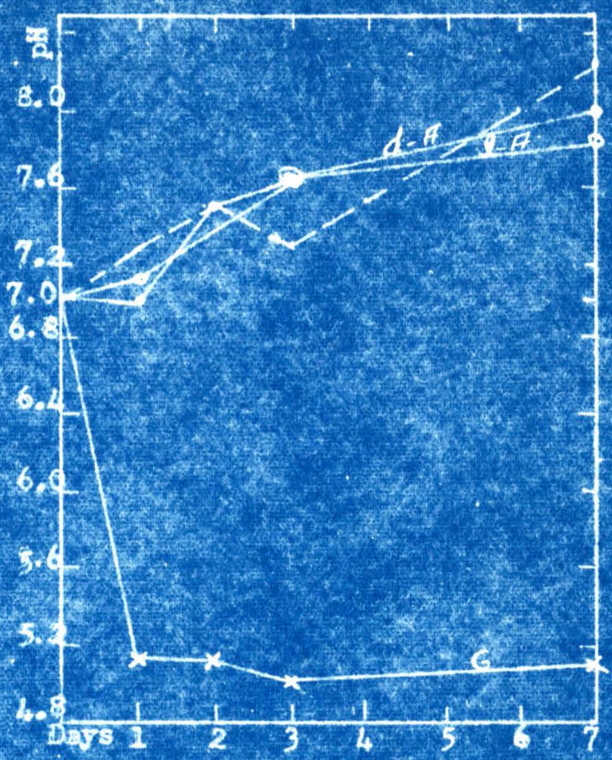
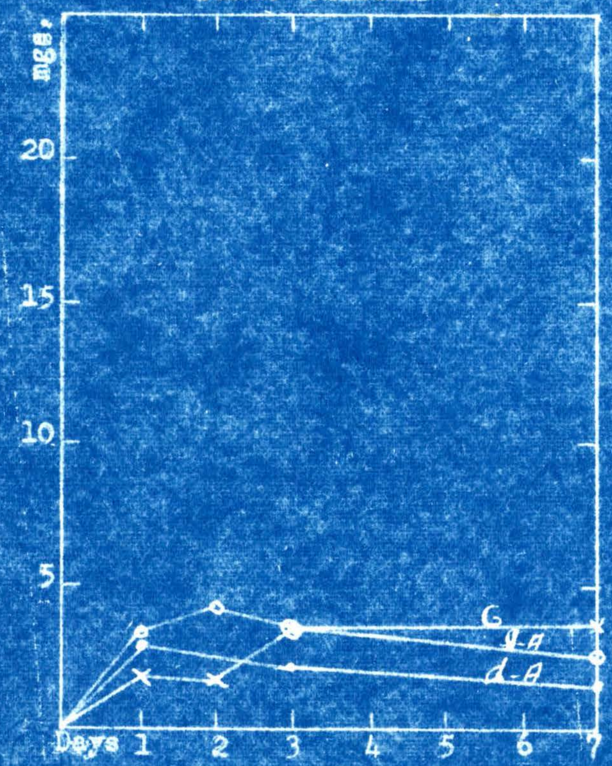


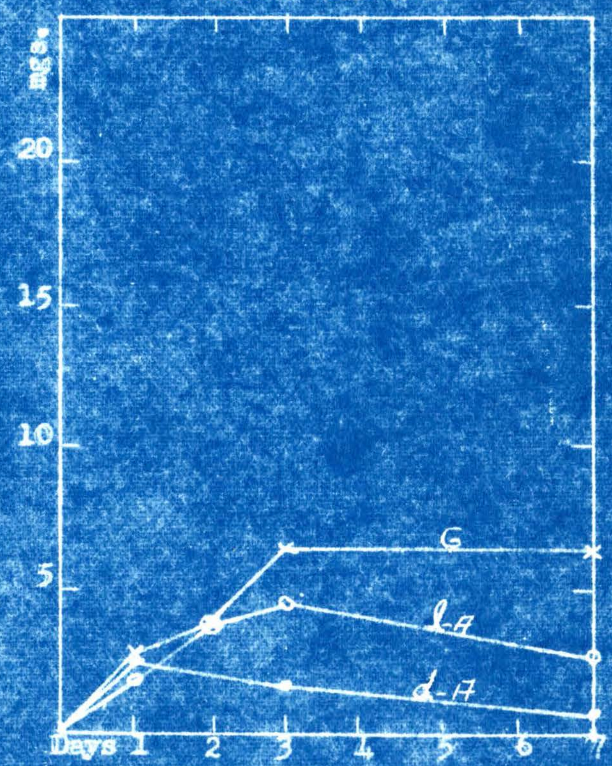
Fig. 60



SUGAR UTILIZED



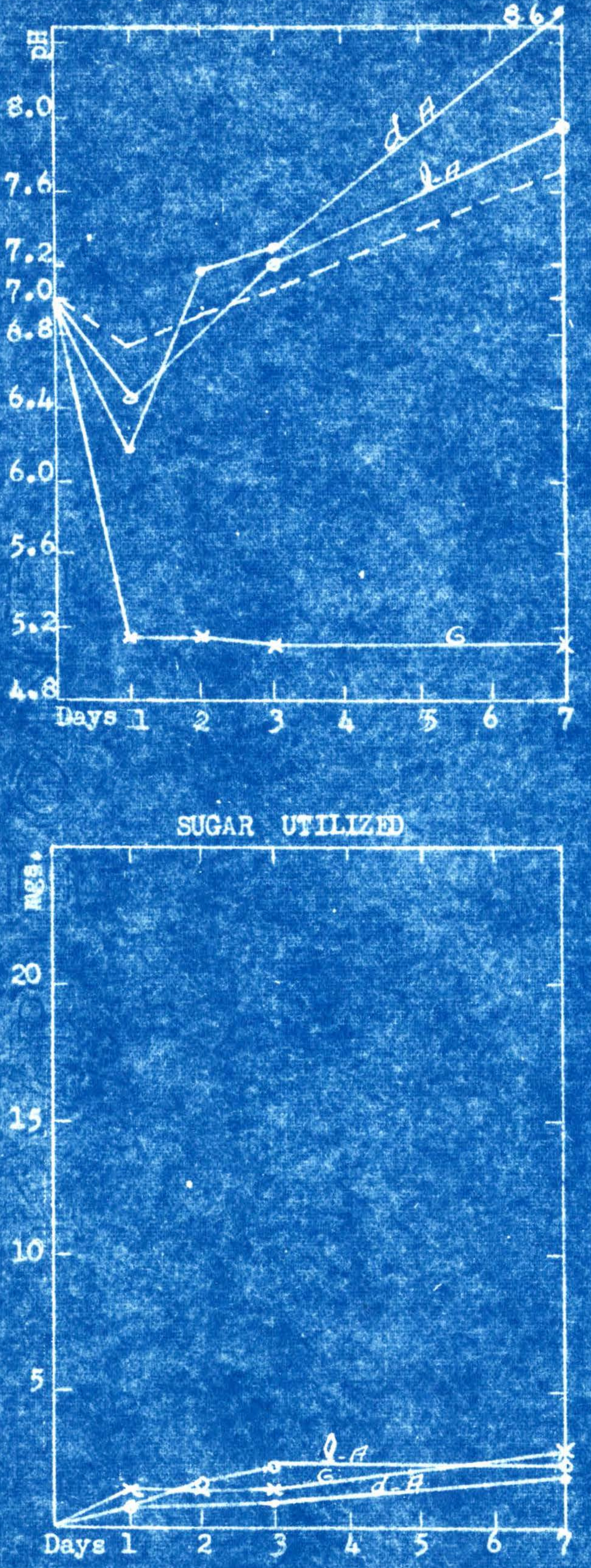
SUGAR UTILIZED



B. megatherium Wis.

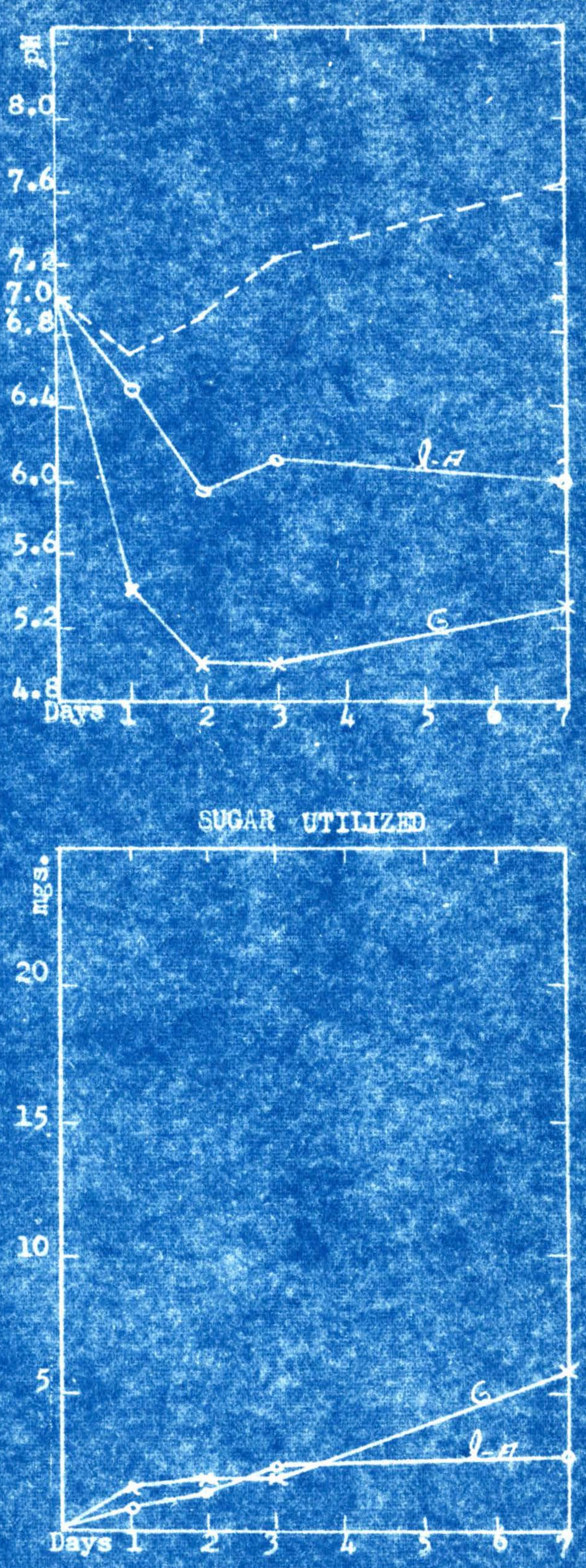
B. megatherium A.T.C. Wis.

Fig. 61



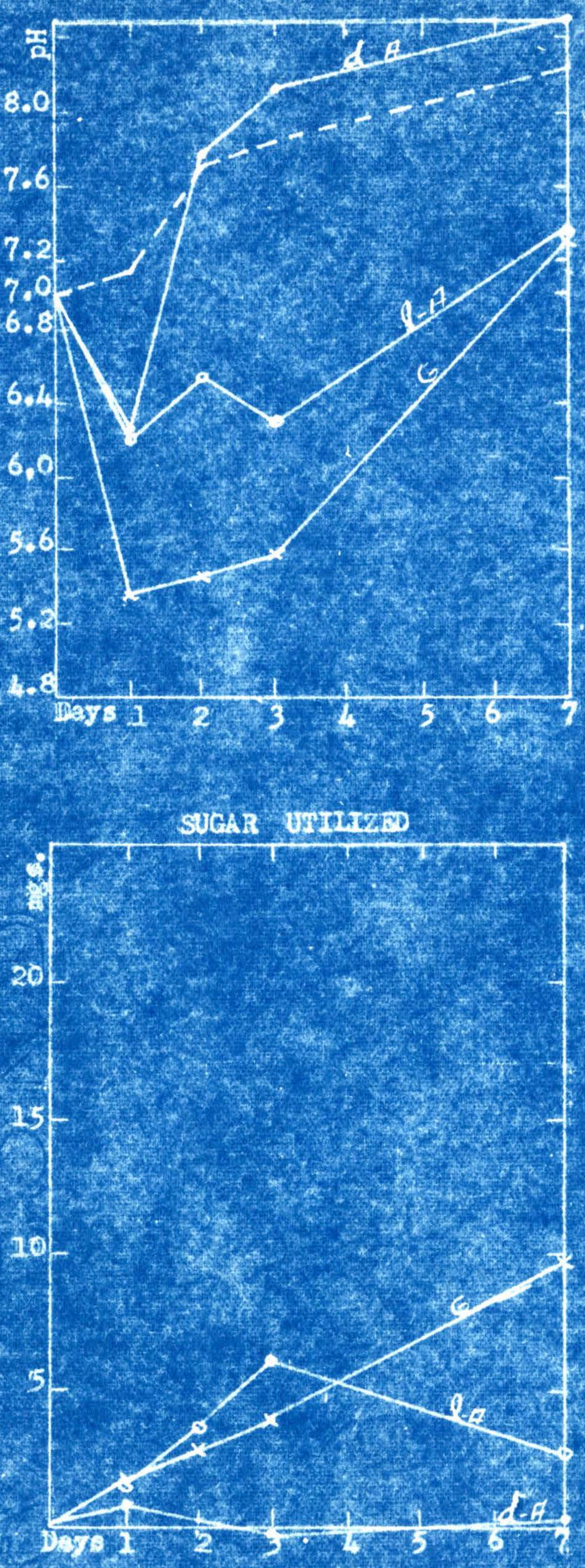
B. megatherium Neb.

Fig. 62



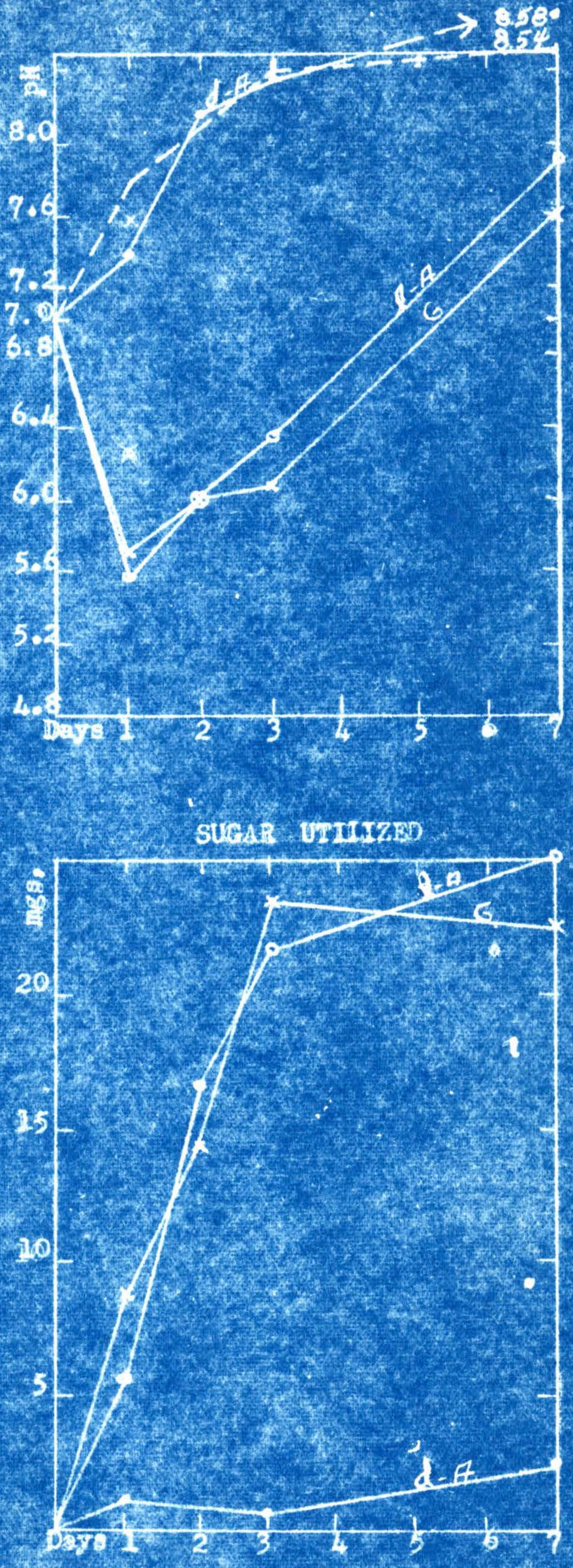
B. mesentericus A.T.C.

Fig. 63



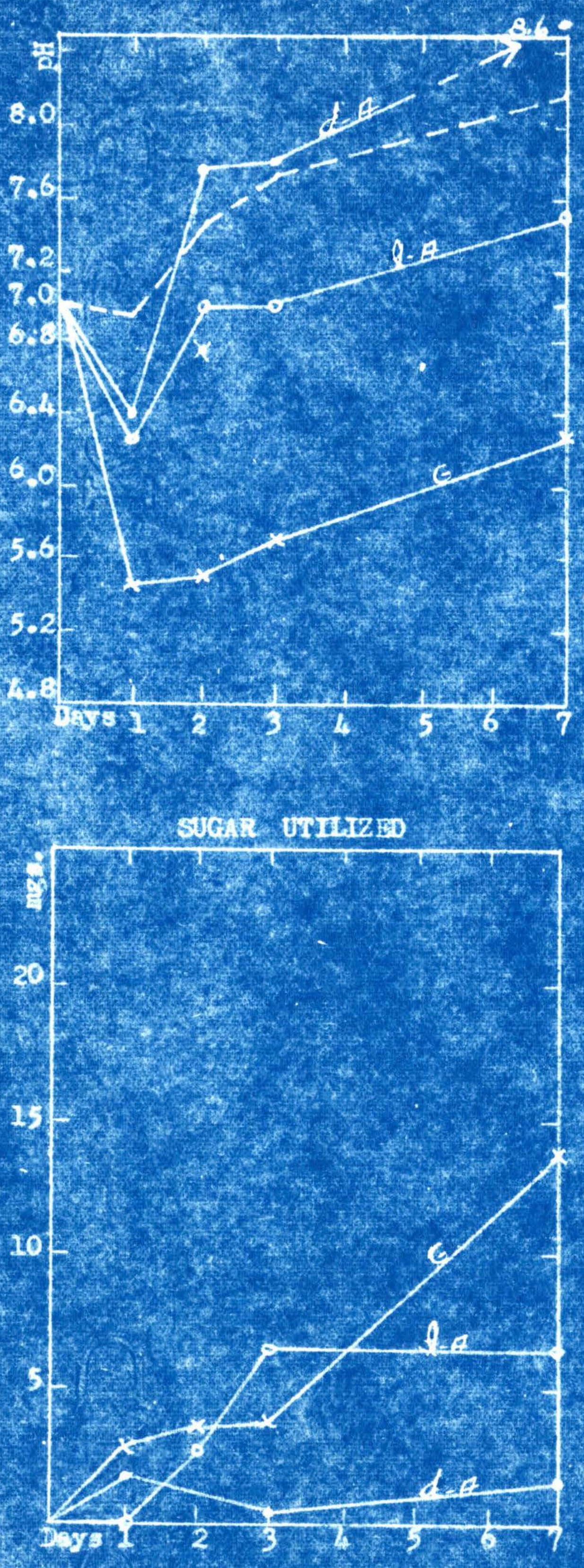
B. mesentericus U.S.D.A.

Fig. 64



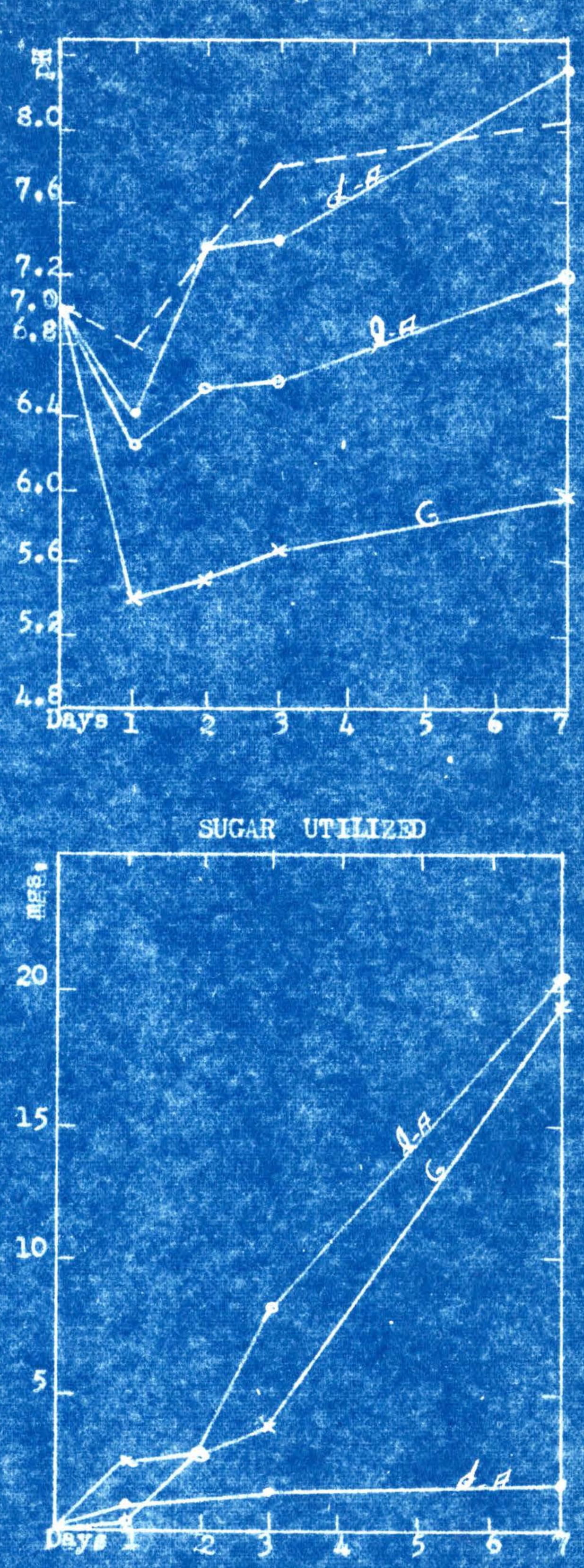
B. mesentericus Tex.

Fig. 65



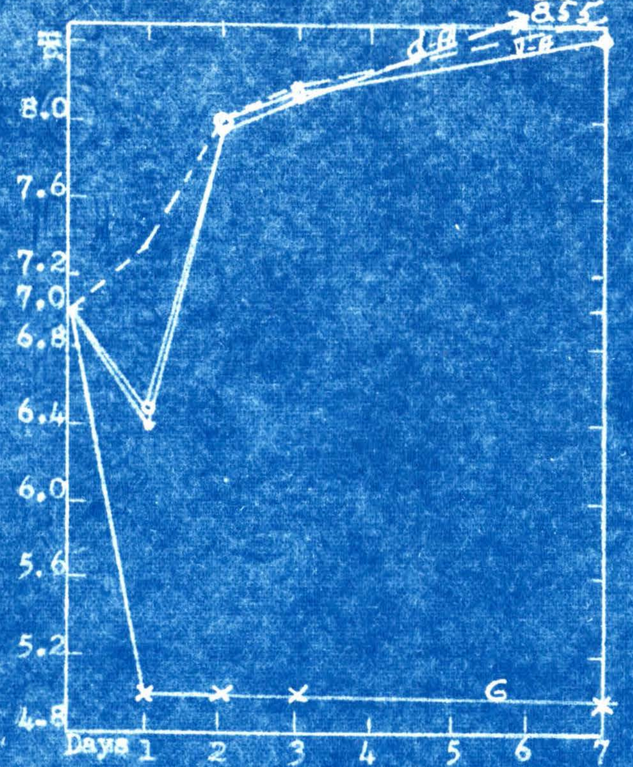
B. mesentericus A.T.C. Wis.

Fig. 66

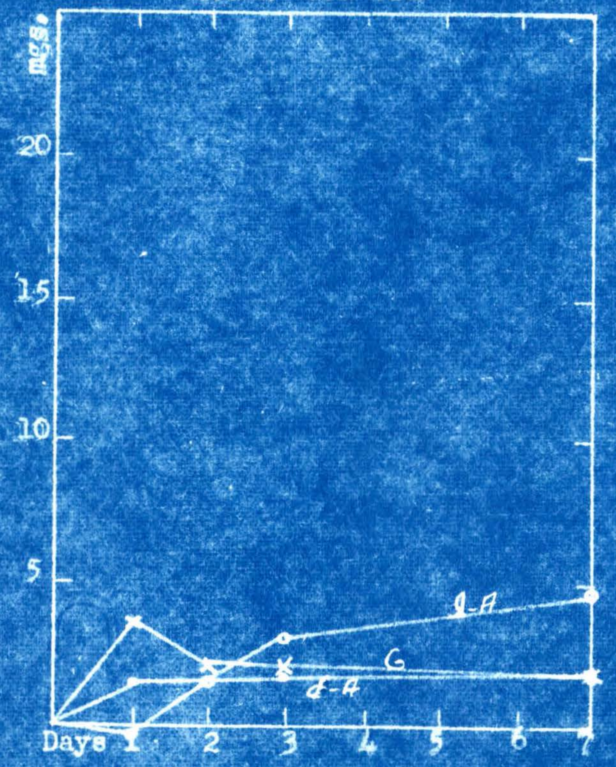


B. mesentericus Frost Wis.

Fig. 67

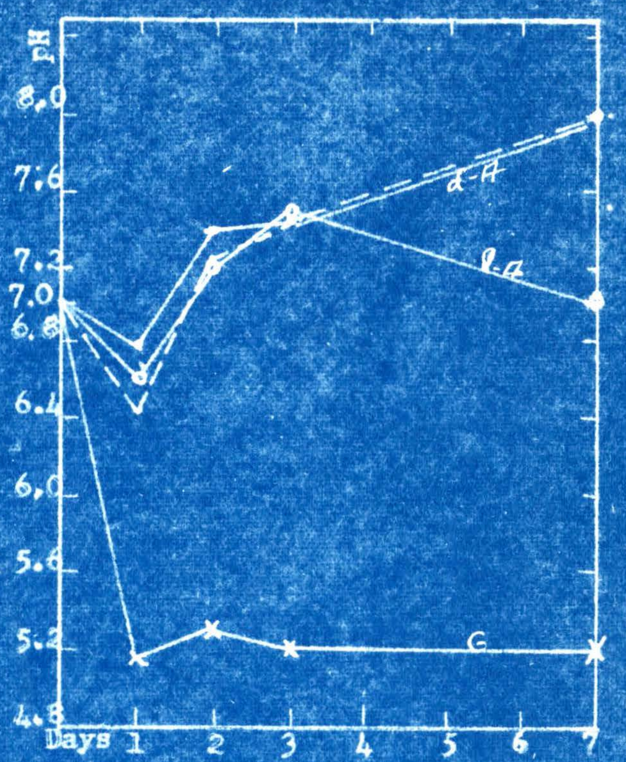


SUGAR UTILIZED



B. mesentericus Neb.

Fig. 68



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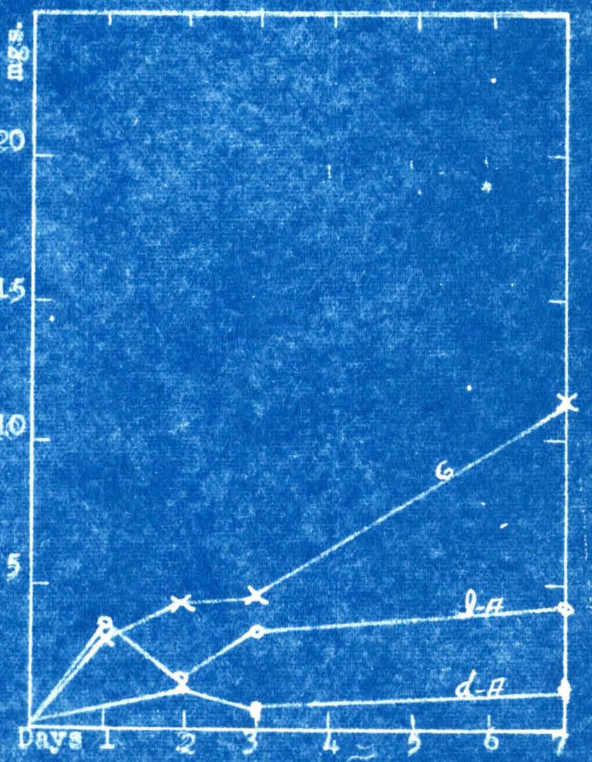
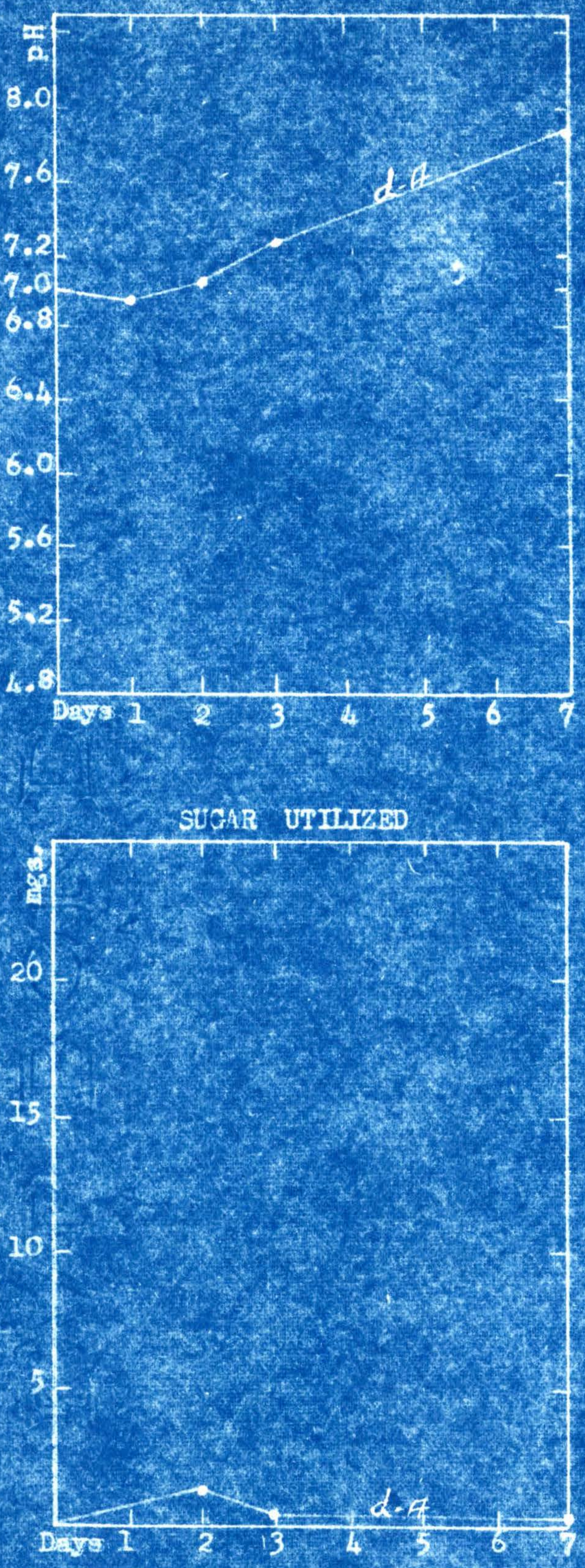


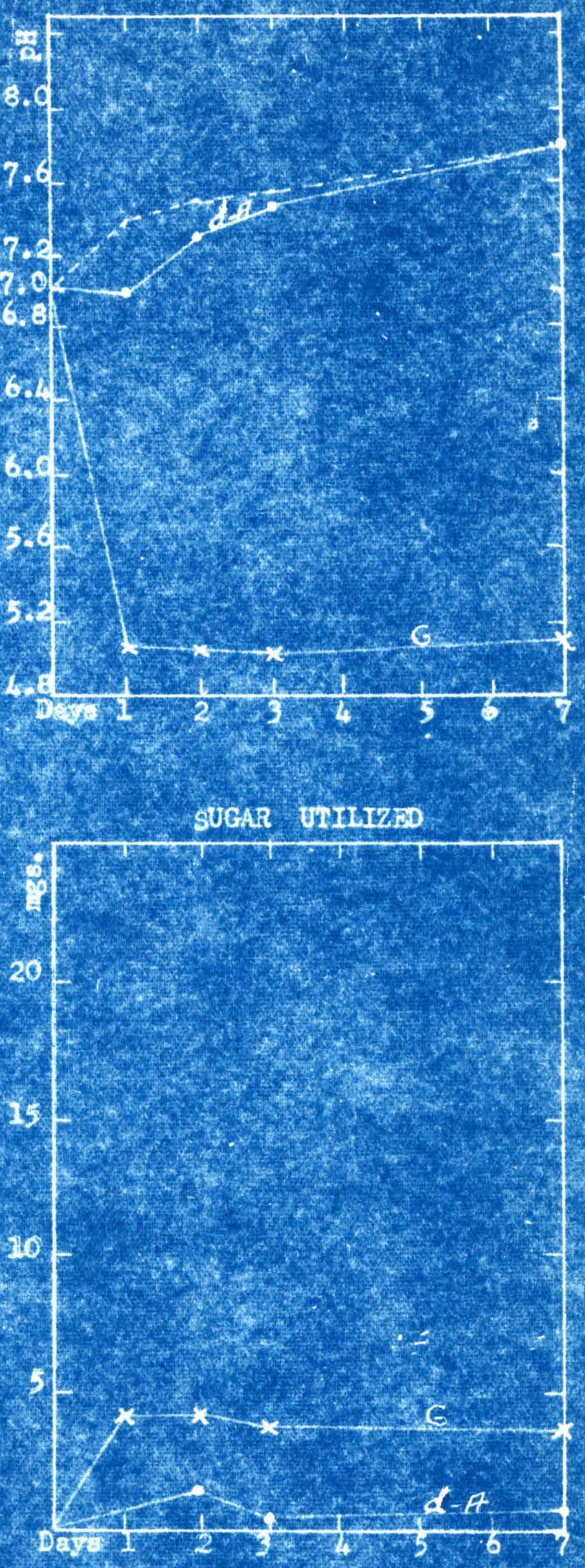
B. mycoides #1 V.P.I.

Fig. 69



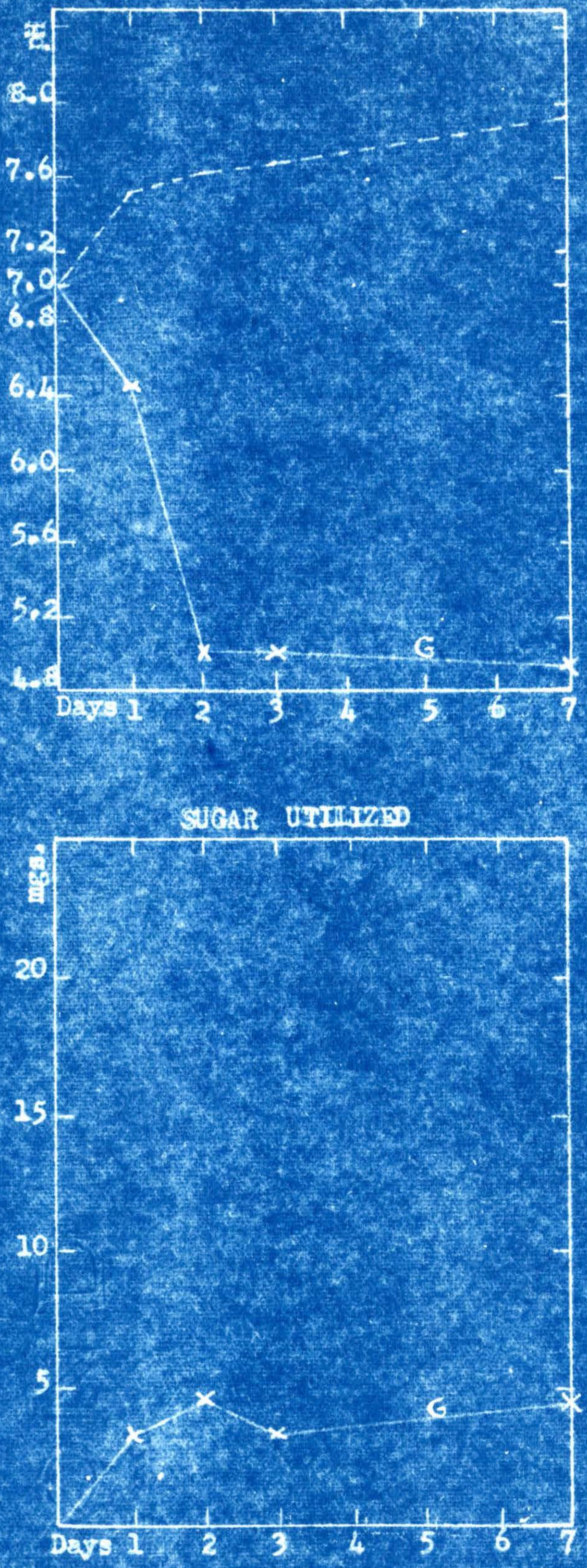
B. mycoides #101 V.P.I.

Fig. 70



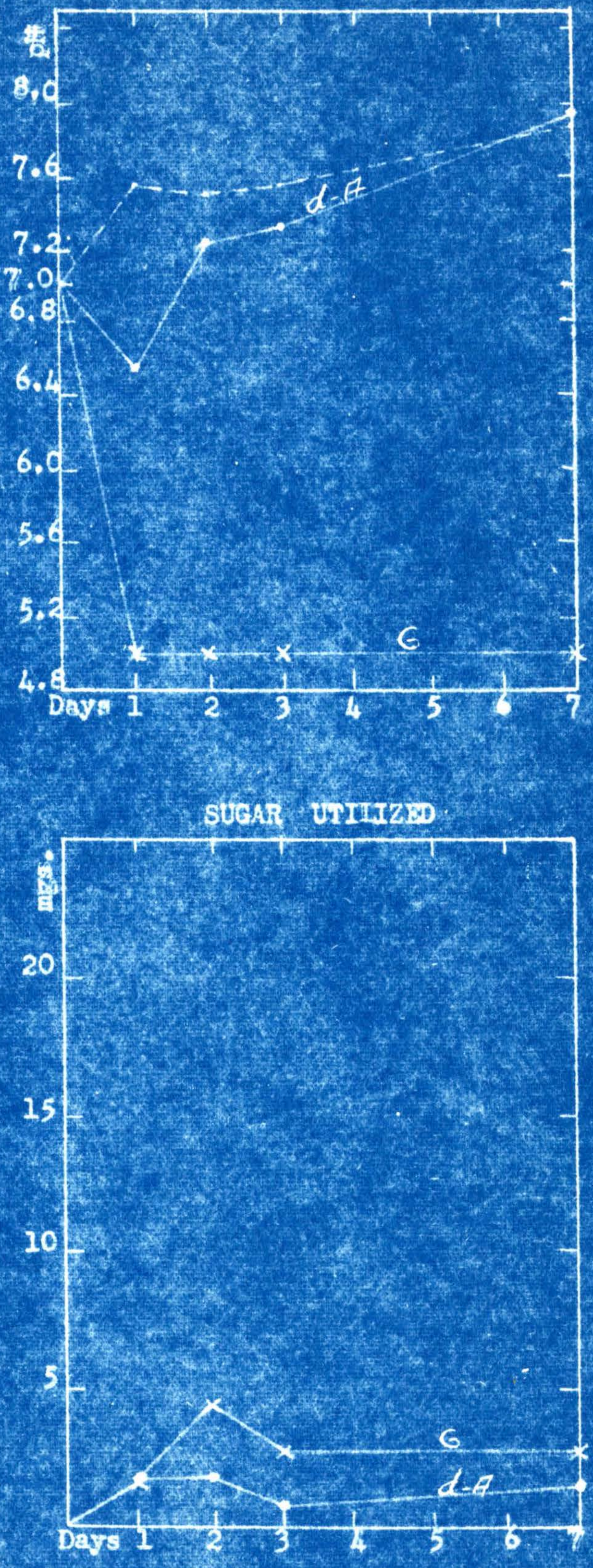
B. mycoides (Beaded) Tex.

Fig. 71



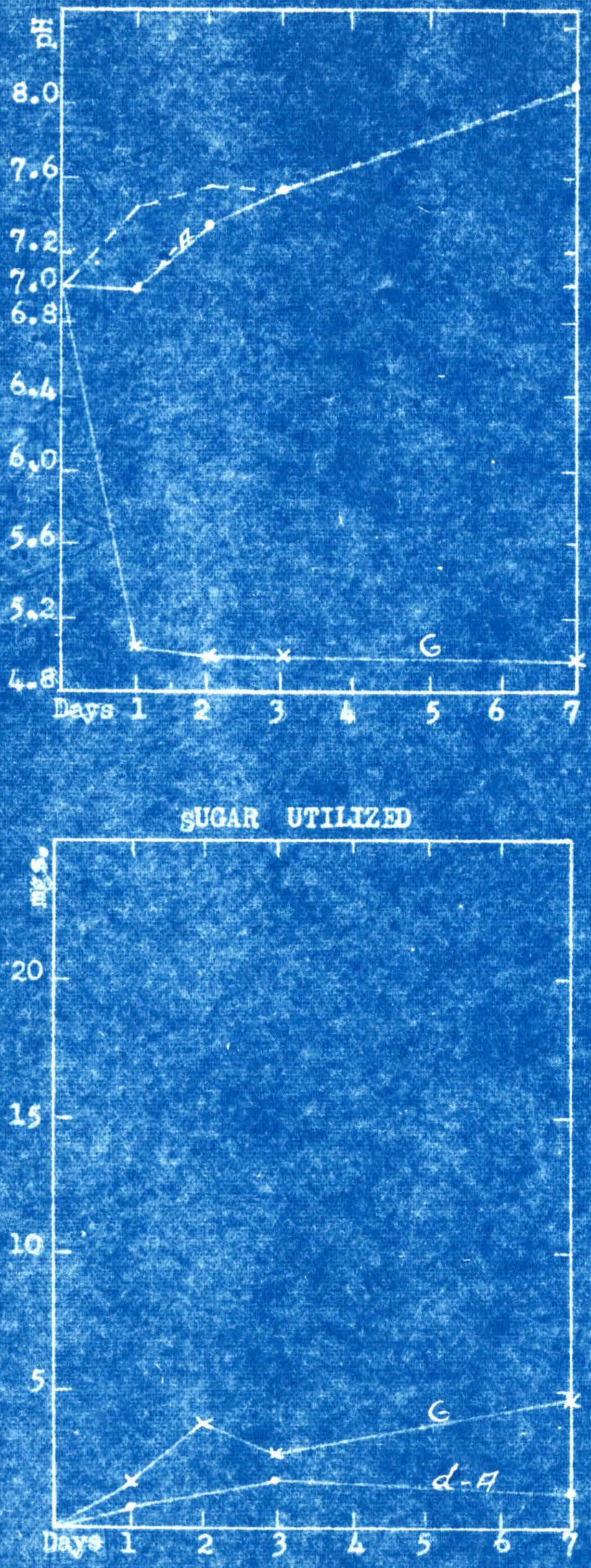
B. mycoides A.T.C. #80 Tex.

Fig. 72



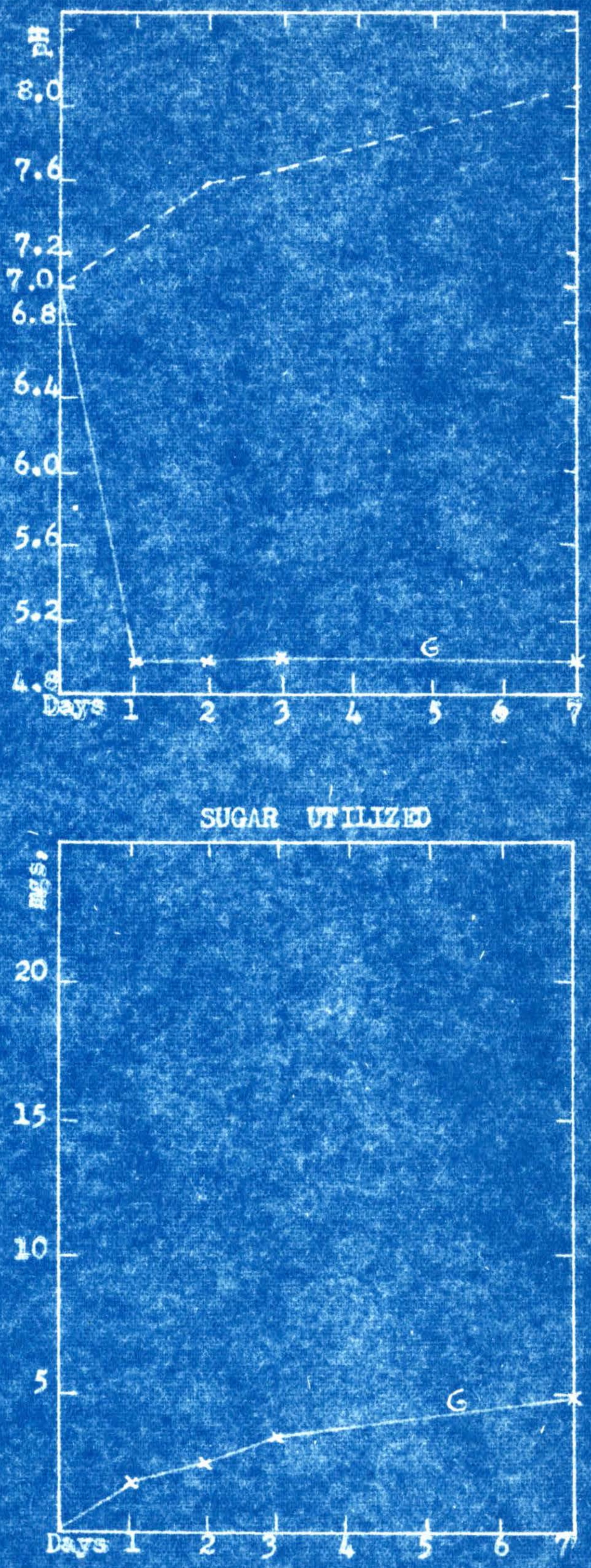
B. mycoides A.2 Tex.

Fig. 73



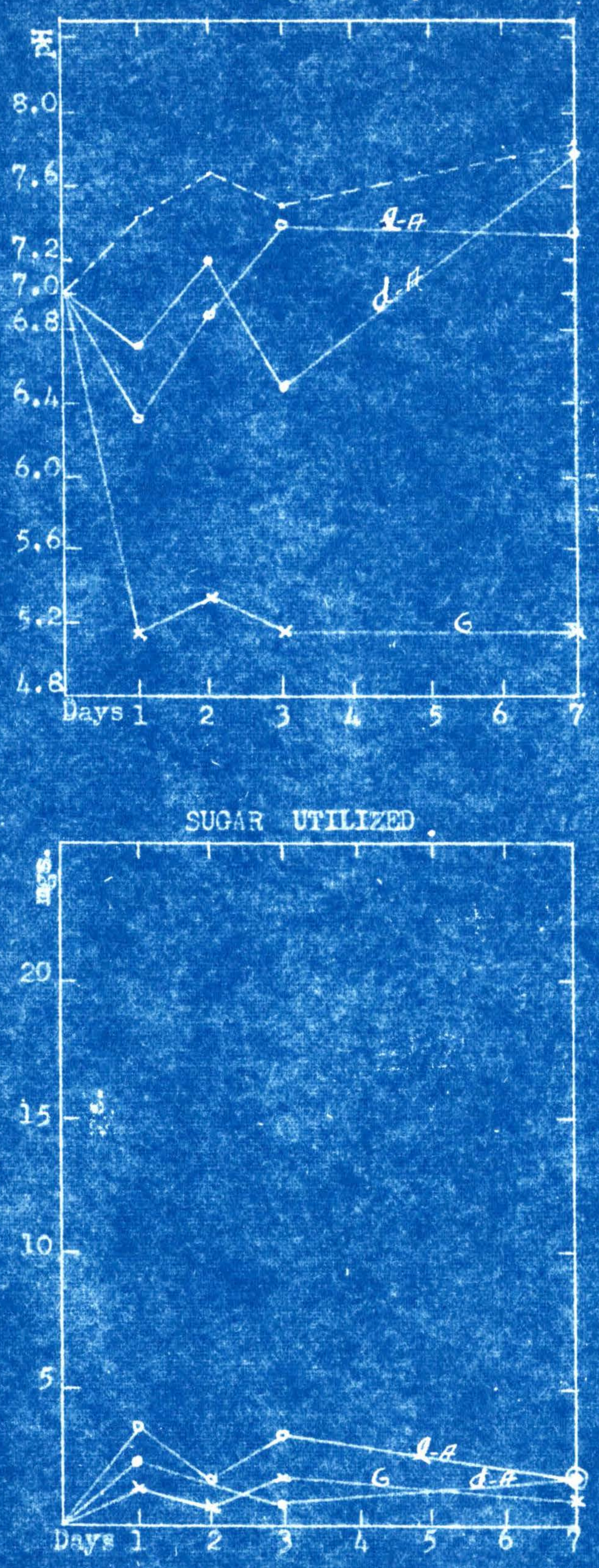
B. mycoides A.6 Tex.

Fig. 74



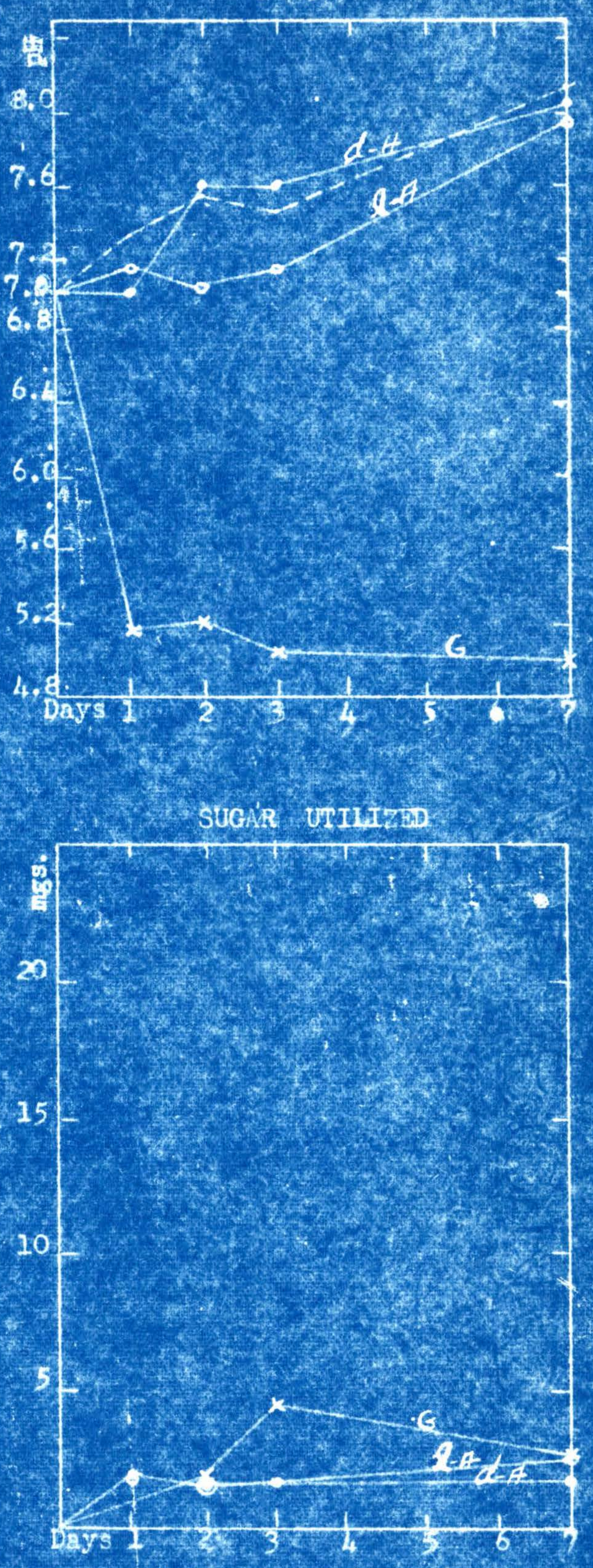
B. mycoides #23 Tex.

Fig. 75



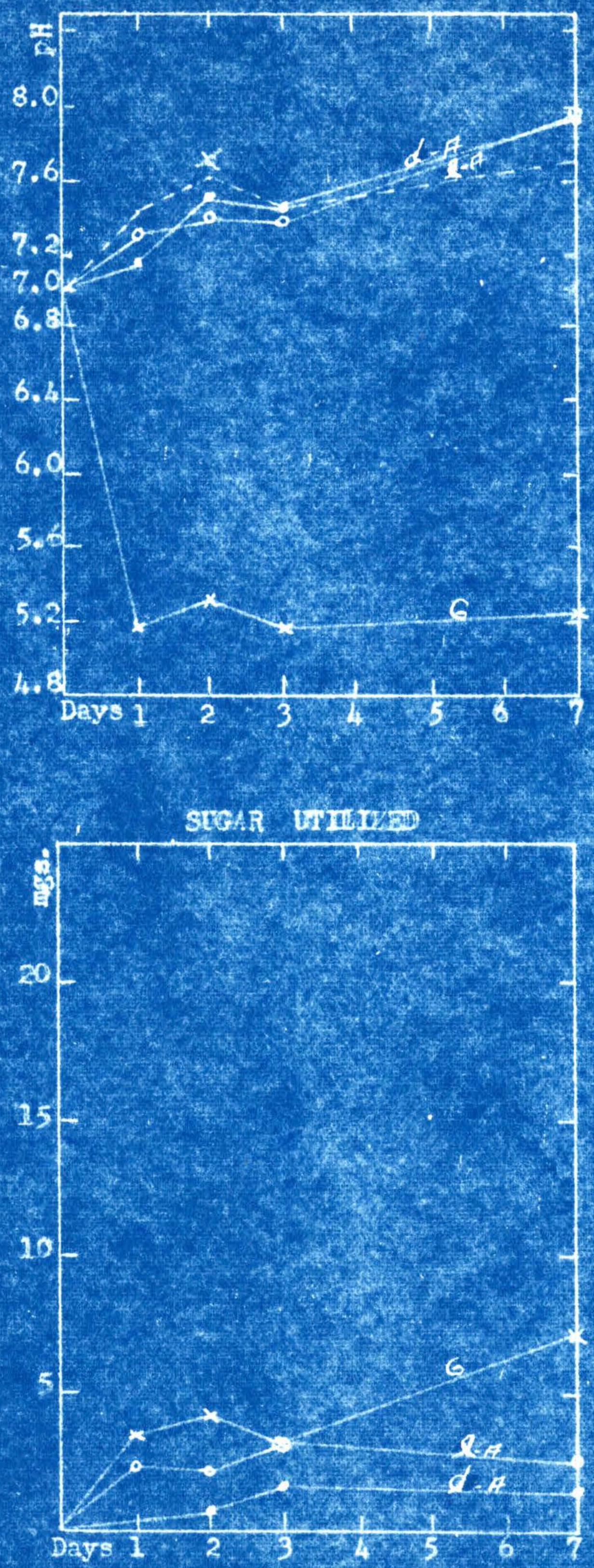
B. mycoides #65 Tex.

Fig. 76



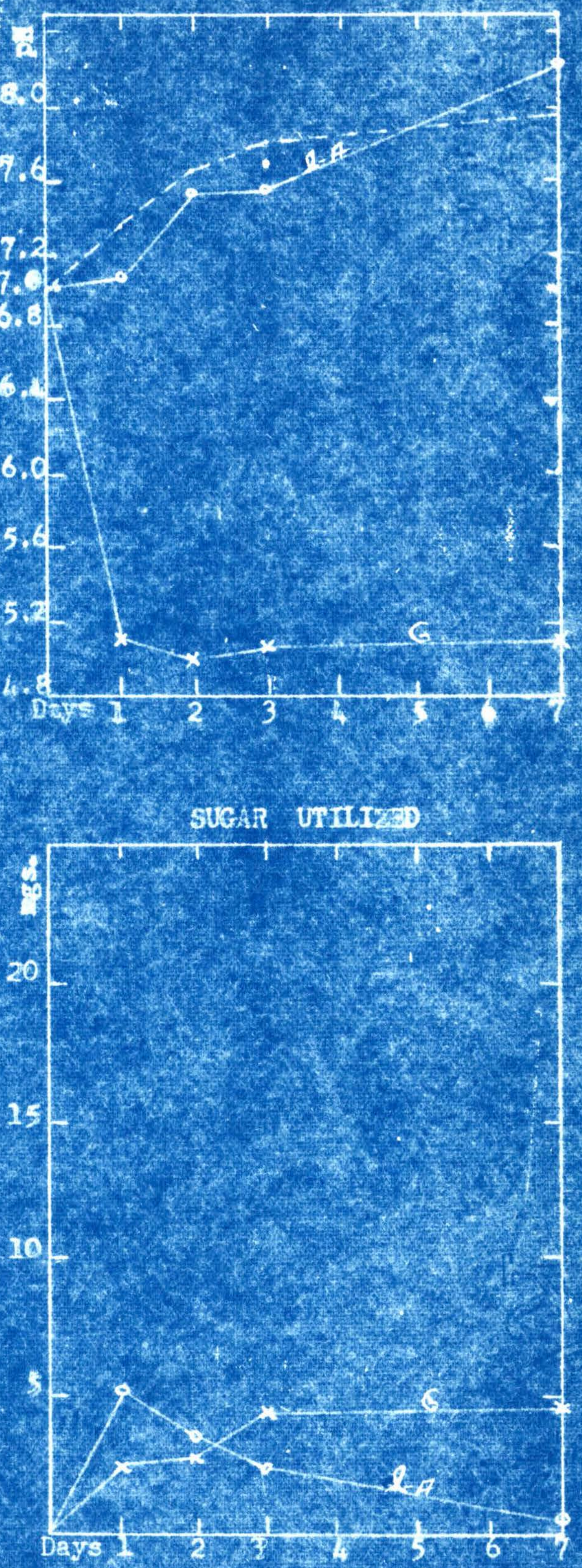
B. mycoides #405 Tex.

Fig. 77



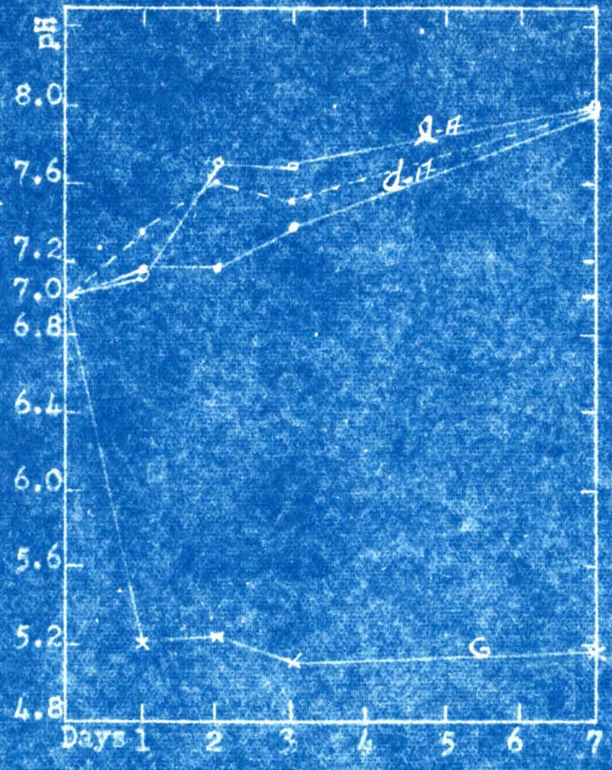
B. mycoides #410 Tex.

Fig. 78

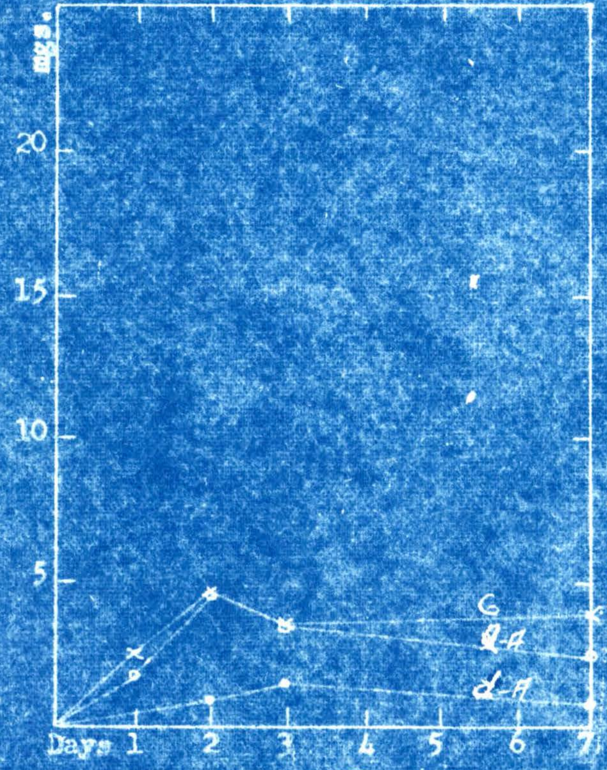


B. mycoides #417 Tex.

Fig. 79

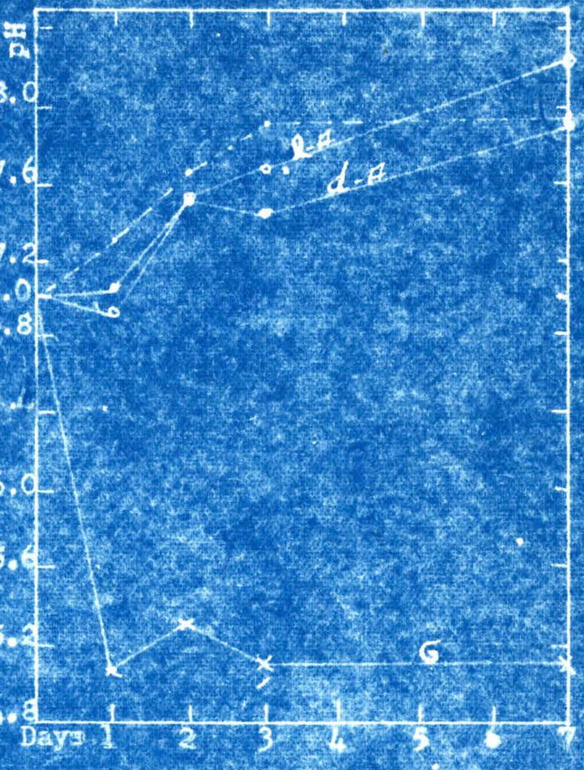


SUGAR UTILIZED



B. mycoides #420 Tex.

Fig. 80



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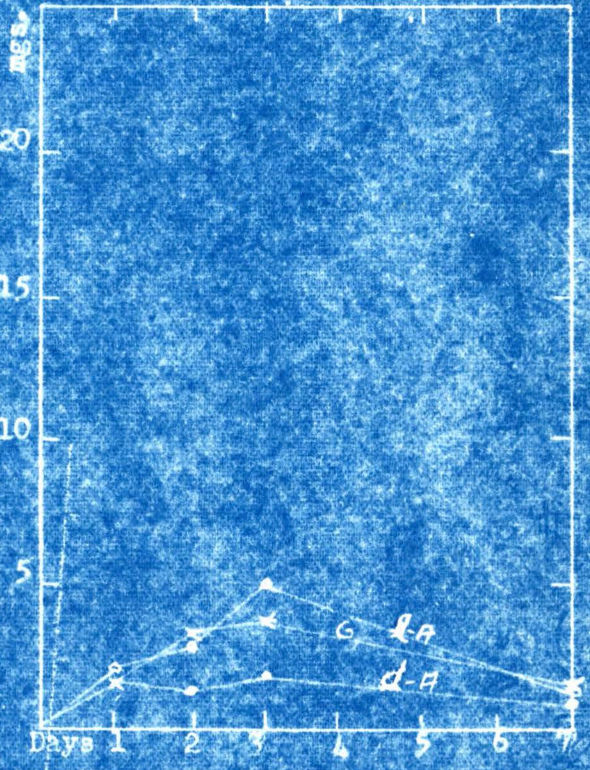
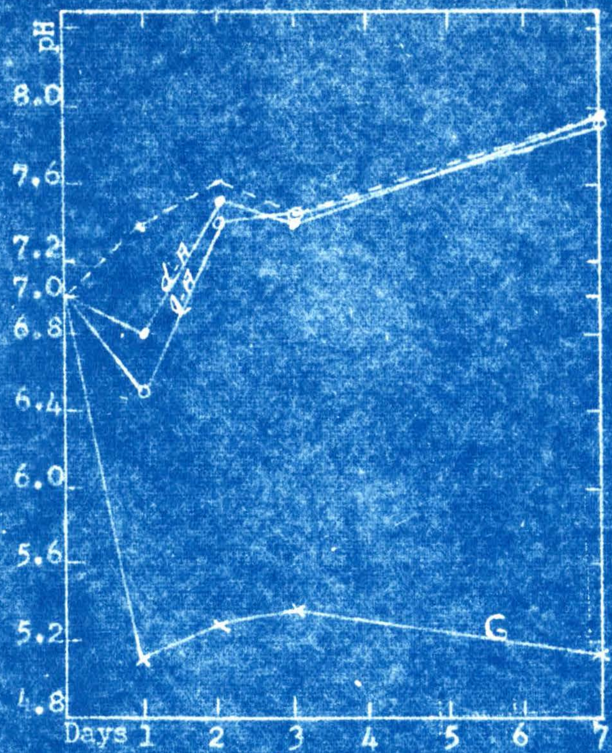


B. mycoides #421 Tex.

Fig. 81



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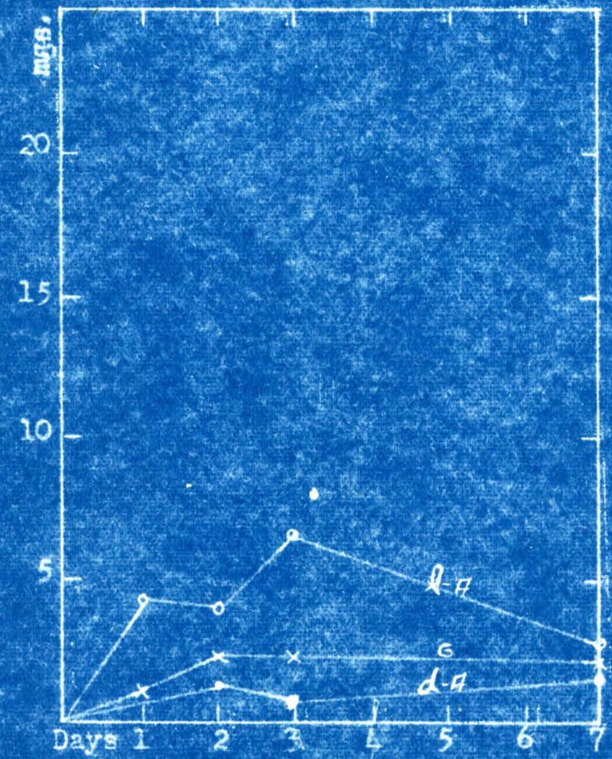
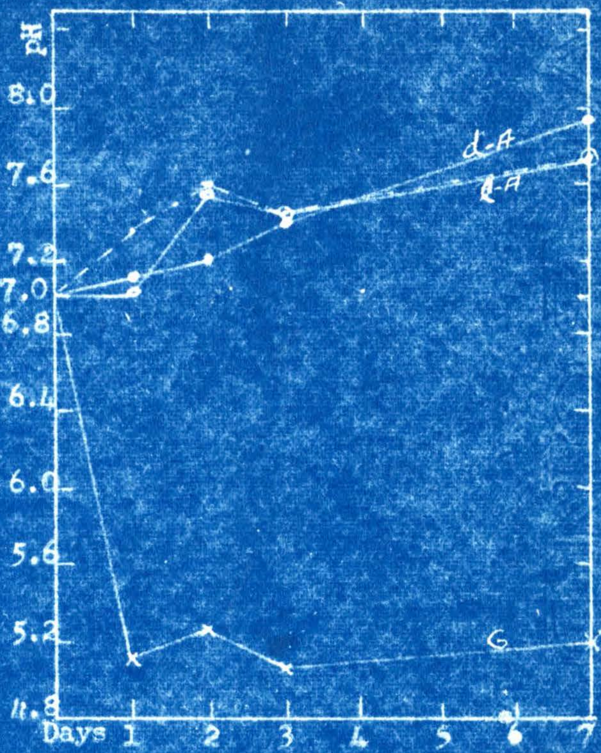
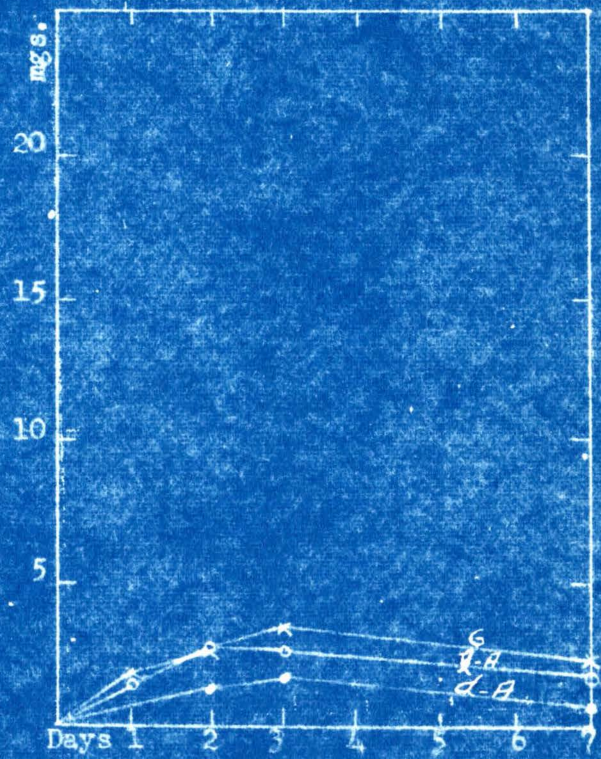


B. mycoides #422 Tex.

Fig. 82

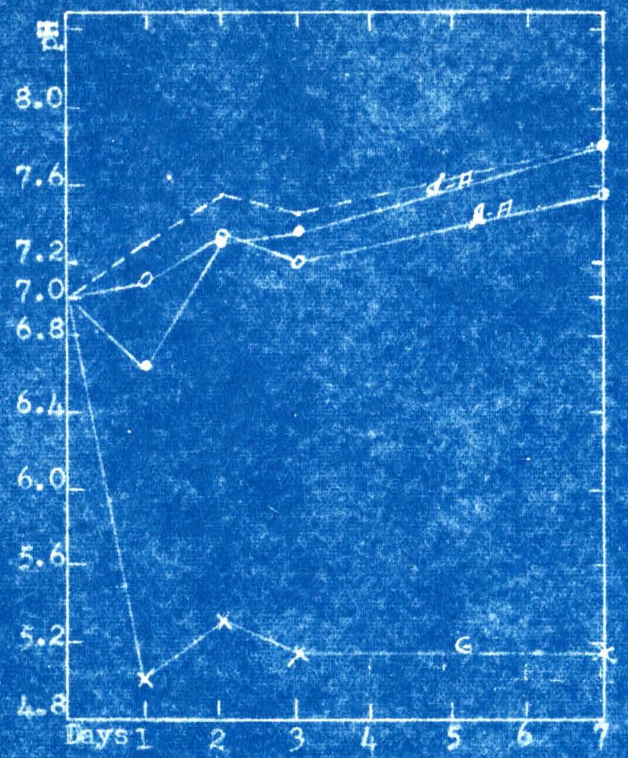


SUGAR UTILIZED



B. mycoides #425 Tex.

Fig. 83



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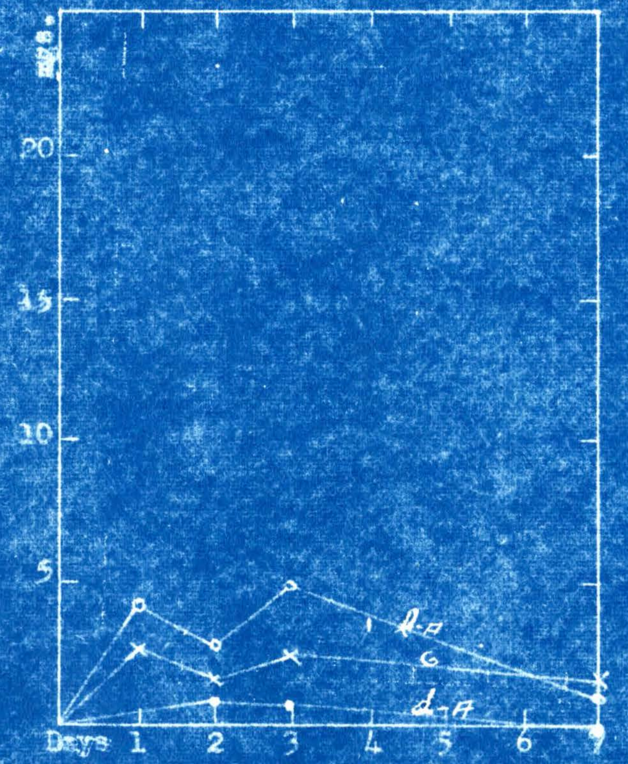
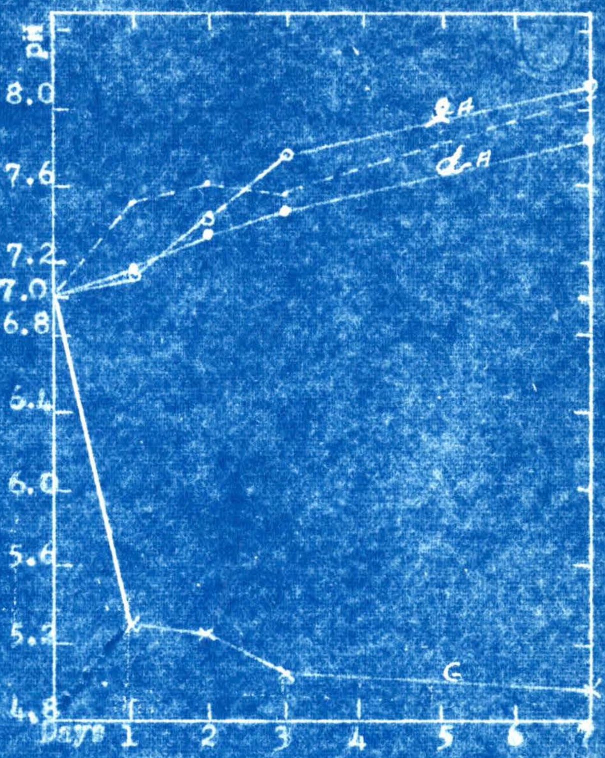
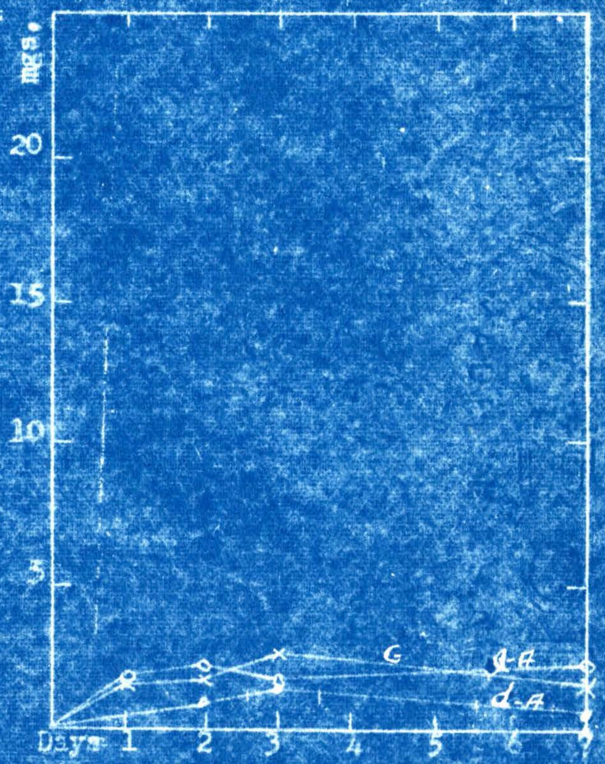


B. mycoides #426 Tex.

Fig. 84

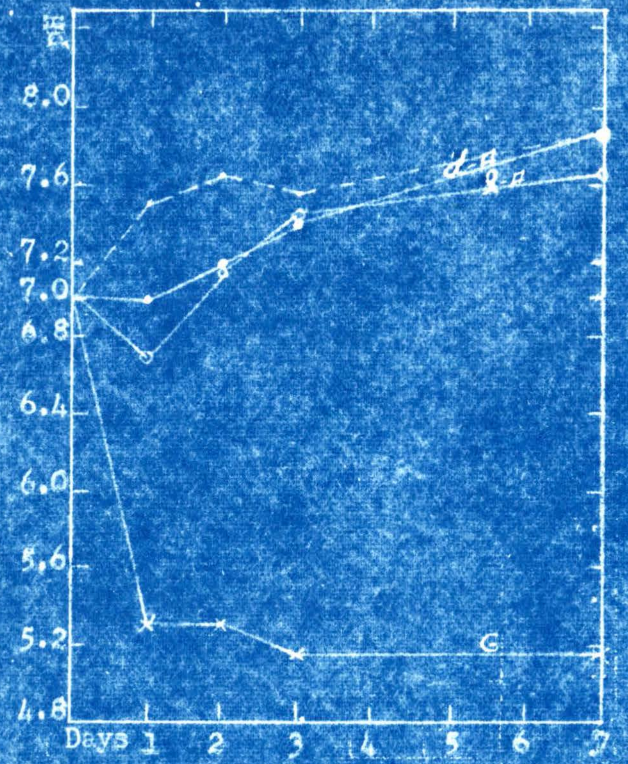


SUGAR UTILIZED



B. mycoides #427 Tex.

Fig. 85



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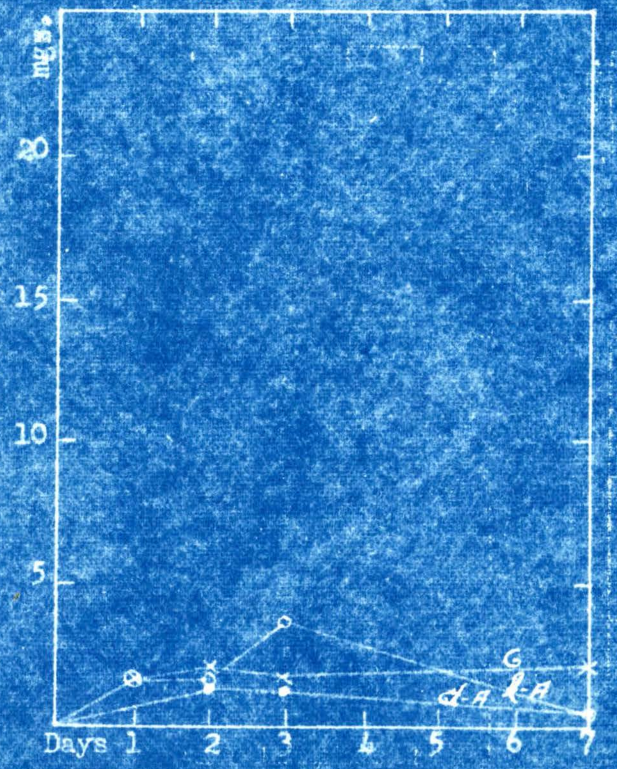
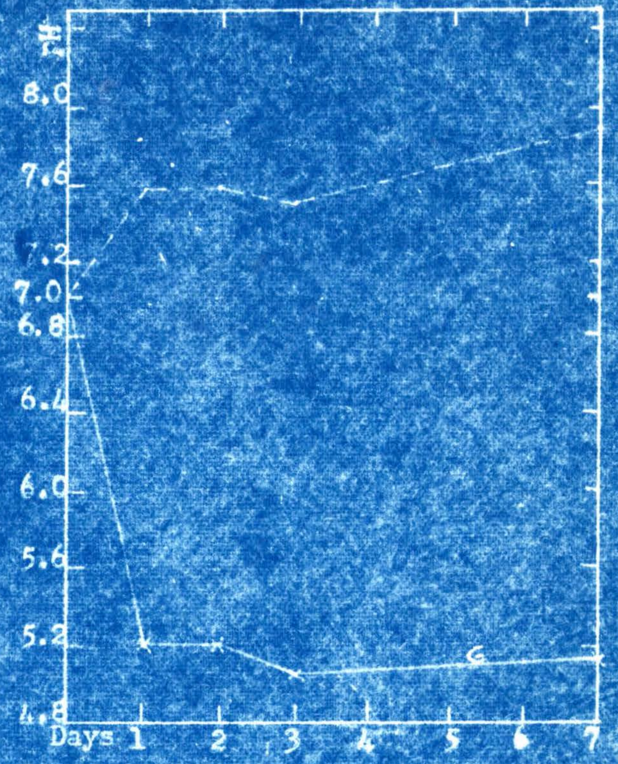


B. mycoides #130 Tex.

Fig. 86



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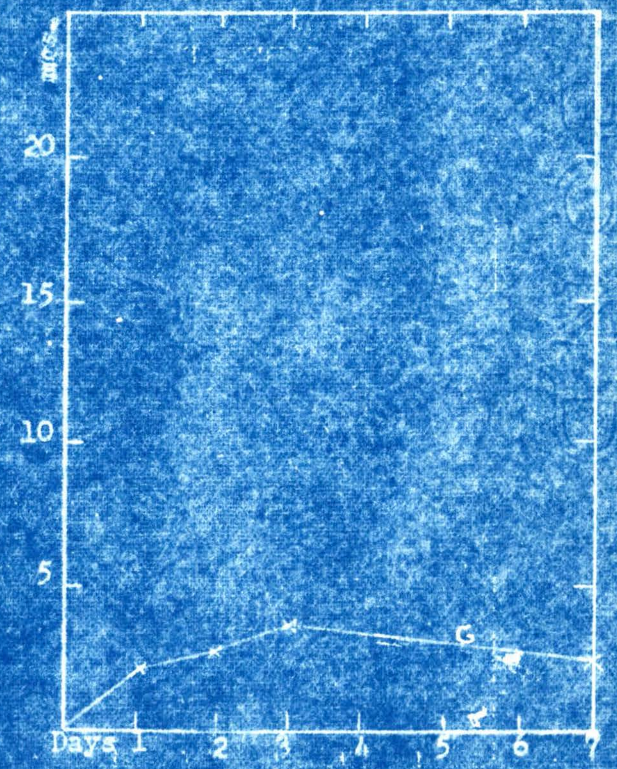


B. mycoides Wright Wis.

Fig. 87

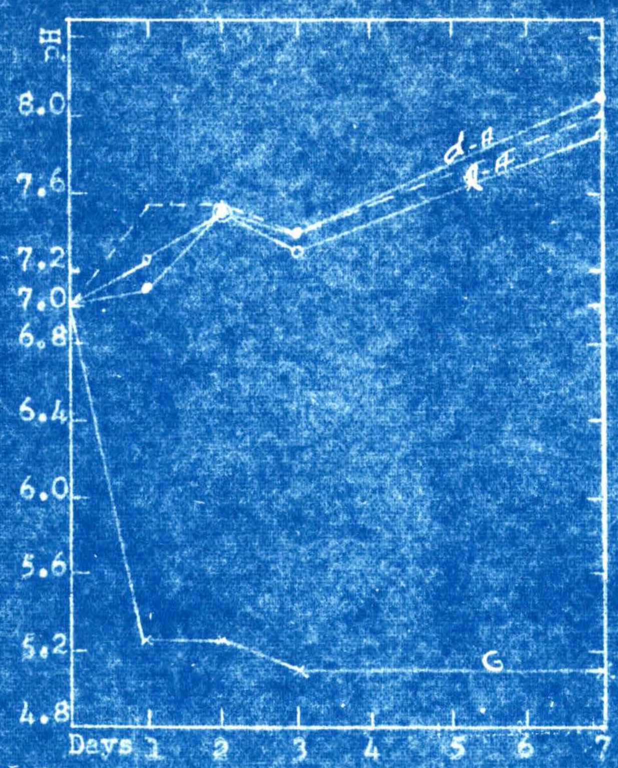
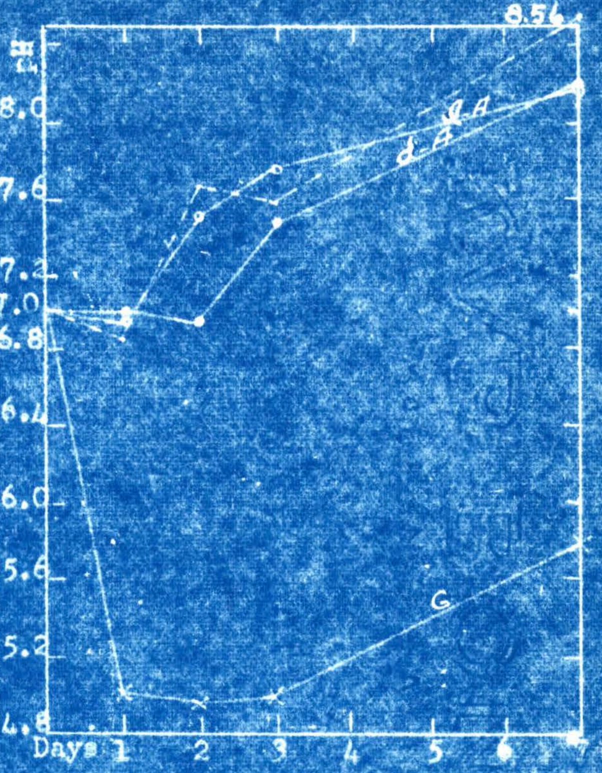


Fig. 88



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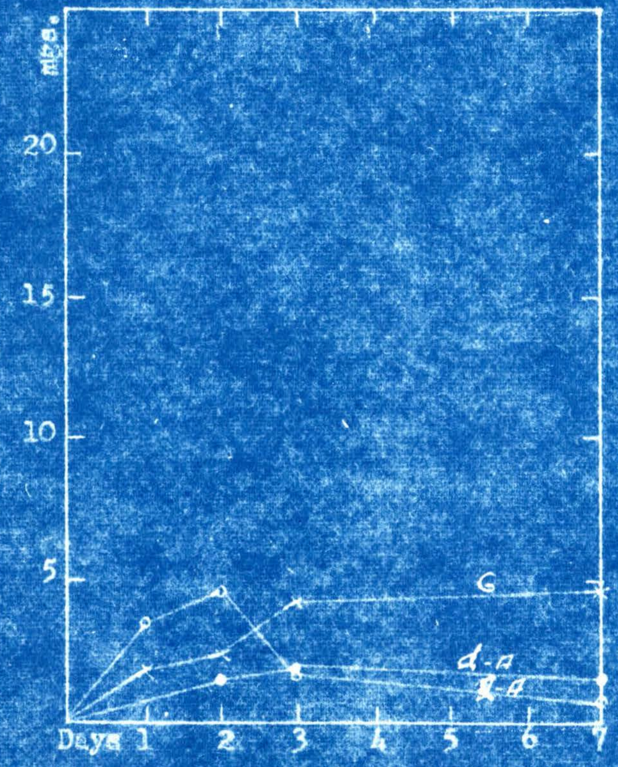

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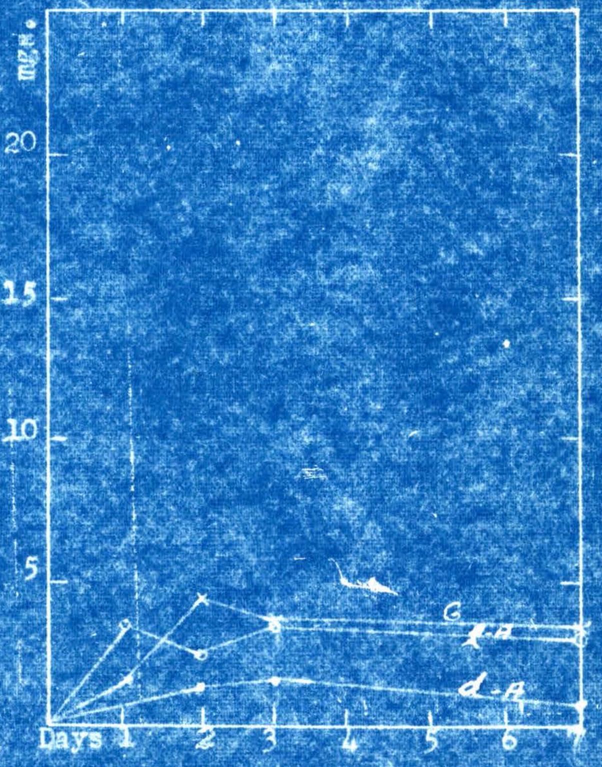
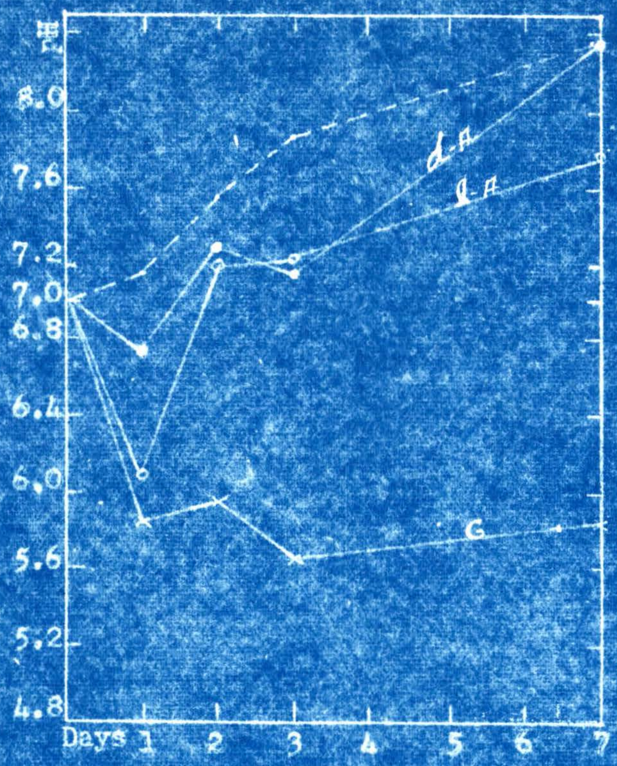


B. mycoides Neb.

B. mycoides (left-hand Sarles) Wis.

Fig. 89



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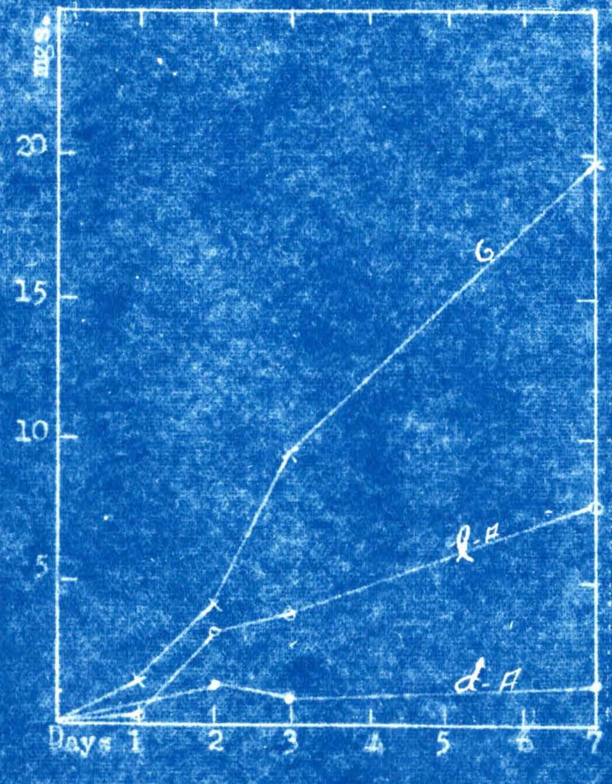
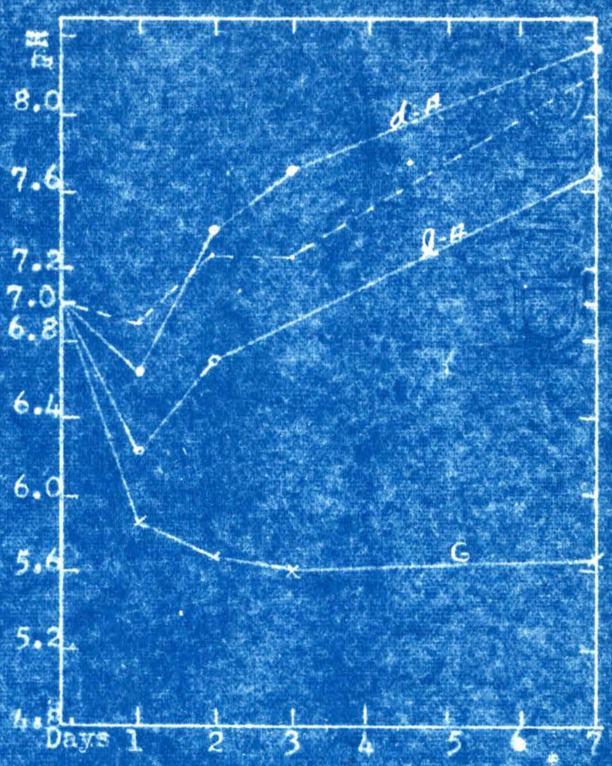


B. *niger* A.T.C.

Fig. 90



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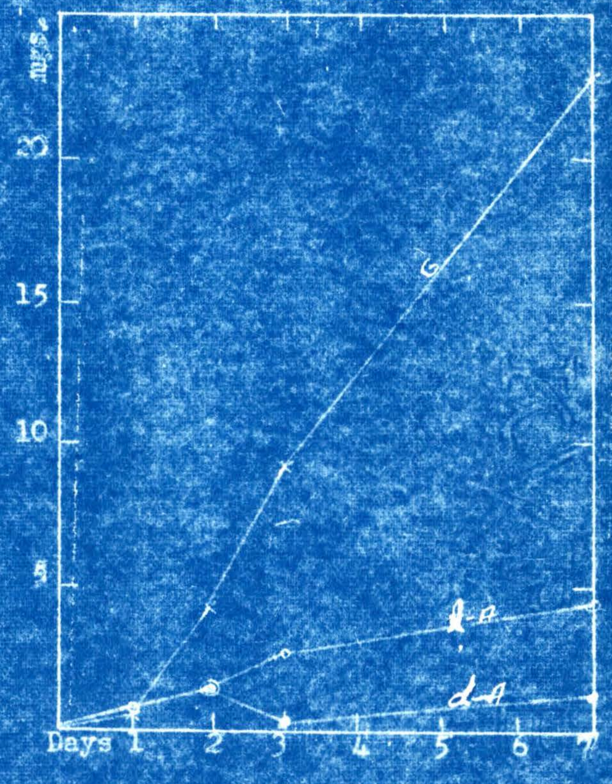


B. *niger* U.S.D.A.

Fig. 91

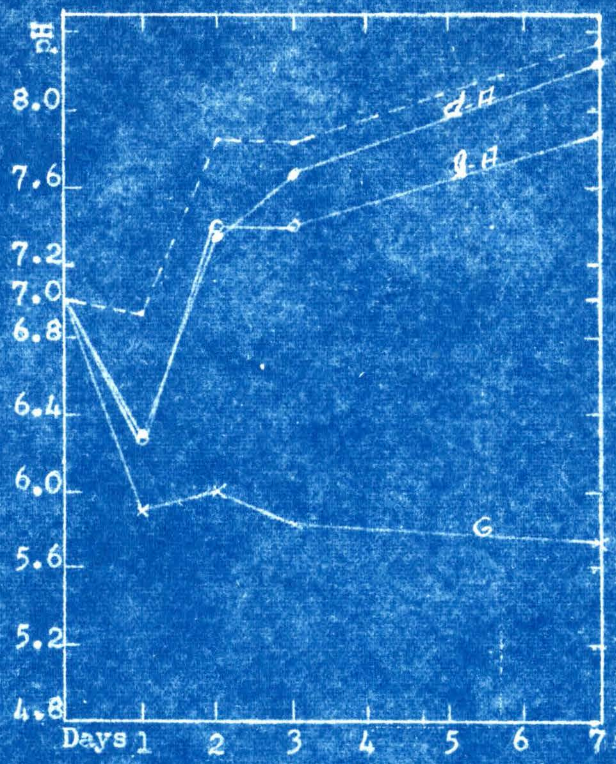
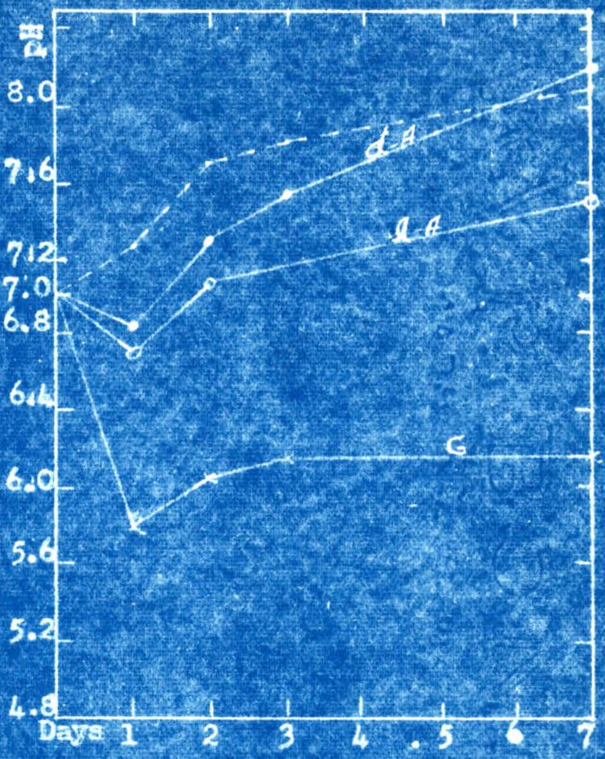


Fig. 92



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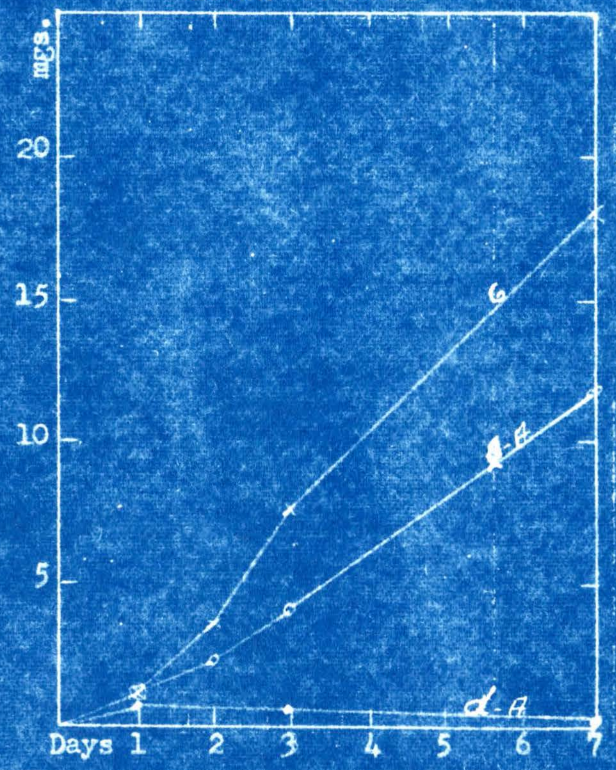
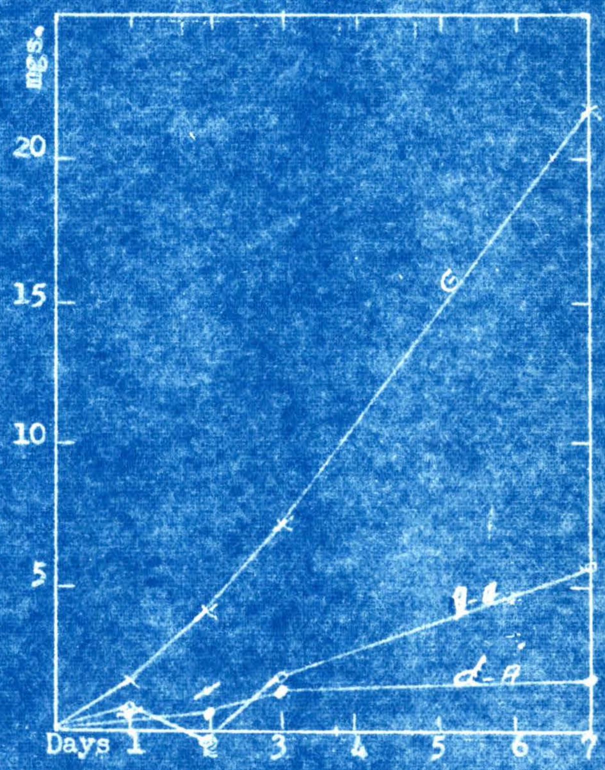


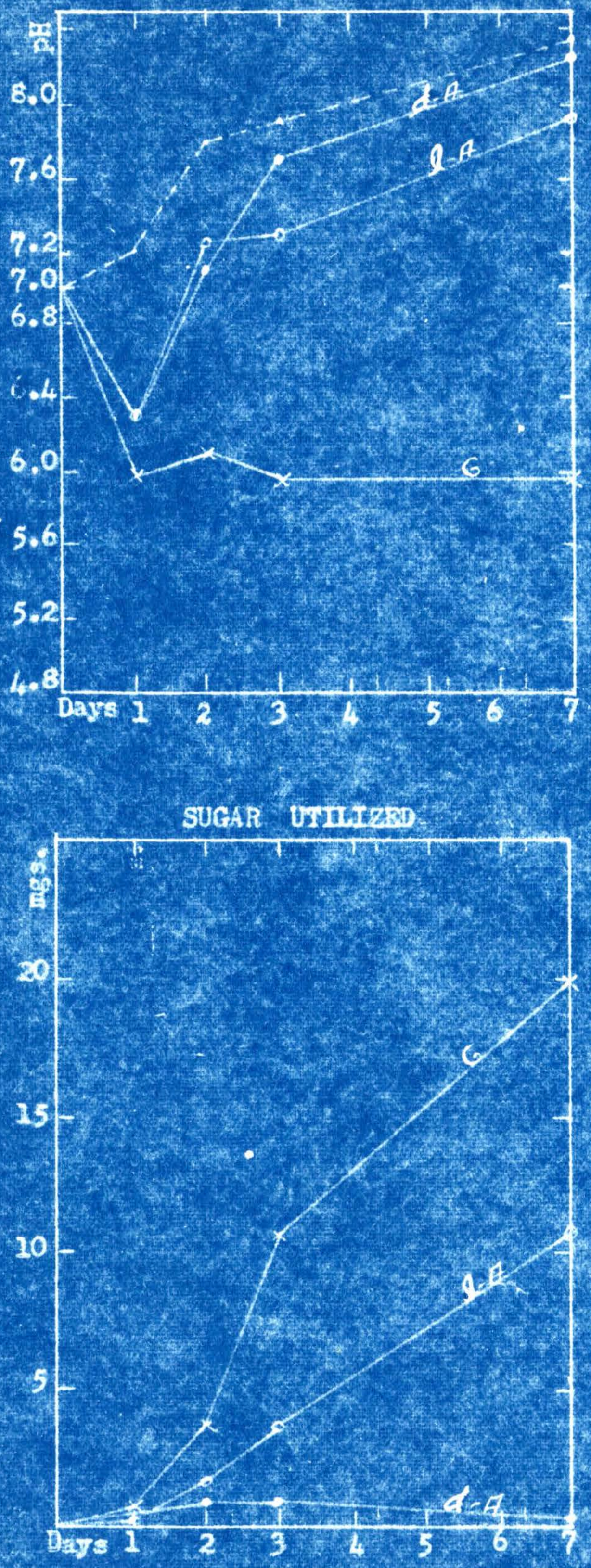
SUGAR UTILIZED



B. *niger* #228 Wis.

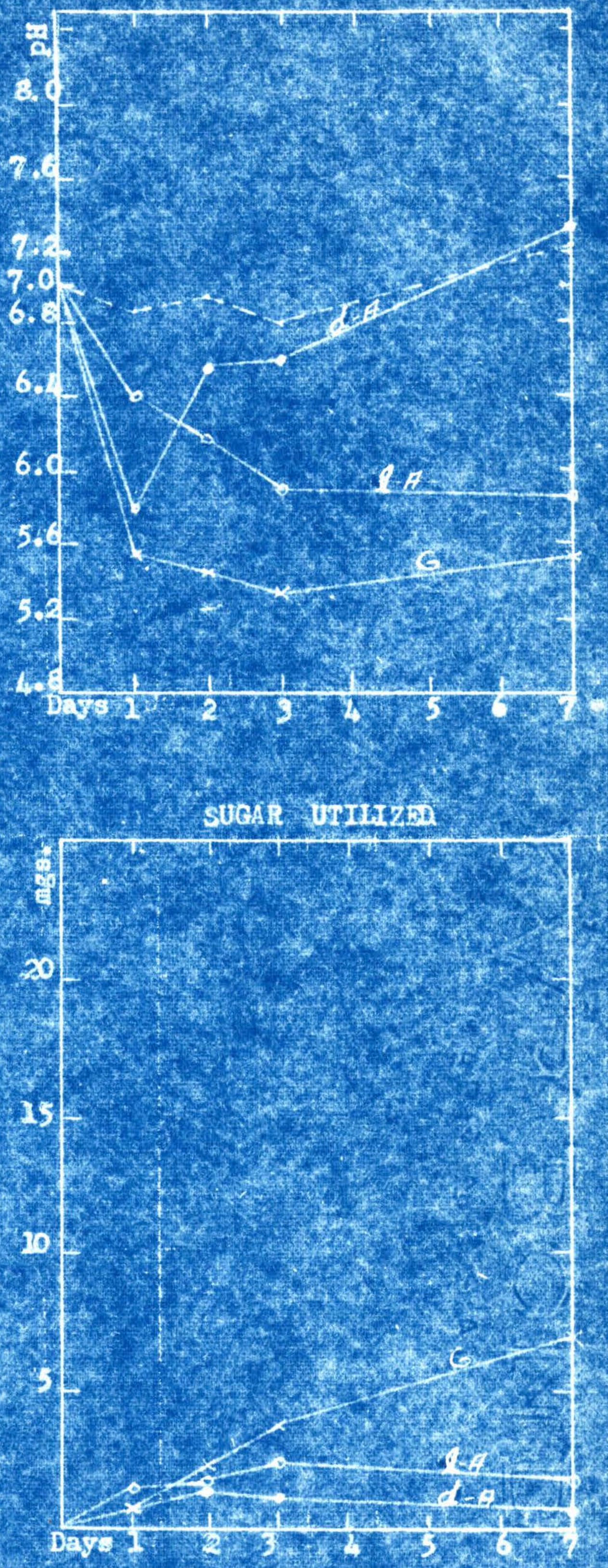
B. *niger* "Old" Wis.

Fig. 93



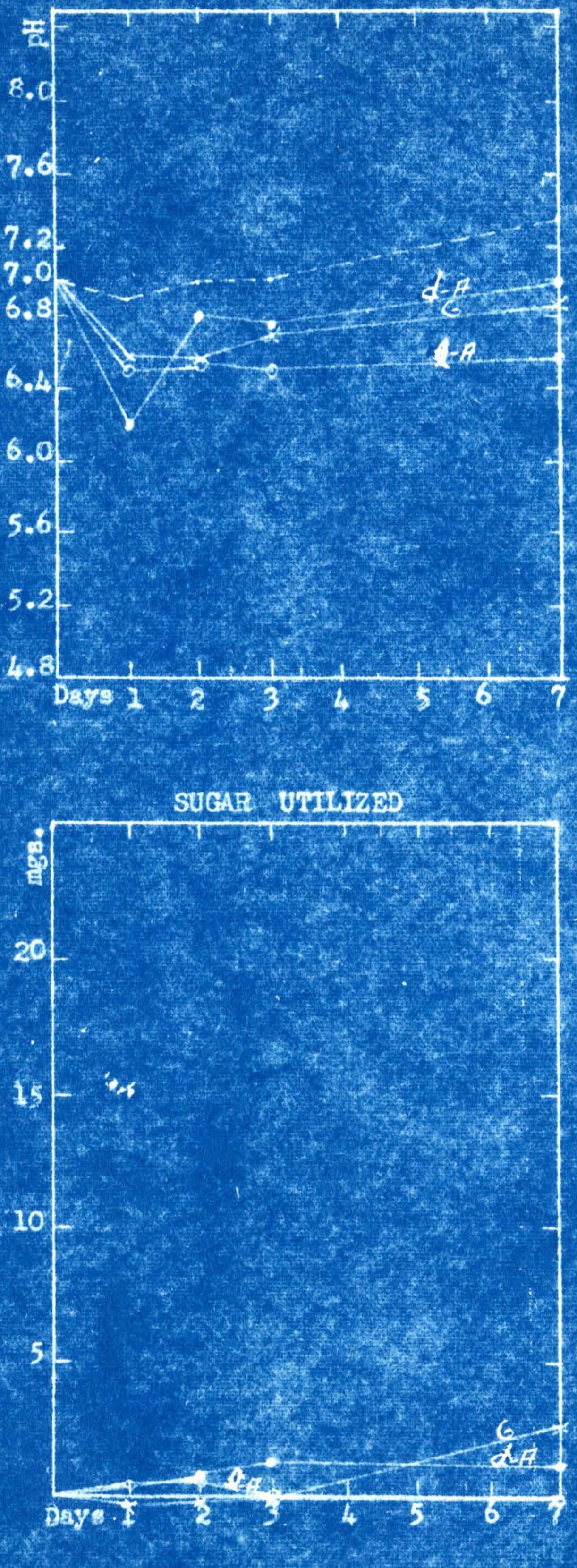
B. niger 'S' Wis.

Fig. 94



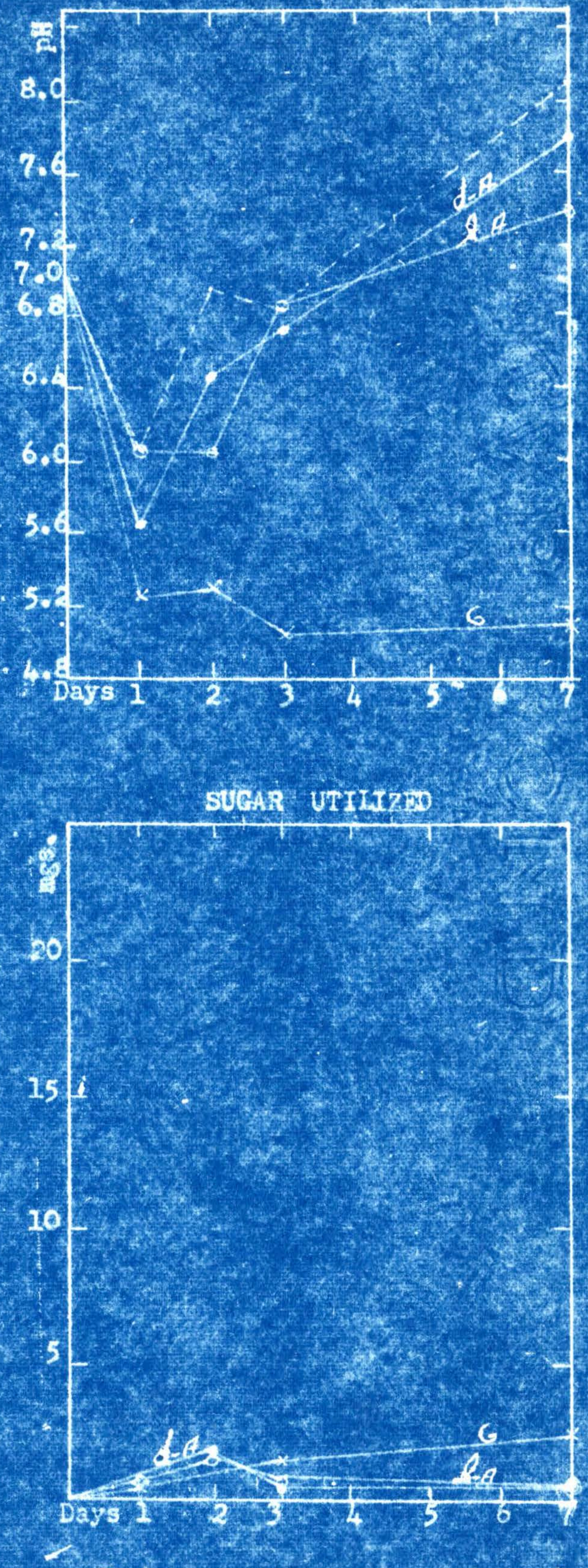
B. panis A.T.C.

Fig. 95



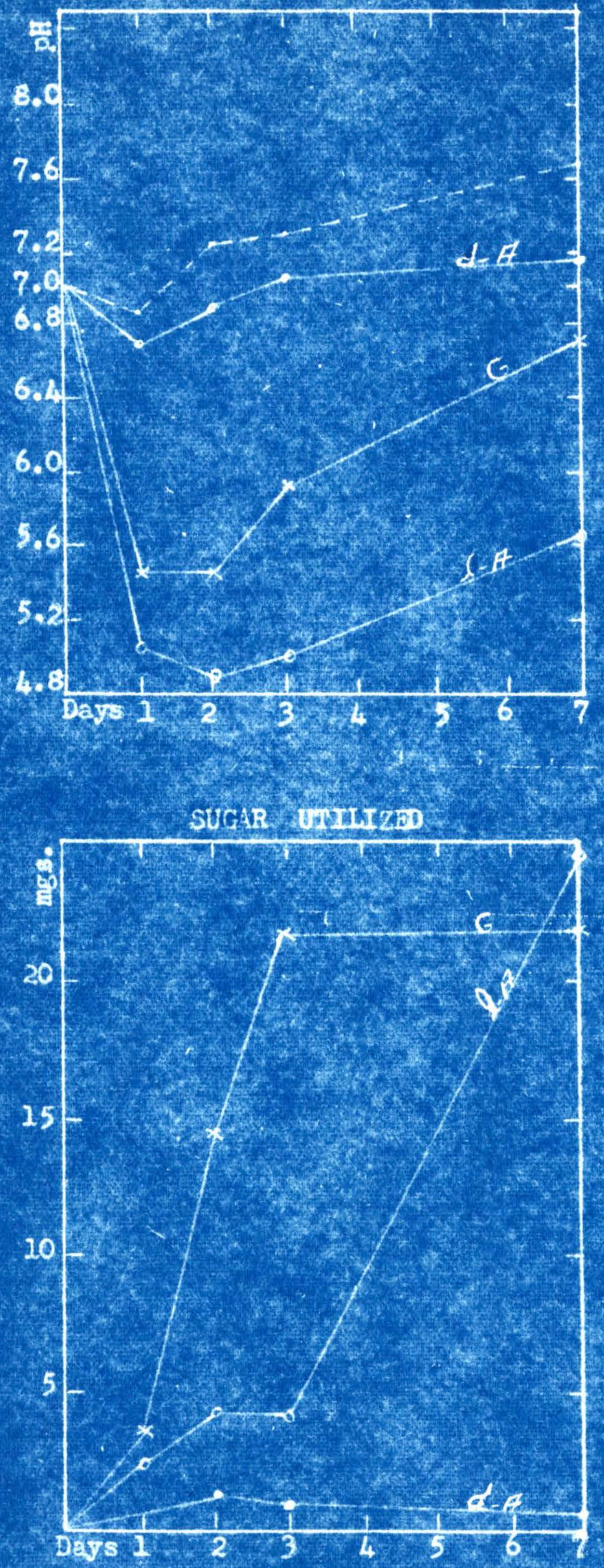
B. panis U.S.D.A.

Fig. 96



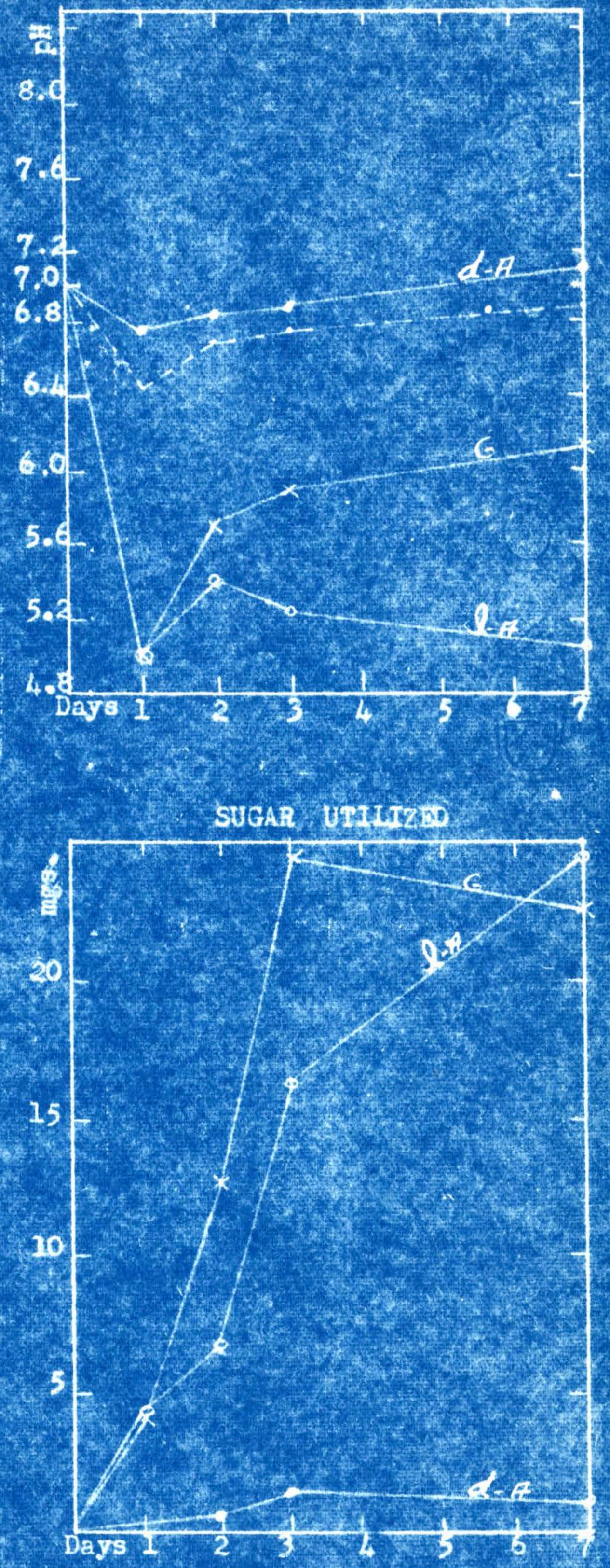
B. paraalvei Tex.

Fig. 97



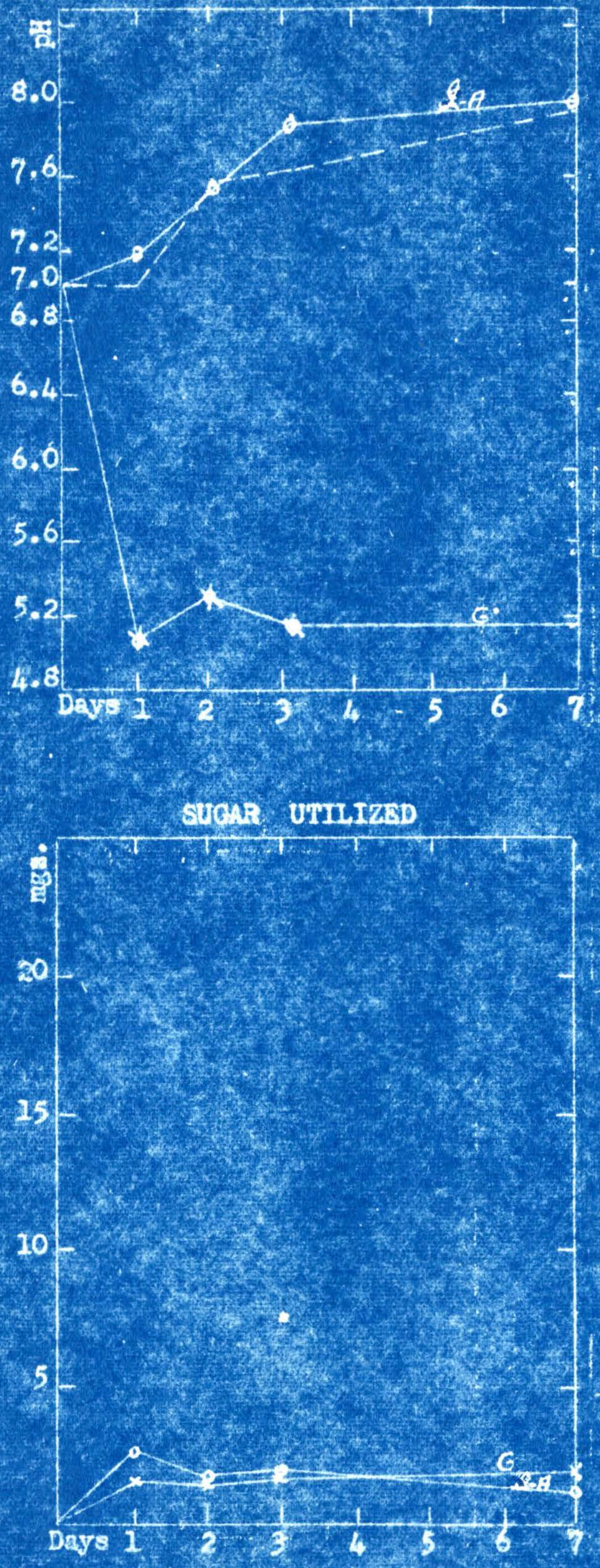
B. polymyxa V.P.I.

Fig. 98



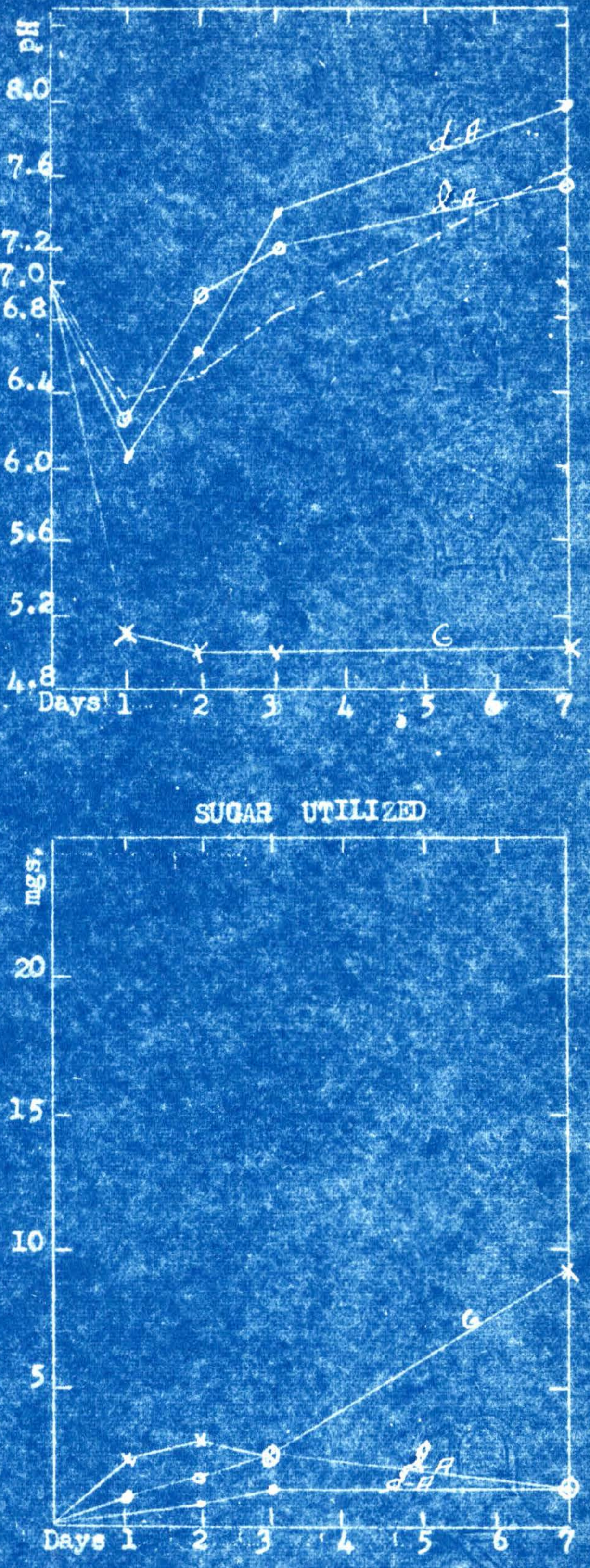
B. polymyxa Tex.

Fig. 99



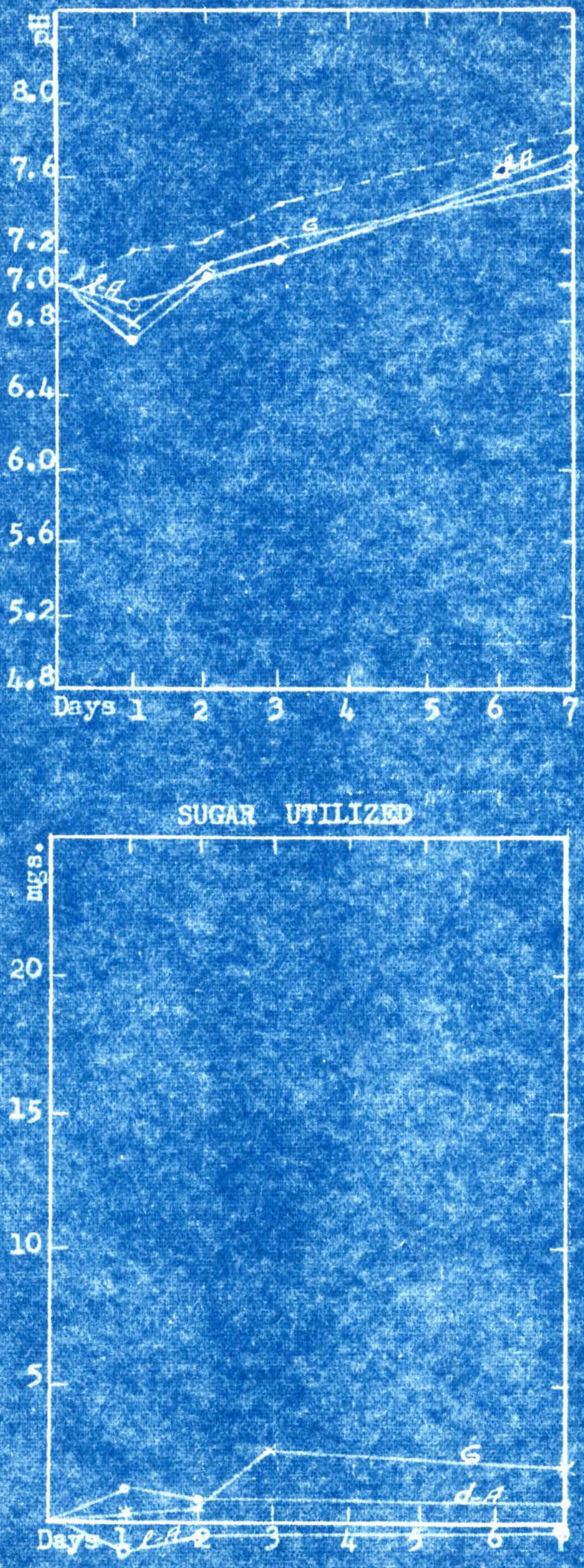
B. prausnitzii Neb.

Fig. 100



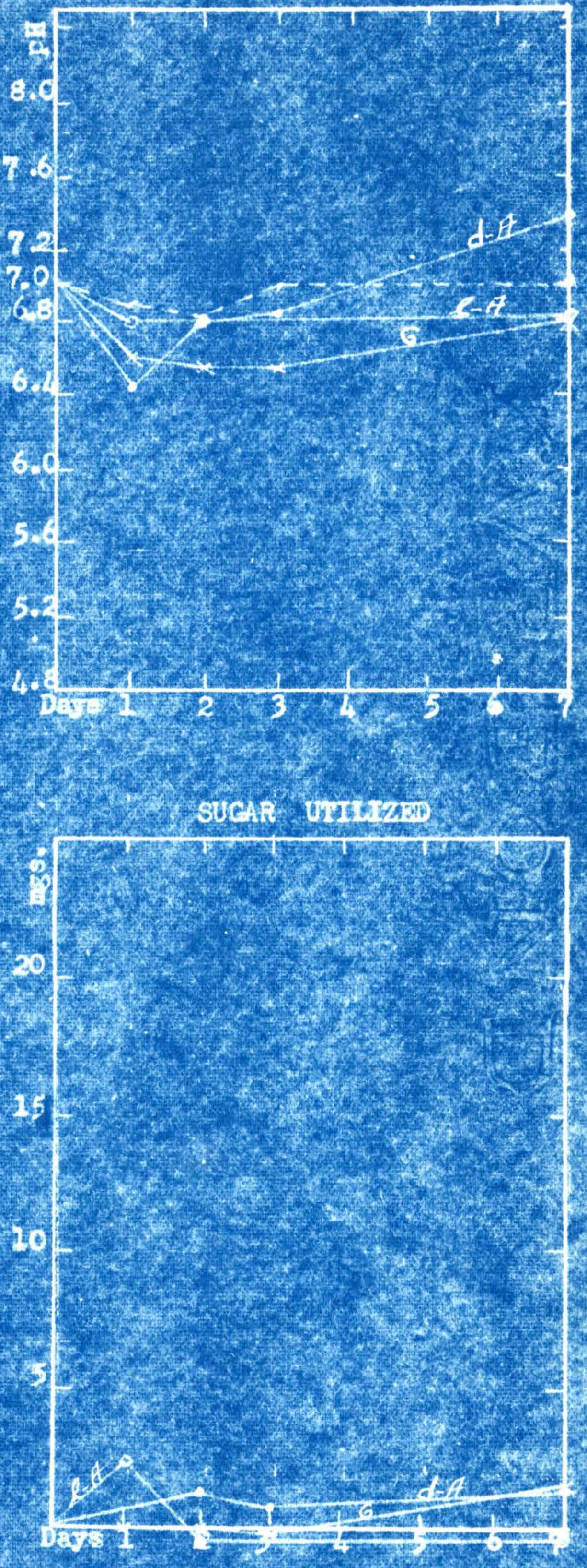
B. robur 946 Tex.

Fig. 101



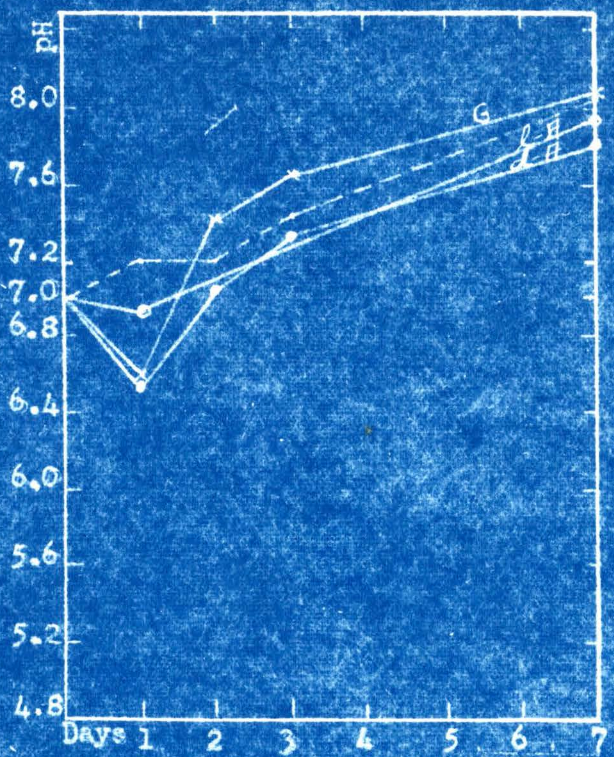
B. rotans A.T.C.

Fig. 102

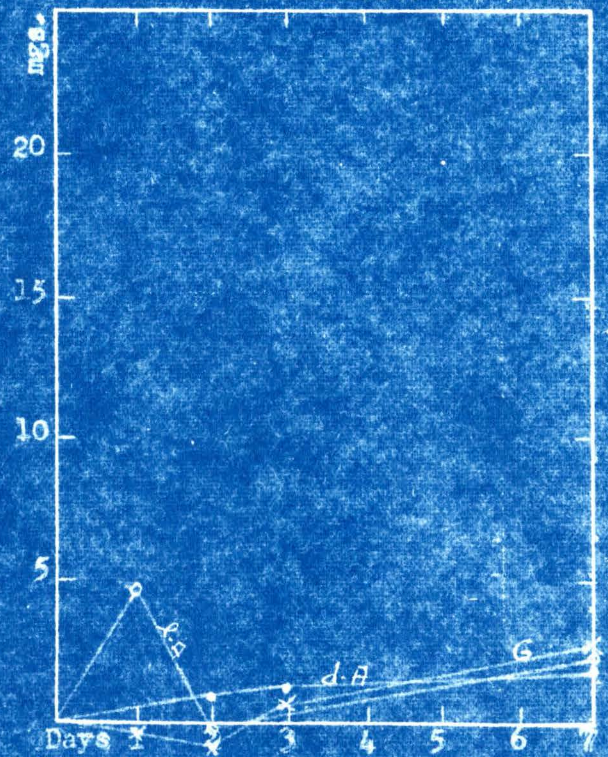


B. rotans Tex.

Fig. 103

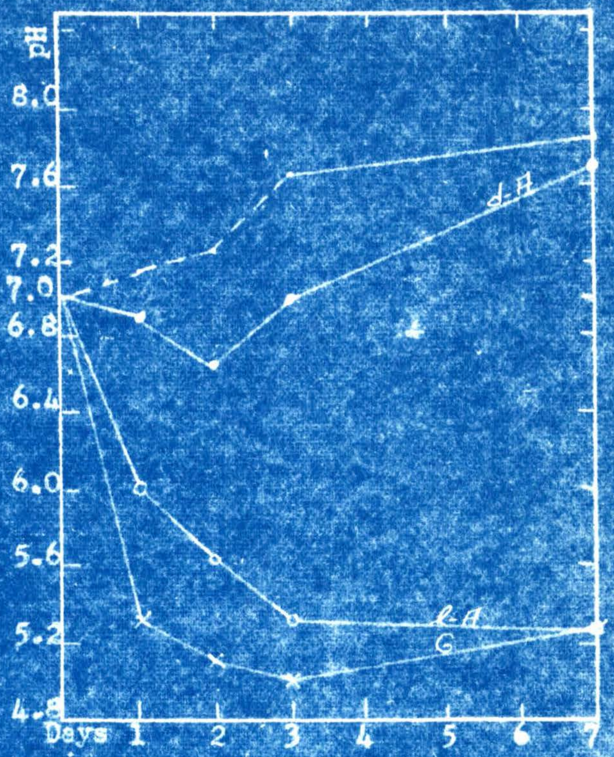


SUGAR UTILIZED

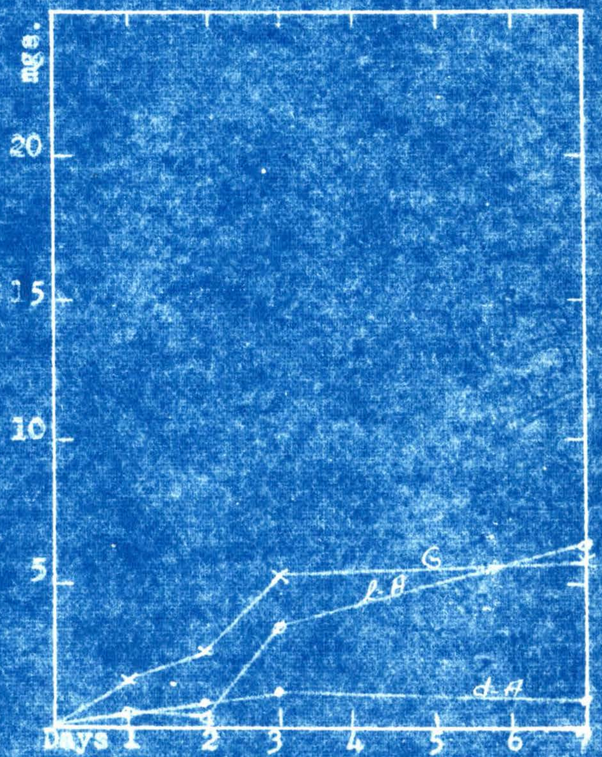


B. rotans (probably Wis.)

Fig. 104

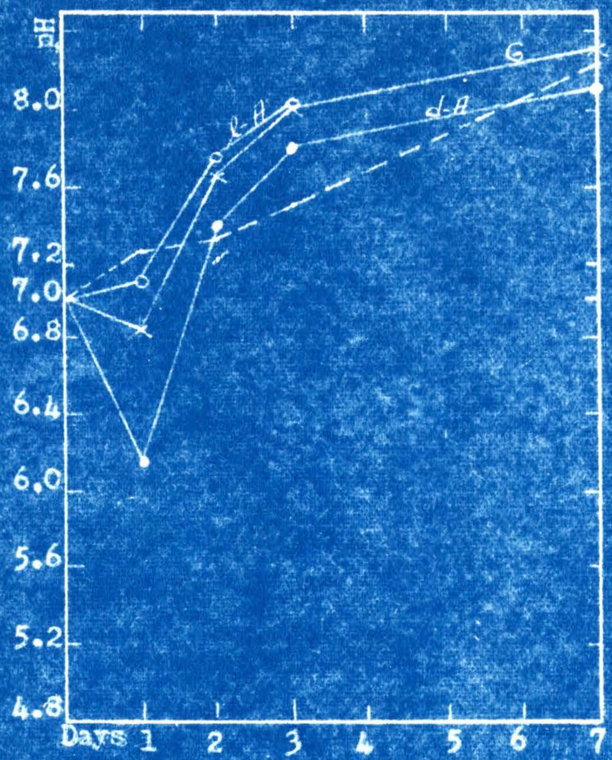


SUGAR UTILIZED

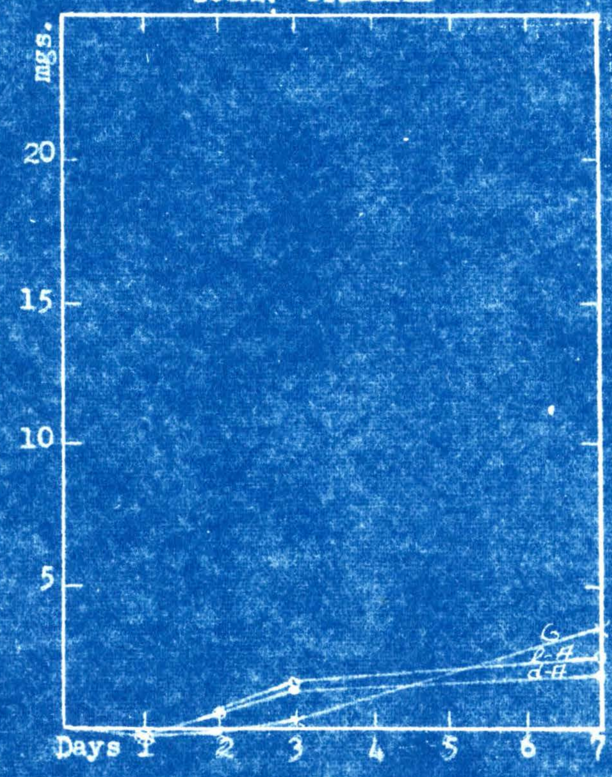


B. ruminatus #952 Tex.

Fig. 105

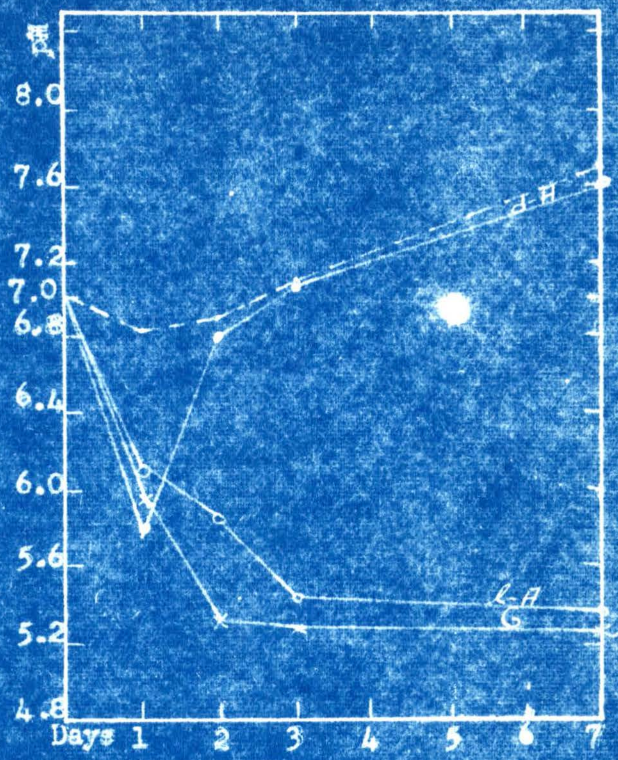


SUGAR UTILIZED

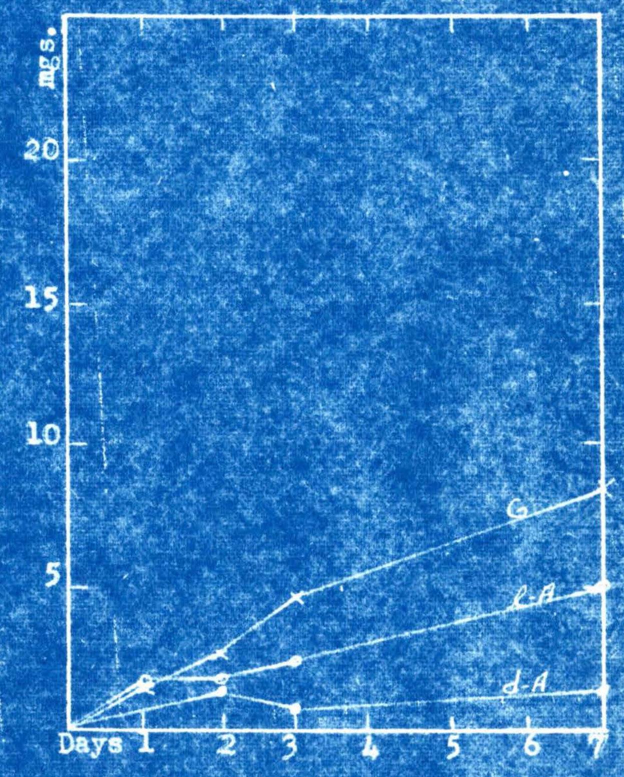


B. serositides Neb.

Fig. 106

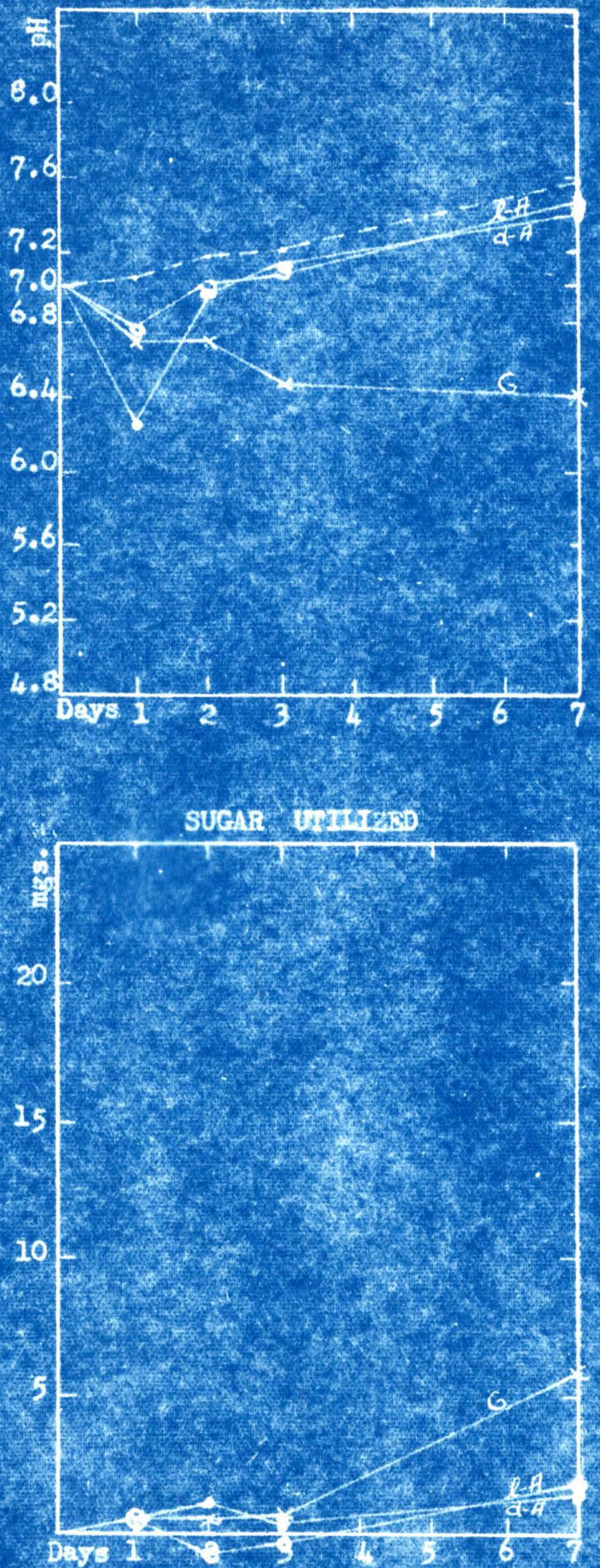


SUGAR UTILIZED



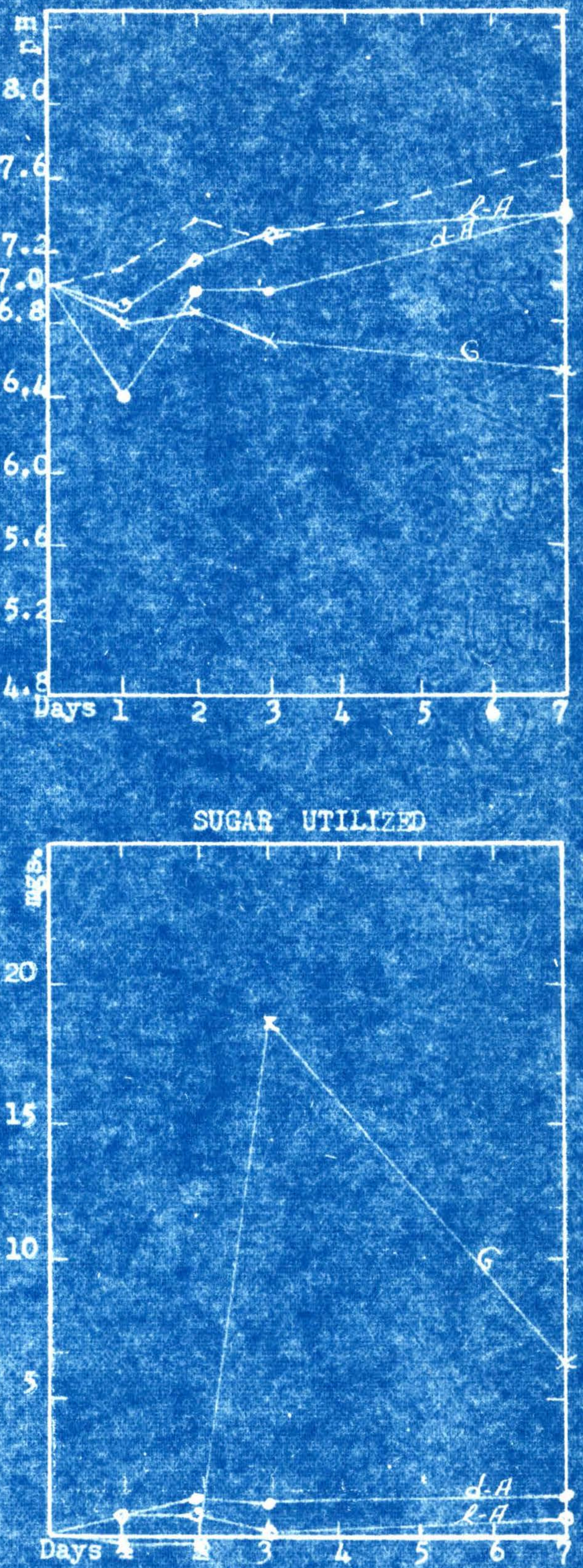
B. silvaticus #957 Tex.

Fig. 107



B. simplex Tex.

Fig. 108



B. simplex #335 Tex.

Fig. 109

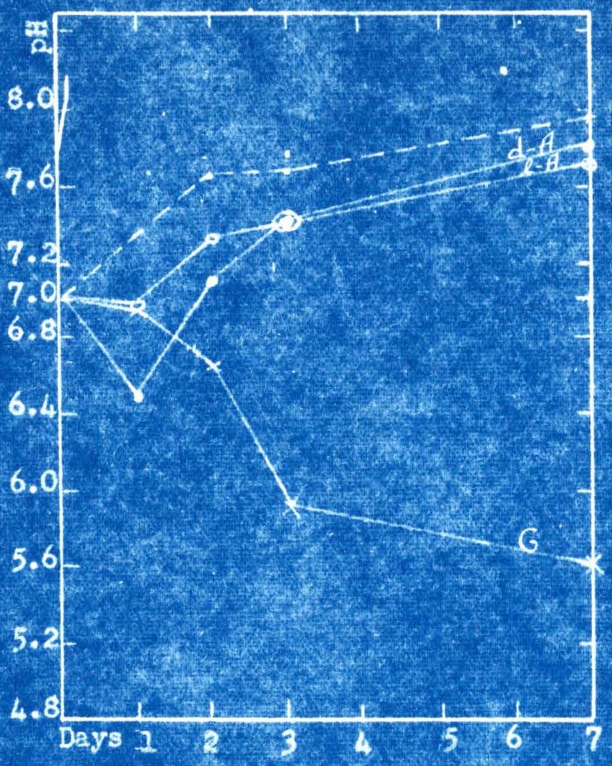
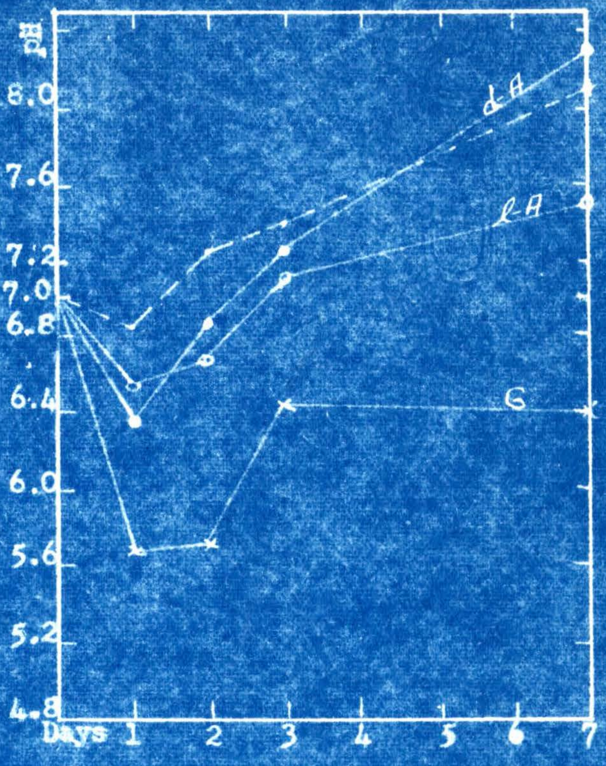


Fig. 110



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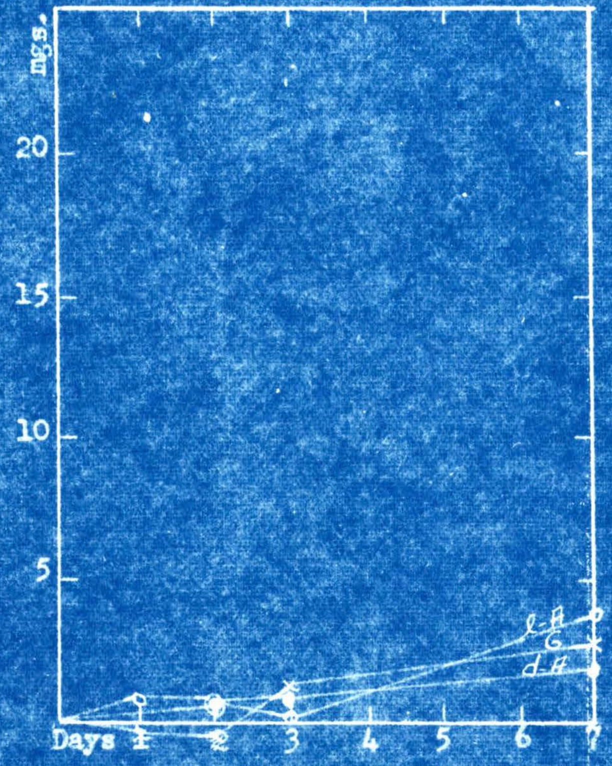

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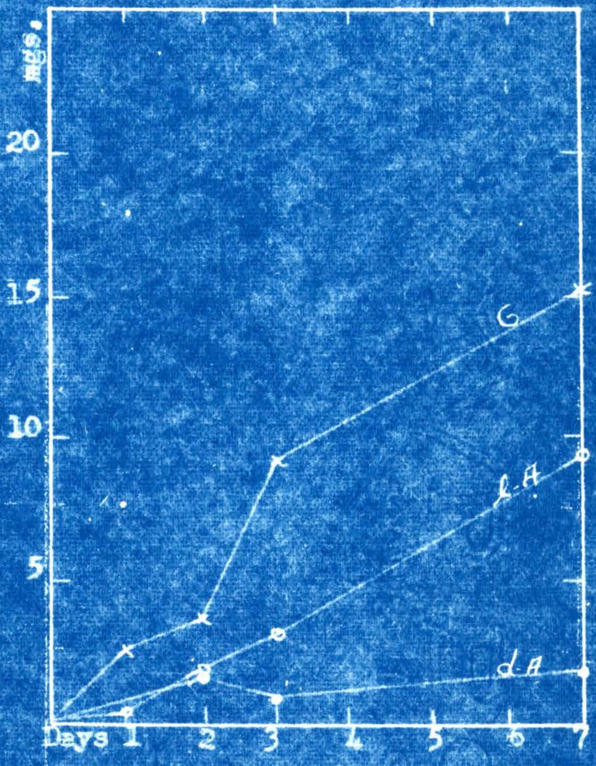
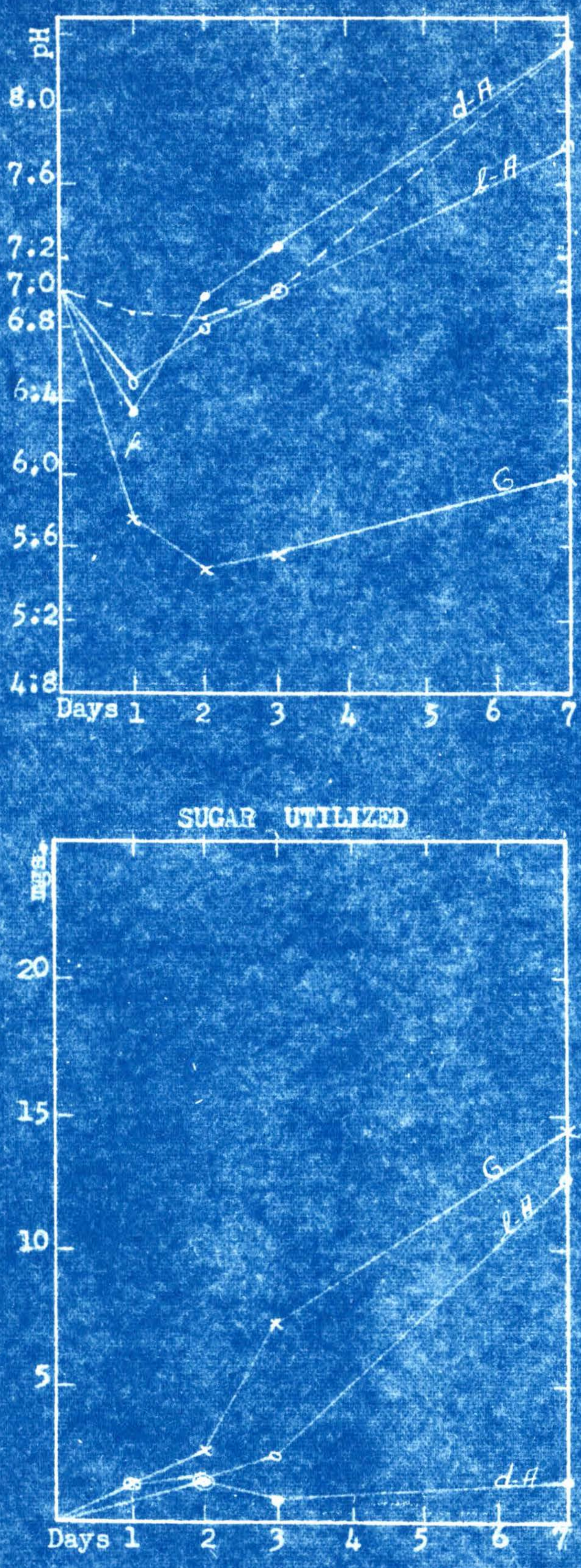


B. sphaericus #348 Tex.

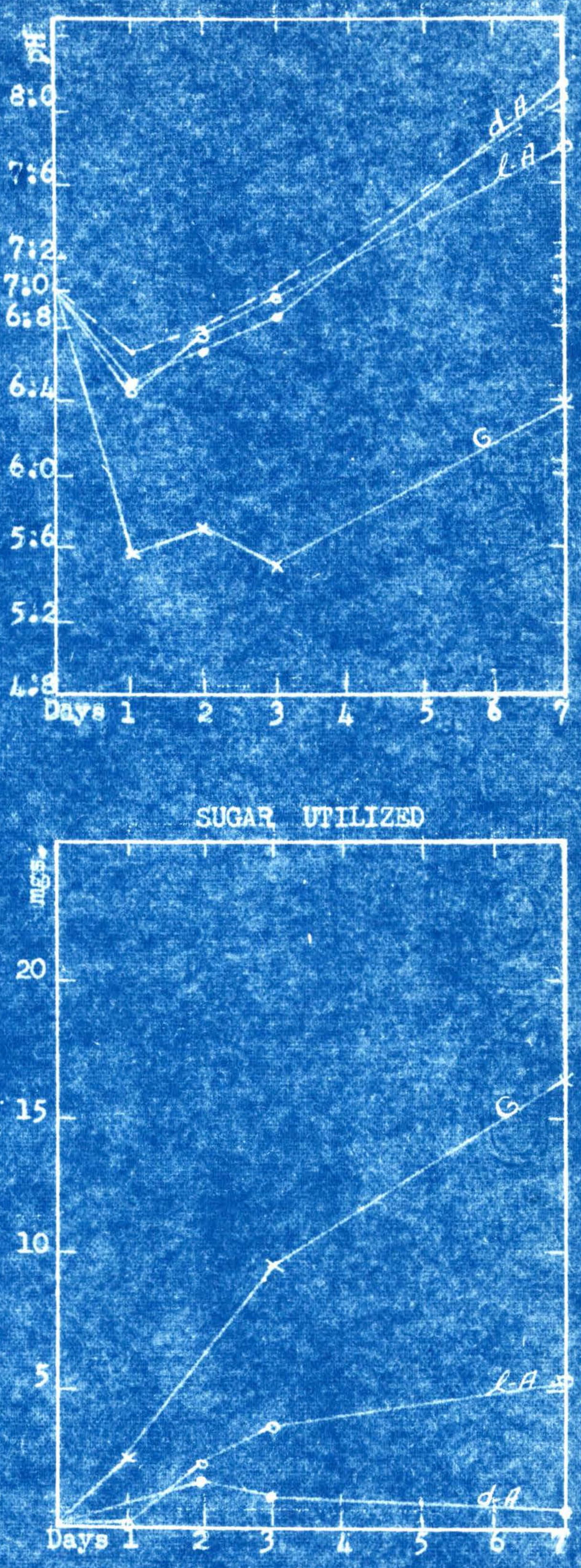
B. subtilis #231 A.T.C.

Fig. 111



B. subtilis Tex.

Fig. 112



B. subtilis Koch-Novy Tex.

Fig. 113

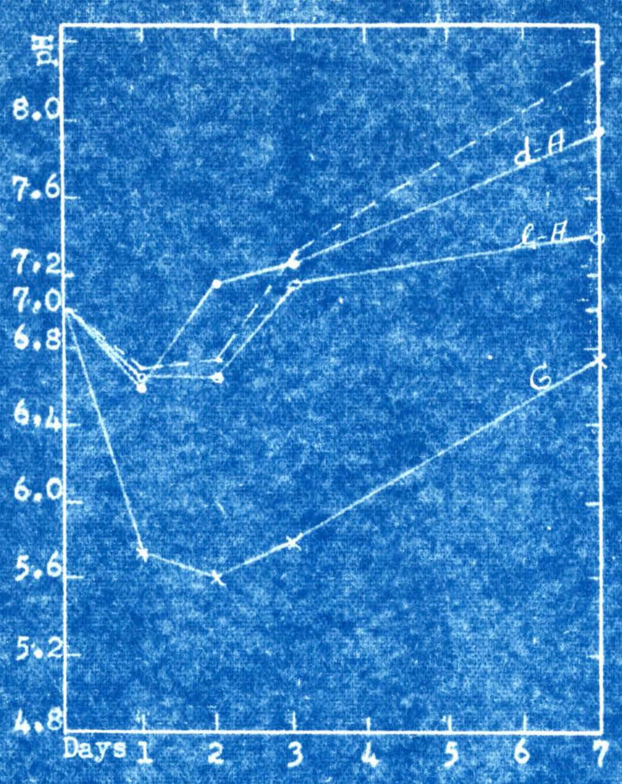
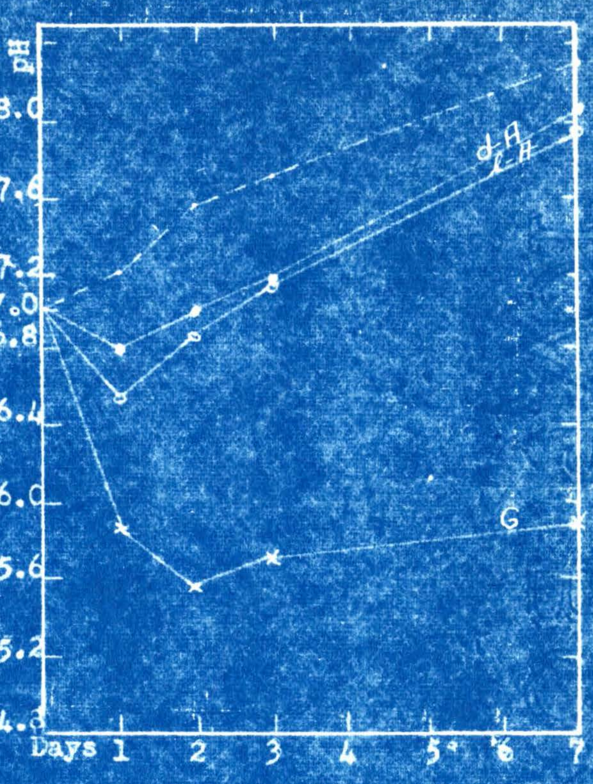


Fig. 114



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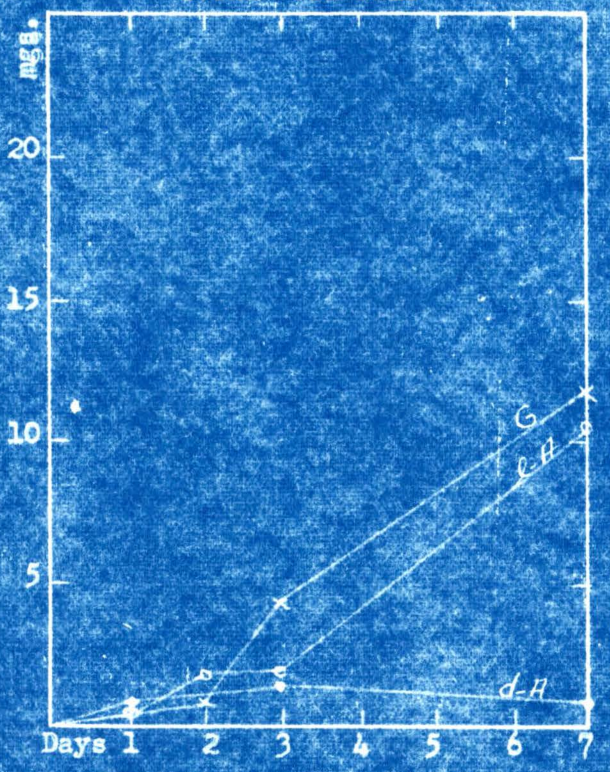

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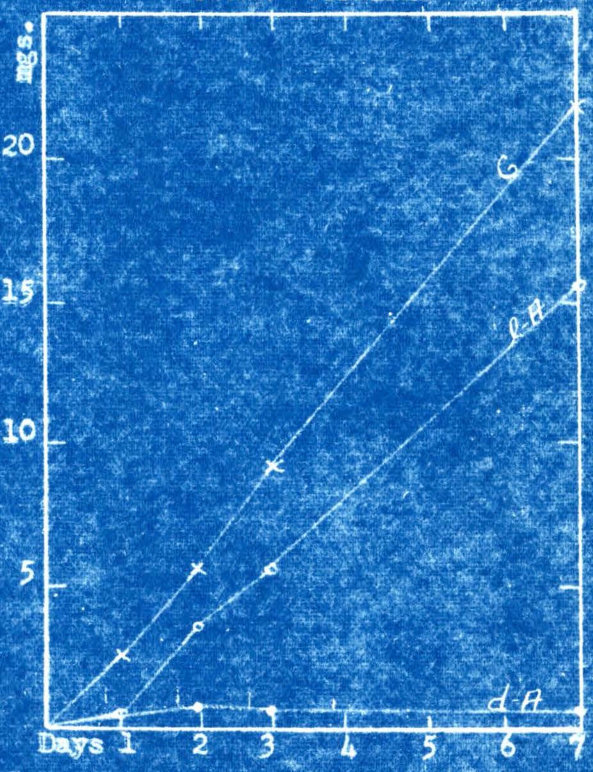


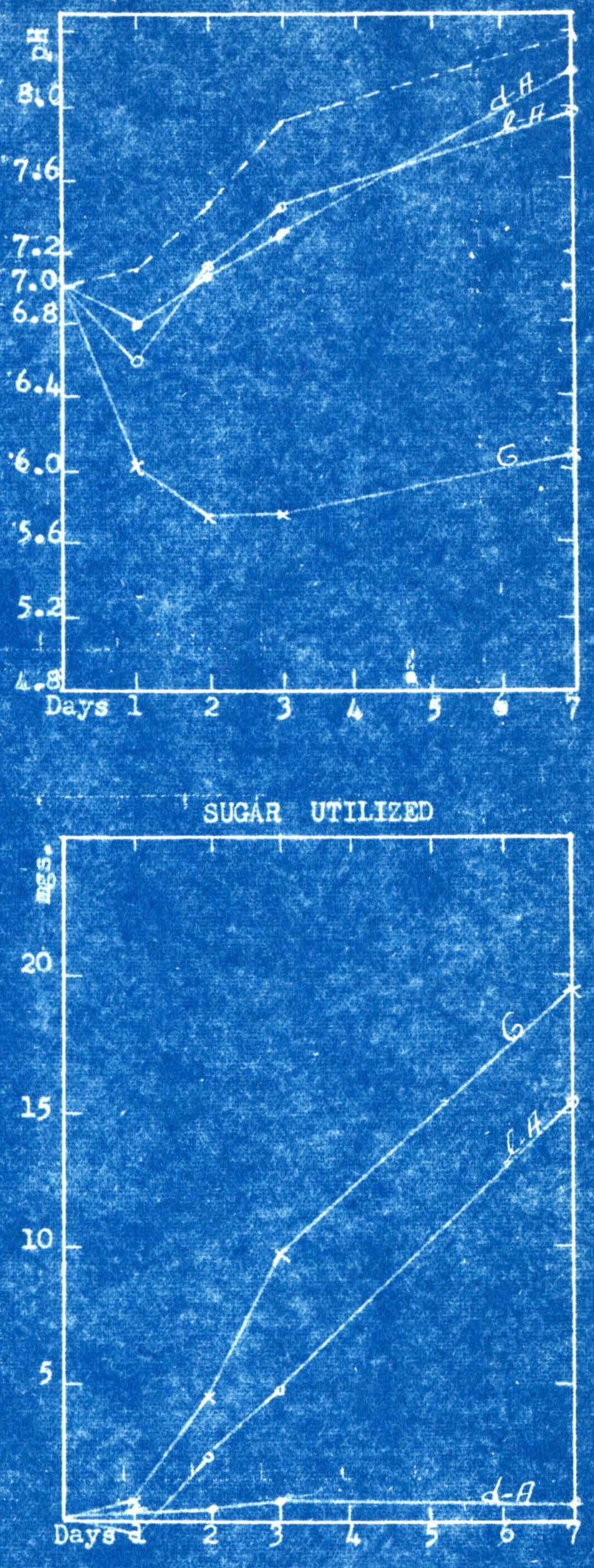
B. subtilis Marburg Tex.

B. subtilis Gram neg. Tex.

Fig. 115

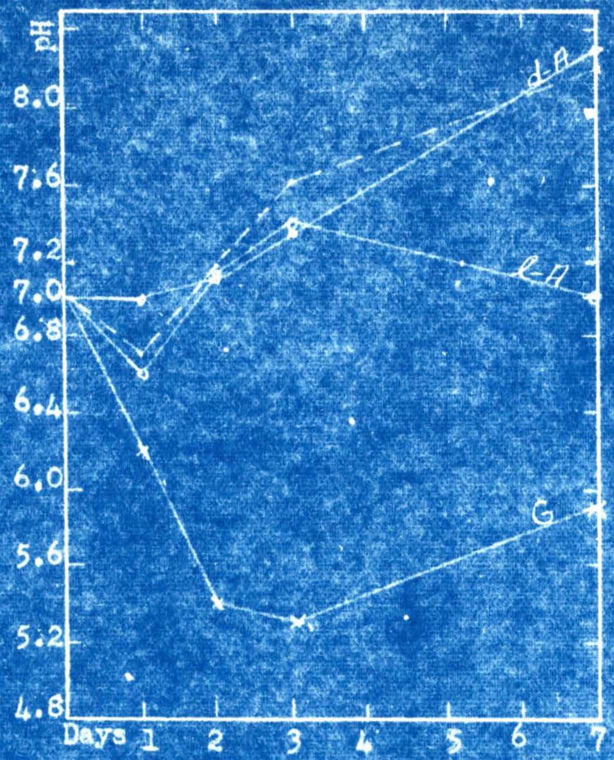
B. subtilis #231 Tex.

Fig. 116

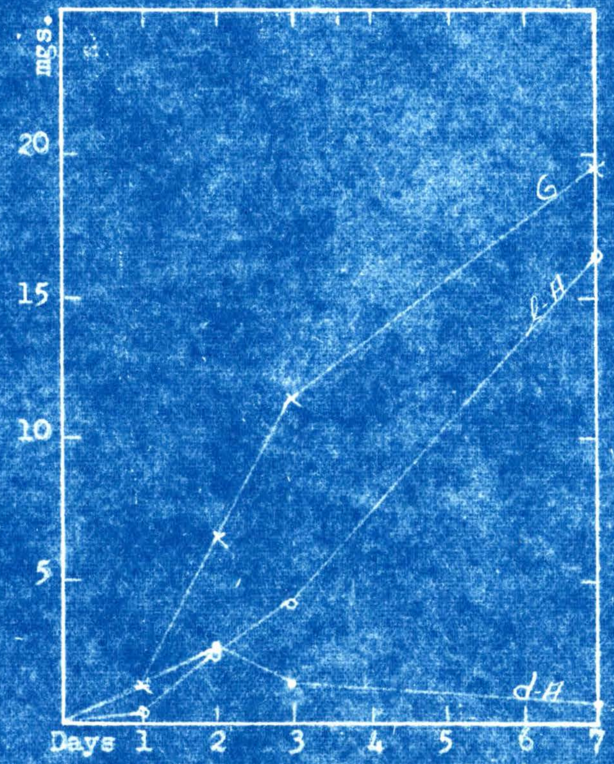


B. subtilis Marburg Wis.

Fig. 117

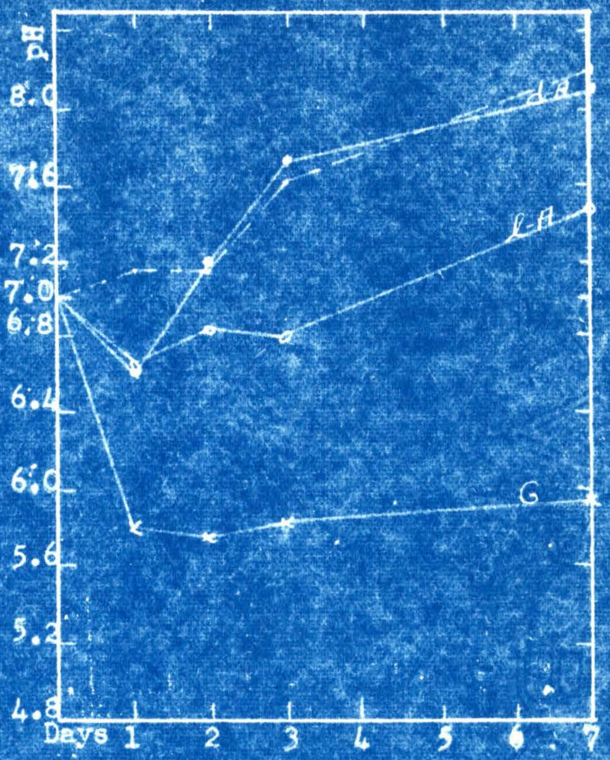


SUGAR UTILIZED

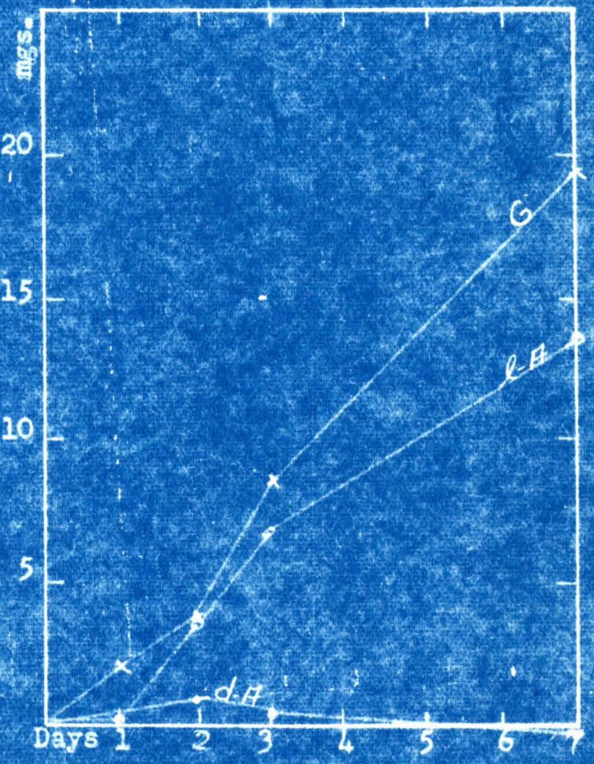


B. subtilis Marburg New York

Fig. 118



SUGAR UTILIZED



B. subtilis Marburg Neb.

Fig. 119

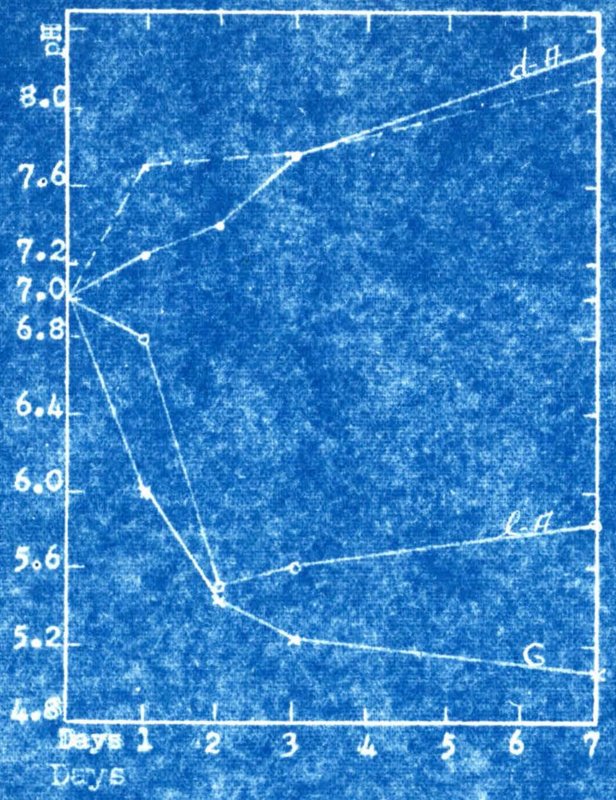
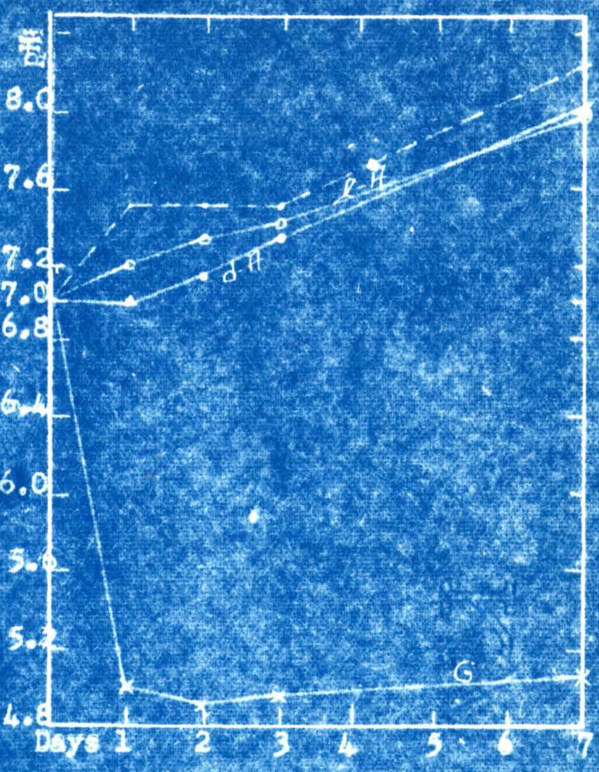
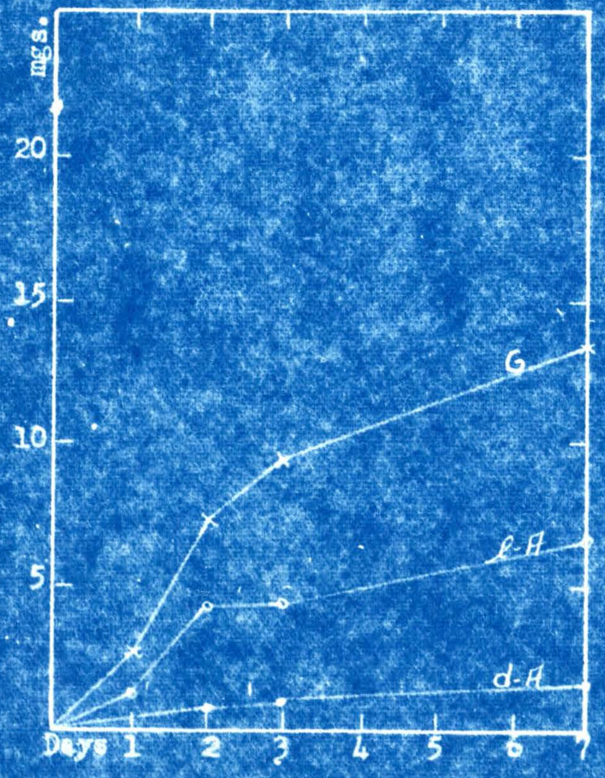


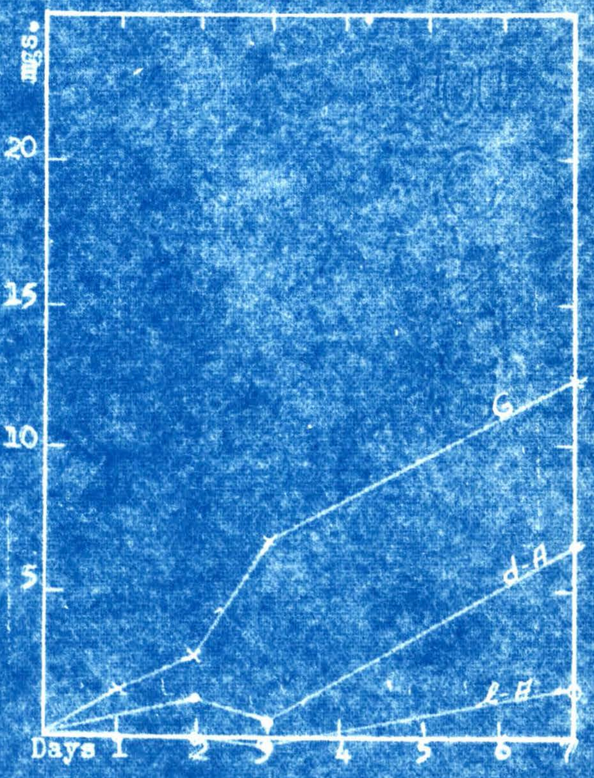
Fig. 120



SUGAR UTILIZED



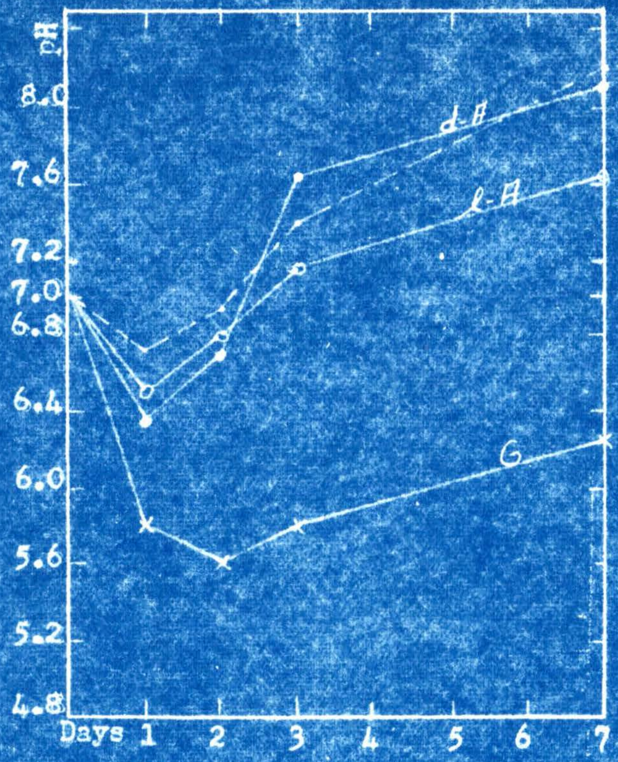
SUGAR UTILIZED



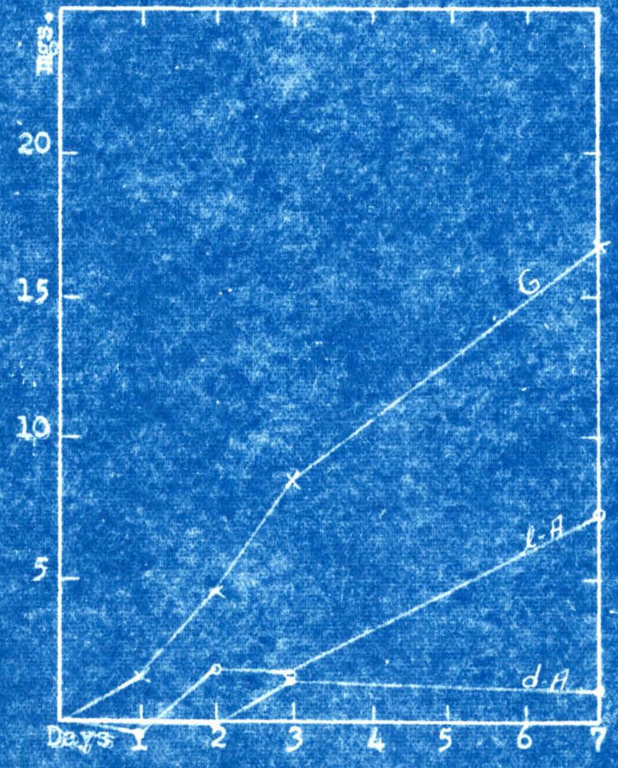
B. subtilis 'L' Neb.

B. subtilis #600 Neb.

Fig. 121

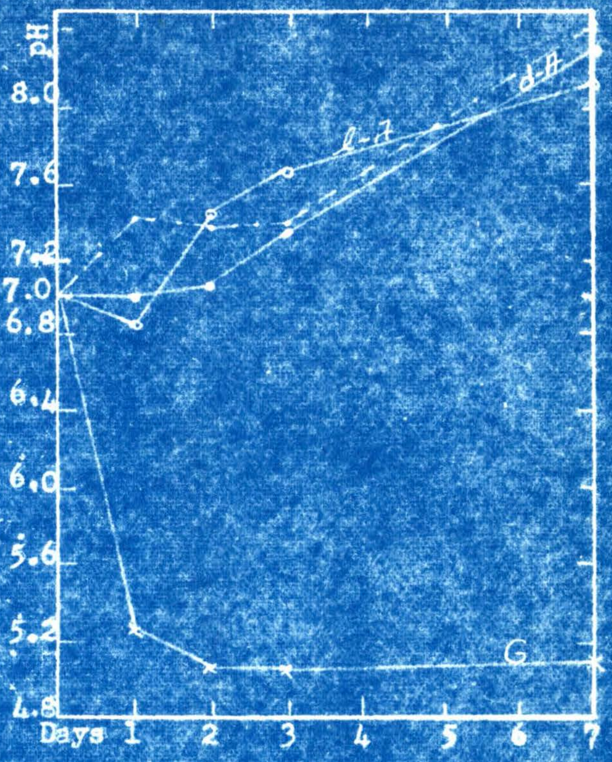


SUGAR UTILIZED

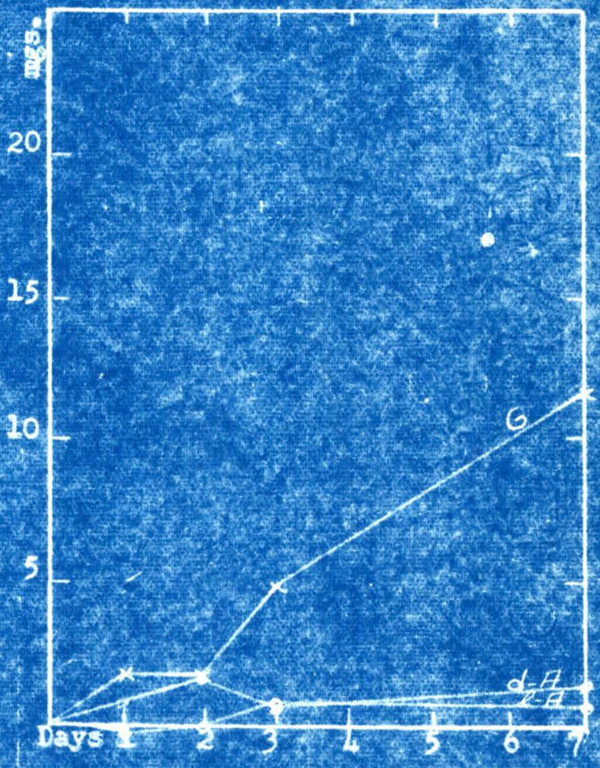


B. subtilis V.P.I. (Milk)

Fig. 122



SUGAR UTILIZED



B. tumefaciens Tex.

Fig. 123

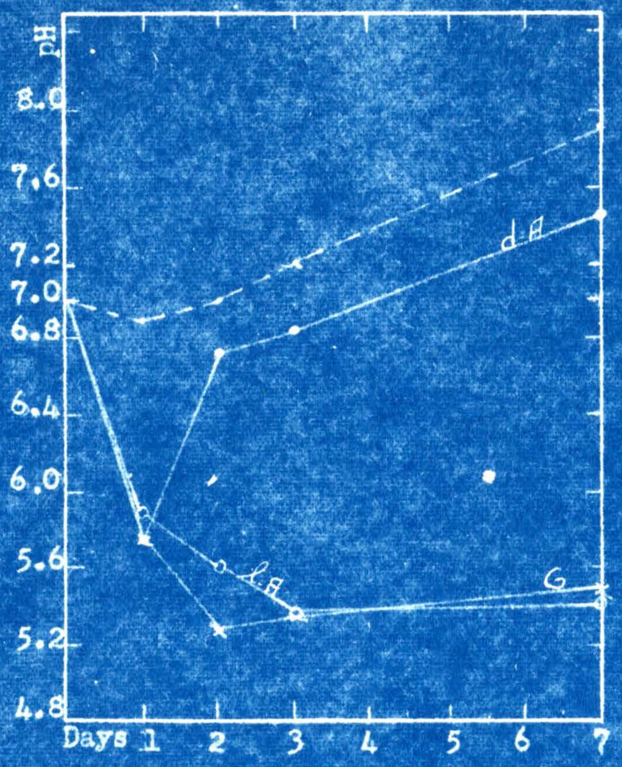
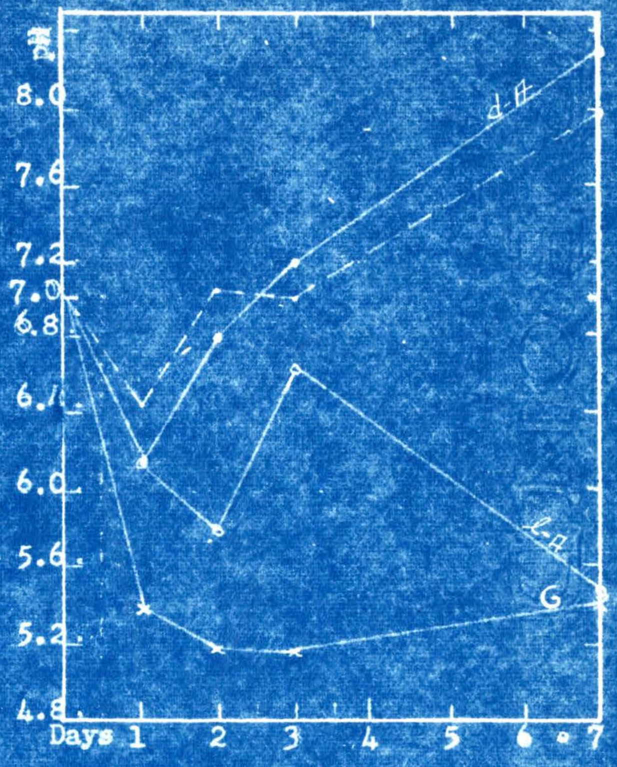
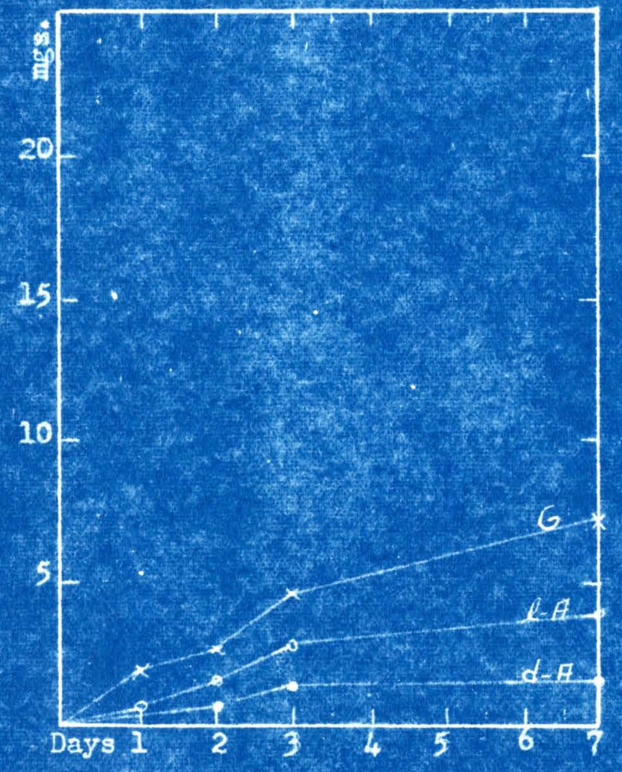


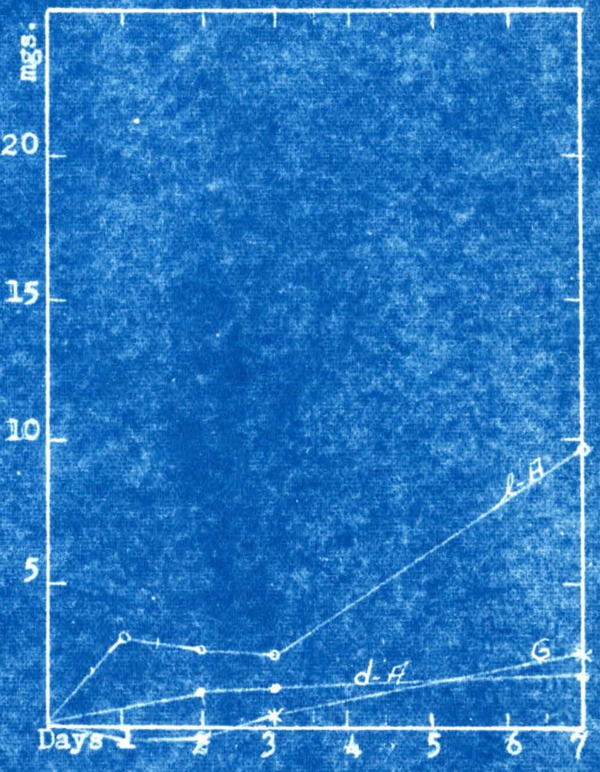
Fig. 124



SUGAR UTILIZED



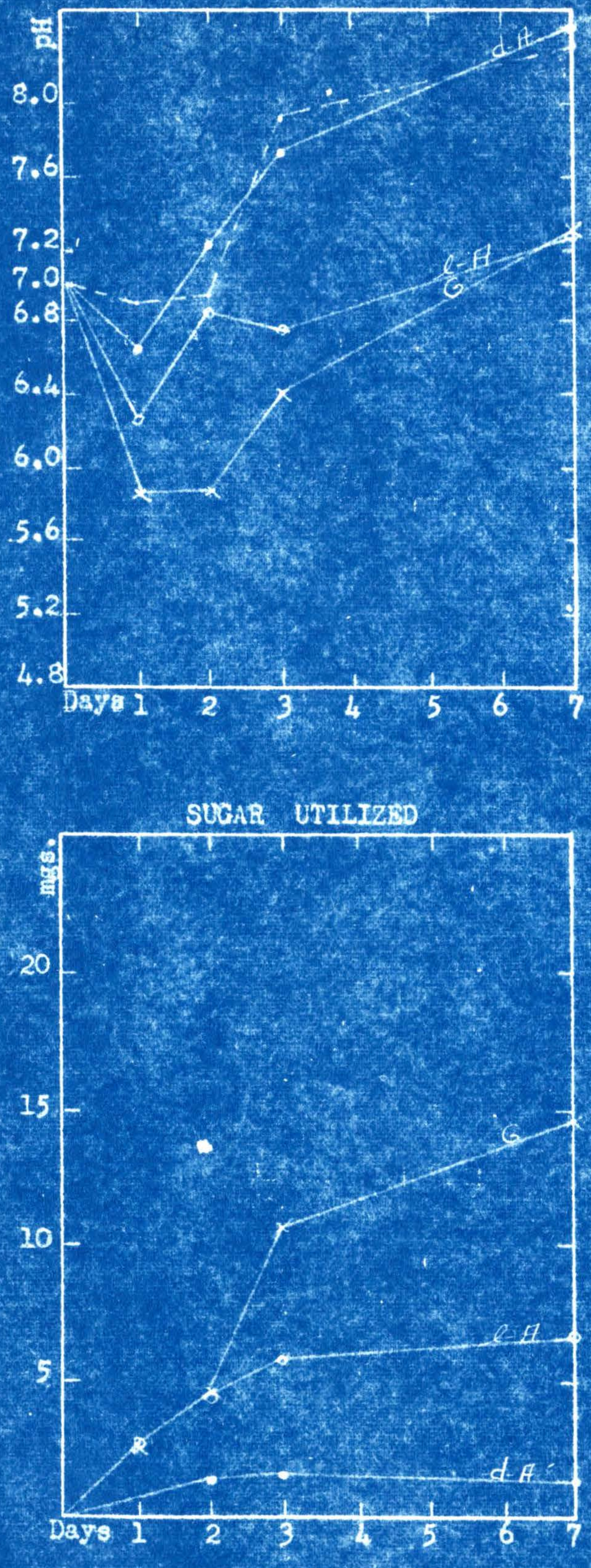
SUGAR UTILIZED



B. tumescens Tex.

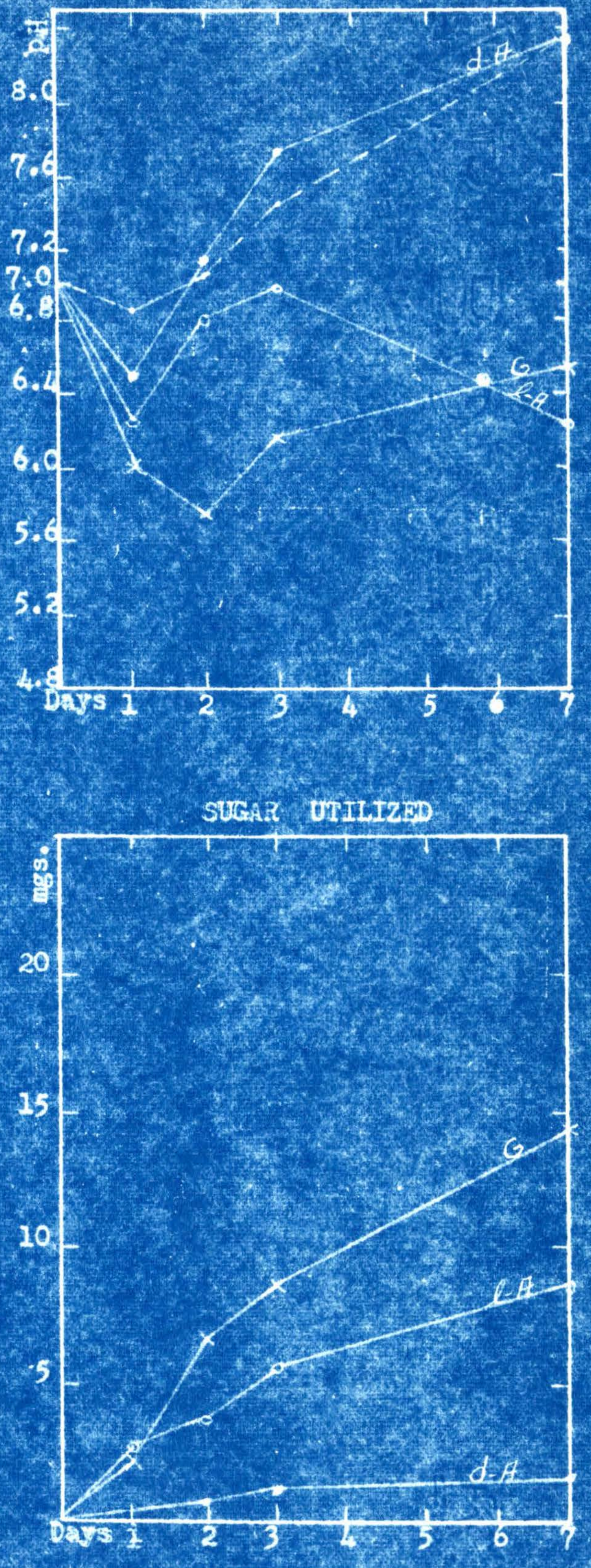
B. tumescens #995 Tex.

Fig. 125



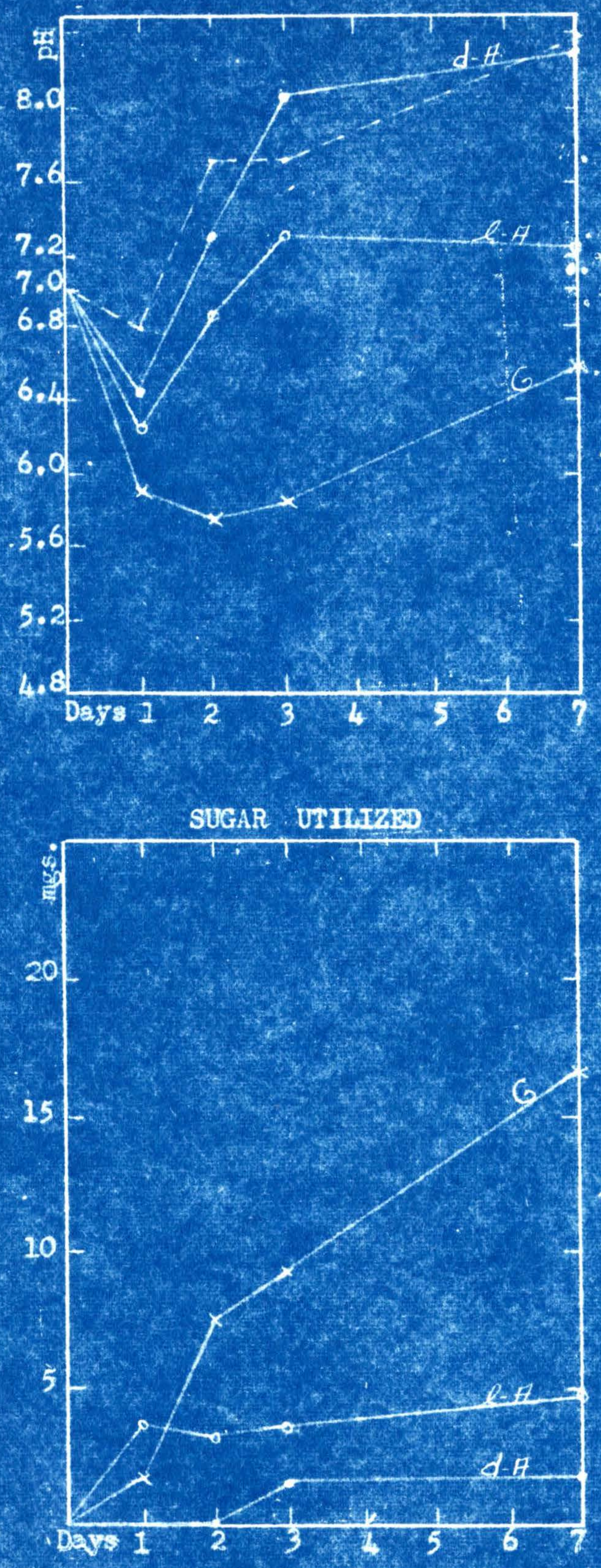
B. vulgatus A.T.C.

Fig. 126



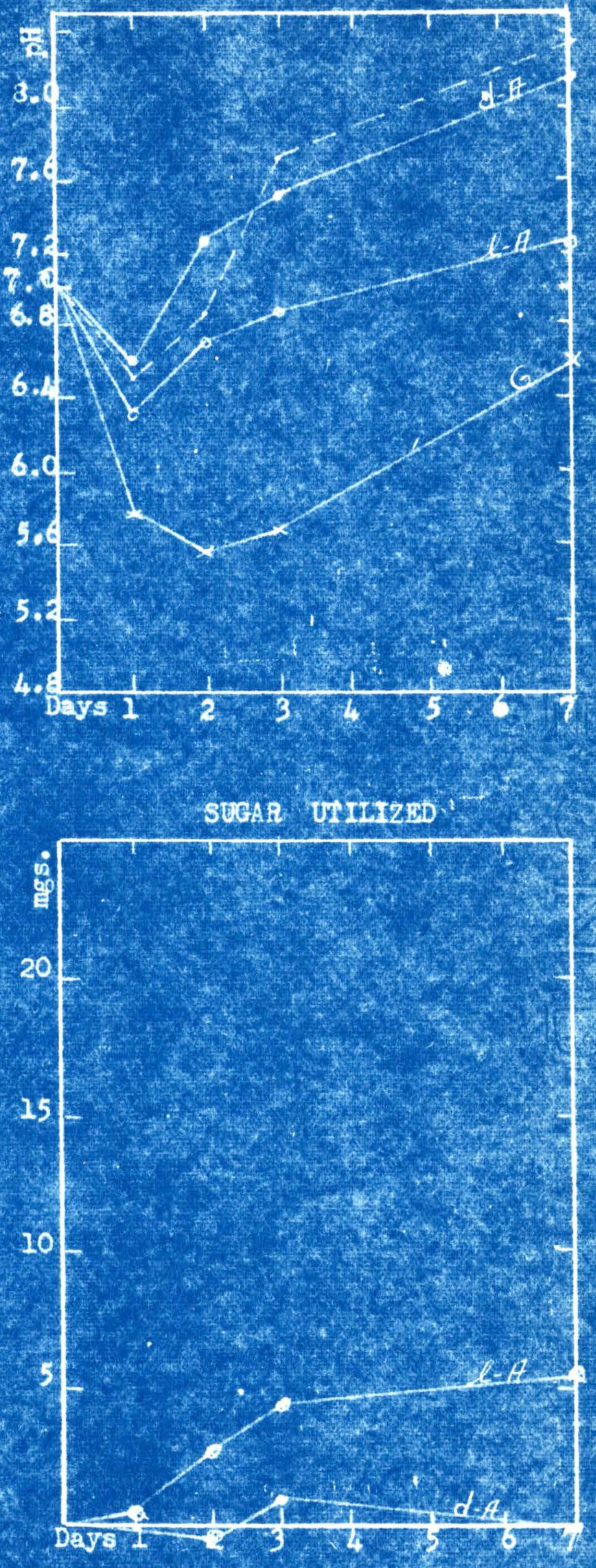
B. vulgatus U.S.D.A.

Fig. 127



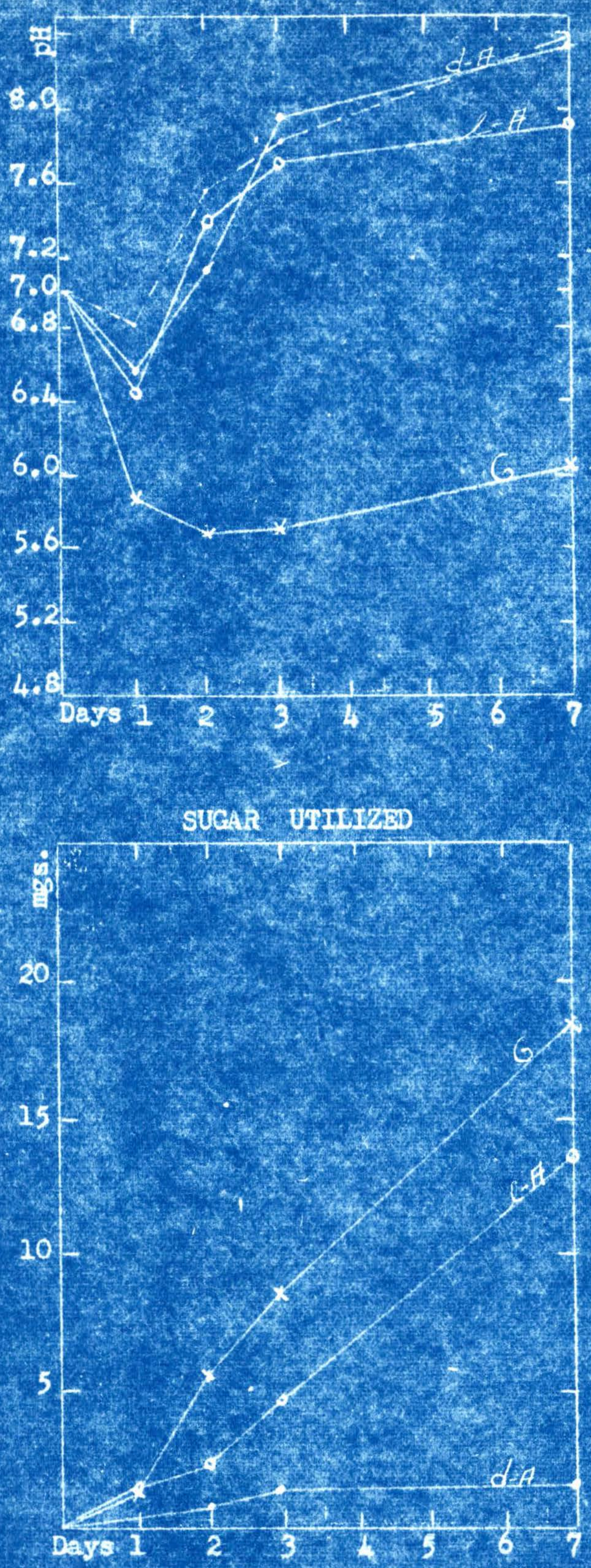
B. vulgatus Neb.

Fig. 128



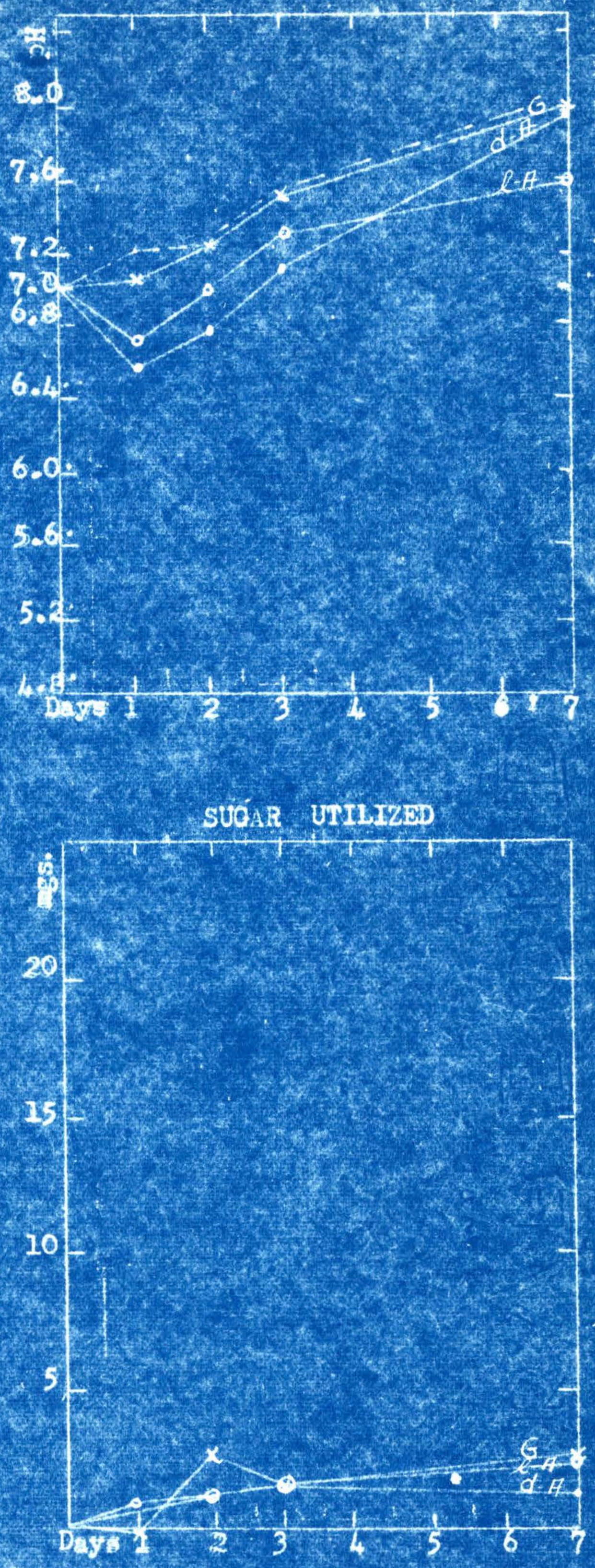
B. vulgatus Tex.

Fig. 129



B. globigii Wis. (Probably Vogel)

Fig. 130



Delong strain pectin fermenter, Wis.

TABLE IV

Interpretation of pH Data from Three Sources to Indicate Dissimilation of Glucose, d-Arabinose and l-Arabinose.

LEGEND:

+	pH within the range 6.0-6.8
++	pH below 6.0
-	either the pH value did not change from its initial 6.8-7.0 or the reaction became more alkaline
?	pH produced just slightly below 6.8-7.0 (Peptone)or, in the case of carbohydrates, pH difference compared to peptone is not great enough to be certain that the acid produced was derived from the carbohydrate. Data taken from Coffee (4) listed by that author as ±. Data of present investigation does not differ by at least 0.2 pH units.

The reactions from Bergey's Manual (1) are listed for species only. The reactions obtained by the author and by Coffee (4) are listed according to both species and strain.

TABLE IV

Interpretation of pH Data from Three Sources to Indicate

Dissimilation of Glucose, d-Arabinose and l-Arabinose

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	a-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. adhaerens										+	
A. T. C.	-	++	+	+							
B. agri										-	
U. S. D. A.	+	++	+	+							
B. albolactis										-	
V. P. I.	-	++	+	-							
Tex.	-	++	++	+	+	++	+	-			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. alvei										-	-
A. T. C.	+	++	-+-	+							
Tex.	+	++	+	+	+	++	+	?			
#622 Tex.	+	++	+	+	+	++	+	+			
#622 Lockheed Tex.	+	++	+	+	+	++	+	+			
B. anthracis										-	
Wis.	+	++	++	++							
Tex.	+	++	++	++	+	++	+	+			
(Wis.) Tex.	+	++	+	++	+	++	+	+			
Rall. Tex.	+	++	++	++	+	++	+	+			
Smooth Tex.	+	++	++	++	+	++	+	+			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. aterrimus										+	
#353 Wis.	+	++	-	++	-	++	-	+			
U. S. D. A.	+	++	-	++							
Pesck Wis.	+	++	-	++	-	++	-	+			
R. C. Wis.	-	++	-	++	-	++	-	+			
B. cereus										+	
#1 A. T. C.	-	++	-	-							
#2 A. T. C.	+	++	++	++							
Tex.	+	++	+	+	-	++	-	-			
Stock Tex.	-	++	-	-	-	++	-	-			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. cereus										+	
Non motile Tex.	-	++	-	-	-	++	-	-			
#244 Tex.	-	++			-	++	-	-			
Clark Tex.	-	++	-	-	-	++	-	-			
Neb.	-	++									
Smith-Thom Tex.	-	++	+	+	-	+	-	?			
Wis.	+	++	+	-	-	++	+	++			
A. T. C. Wis.	-	++	-	-	-	++	-	-			
B. brevis											
#604 A. T. C.	-	-	-	-							
U. S. D. A.	-	-	-	-							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. circulans										+	
A. T. C.	+	++	++	++							
B. cohaerens										+	
804 Tex.	-	++	-	+	-	++	+	-			
B. danicus											
U. S. D. A.	+	++	+	+							
1 A. T. C.	-	++	+	+							
B. firmus											
U. S. D. A.	+	+	+	+							
B. flexus										+	
V. P. I.	+	+	+	+							
B. freudenreichii											
A. T. C.	-	+	+	+							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. fusiformis										-	-
A. T. C.	-	-	+	-							
Tex.	-	-	+	-							
339 Tex.	-	-	+	-							
Wright Wis.	-	-	+	-	-	-	-	-			
B. globigii										+	
A. T. C.	+	++	+	++							
Vogel Wis.	-	++	+	+							
356 Wis.	-	++			-	++	+	-			
B. graveolens										+	
U. S. D. A.	-	++	+	++							
615 Tex.	-	++	+	++	-	++	+	++			
Wright Wis.	-	++	-	-	-	++	-	-			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. graveolens Neb.	-	++	+	-							
B. lacticola									+		
Tex.	-	++	-	-							
876 Tex.	-	++	-	-	-	++	-	-			
B. lactis									+	+	
618 Tex.	+	++	-	-	-	++	-	-			
B. laterosporus										+	
V. P. I.	+	++	+	+							
B. lautus										+	
V. P. I.	-	++	-	+							
B. macerans										+	+
U. S. D. A.	+	++	+	++							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. megatherium									+	+	
U. S. D. A.	+	++	+	++							
#1 A. T. C.	+	++	+	++							
#2 A. T. C.	+	++	-	+							
Tex.	+	++	+	++	-	++	+	++			
#240 Tex.	+	++	+	+	-	++	+	++			
Wis.	-	++	+	-	-	++	-	-			
A. T. C. Wis.	-	++	-	-	-	++	-	-			
Neb.	+	++	+	+							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. mesentericus									-	+	-
A. T. C.	?	++		+							
U. S. D. A.	-	++	+	+							
Tex.	-	++	-	++	-	++	-	++			
A. T. C. Wis.	-	++	+	+	-	++	?	?			
Frost Wis.	-	++	+	+	-	++	?	?			
Neb.	-	++	+	+							
B. mycoides										+	-
#1 V. P. I.	+	++	?	+							
#101 V. P. I.	No Peptone data, therefore cannot interpret results.										

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. mycoides										+	-
Beaded Tex.	-	++			-	++	-	-			
#417 Tex.	-	++									
A. T. C. 80 Tex.	-	++			-	++	?	-			
#A2 Tex.	-	++			-	++	-	+			
#A6 Tex.	-	++			-	++	-	-			
#23 Tex.	-	++			-	++	?	-			
#65 Tex.	-	++	?	+	-	++	?	+			
#405 Tex.	-	++	-	-	-	++	-	-			
#410 Tex.	-	++	-	-	-	++	?	-			
#420 Tex.	-	++	-	-	-	++	-	-			
#421 Tex.	-	++	-	-	-	++	-	-			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. mycoides										+	-
#422 Tex.	-	++	?	+	-	++	-	-			
#425 Tex.	-	++	-	-	-	++	-	-			
#426 Tex.	-	++	-	-	-	++	-	-			
#427 Tex.	-	++	-	-	-	++	-	-			
#430 Tex.	-	++	-	?	-	++	?	-			
Wright Wis.	-	++	-	-	-	++	?	-			
Left-Hand Sarles Wis.	-	++			-	++	-	-			
Neb.	-	++	-	-							
B. niger									-	+	-
A. T. C.	-	++	+	+							
#228 Wis.	-	++	+	+	-	++	-	-			
U. S. D. A.	-	++	+	+							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. niger									-	+	-
'Old' Wis.	-	++	?	?	-	++	-	-			
'S' Wis.	-	++	+	+	-	++	-	-			
B. panis										+	
A. T. C.	-	++	++	+							
U. S. D. A.	-	++	+	+							
B. paraalvei											
Tex.	+	++	++	+	+	++	++	++			
B. polymyxa										+	+
V. P. I.	-	++	+	++							
Tex.	+	++	?	++							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. prausnitzii										+	-
Neb.	-	++		-						+	-
B. robur										+	
#946 Tex.	+	++			?	++	+	+			
B. rotans										-	-
A. T. C.	-	-	-	?							
Tex.	-	-	+	?	+	+	+	+			
Wis.	-	-	+	-	-	-	-	-			
B. ruminatus											
#952 Tex.	-	++	-	+	?	++	+	++			
B. serositidis Neb.	-	-	+	-							

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. silvaticus									+		
#957	-	++	++	+	-	++	?	++			
B. simplex											
Tex.	-	?	+	-							
#335 Tex.	-	?	+	-	-	+	+	-			
B. sphaericus											
#348 Tex.	-	?	+	-	-	-	-	-			
B. subtilis									-	+	+
#231 A. T. C.	-	++	+	-							
Tex.	-	++	+	-							
Koch-Novy Tex.	?	++	+	+	-	++	+	+			
Marburg Tex.	?	++	+	+	-	++	?	-			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. subtilis									-	+	+
Gram-Neg. Tex.	-	++	-	+	-	++	-	?			
#231 Tex.	+	++	+	+	+	++	?	++			
Marburg Wis.	-	++	-	+	?	++	-	+			
Marburg N. Y.	+	++	-	+							
Marburg Neb.	-	++	+	+							
'L' Neb.	-	++	-	?							
#600 Neb.	-	++	-	-							
V. P. I. (Milk)	-	++	-	-							
B. tumefaciens Tex.	-	++	-	-							
B. tumescens Tex.	-	++	++	++						+	
#995 Tex.	-	++	++	++	-	++	+	++			

TABLE IV (Continued)

	Investigations by the Author				Investigations by Coffee				Reactions listed in Bergey's Manual		
	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	d-Arabinose	l-Arabinose	Peptone	Glucose	Arabinose
B. vulgatus									-	+	-
A. T. C.	-	++	+	+							
U. S. D. A.	-	++	+	+							
Neb.	-	++	+	+							
Tex.	+	++			-	++	-	-			
Delong strain Pectin Fermenter Wis.	-	-	+	+	-	++	-	++			

TABLE V

A Summary of Strain Reactions as They Appear to be of Value for Species Identification in Indicating Sugar Dissimilation. (Summary of pH Data in Figures 1-130 and Tables III and IV.)

Legend:

Glucose media:	to be of value taxonomically the pH of this medium must be converted to a pH of 6.0 or less. Plus (+) marks indicate such reactions.
d- and l- Arabinose media:	pH values have taxonomic importance only when peptone is tested concurrently. Plus (+) marks indicate a difference of acidity greater than 0.2 pH units lower than that produced by the same organism in peptone medium below 6.8. Admittedly a scheme of interpretation such as that employed for this media is open to question. Evidence for such an interpretation may be found under "Discussion of Results."
? :	Reaction of questionable value because of strain variation.
+* or -* :	indicate disagreement with the results listed by Bergey's Manual (1).

TABLE V

A Summary of Strain Reactions as They Appear to be of Value for Species Identification in Indicating Sugar Dissimilation. (Summary of pH Data in Figures 1-130 and Tables III and IV.)

Organism	Glucose	l-Arabinose	d-Arabinose
B. adhaerens	+	+	+
B. agri	+*	+*	+*
B. albolactis	+*	-*	+
B. alvei	+*	-	-
B. anthracis	+*	-	?
B. aterrimus	+	+	-
B. brevis	-	-	-
B. cereus	+*	-	?
B. circulans	+	+	+
B. cohaerens	+	+	-
B. danicus	+	+	+
B. firmus	-	+	+
B. flexus	-*	+	-
B. freudenreichii	-	-	+
B. fusiformis	-	-	+*
B. globigii	+	?	+
B. graveolens	+	+	-
B. lacticola	+	-	-
B. lactis	+	-	-
B. laterosporus	+	+	+
B. lautus	+	+	-

TABLE V (Continued)

Organism	Glucose	l-Arabinose	d-Arabinose
B. macerans	+	+	+
B. megatherium	+	?	?
B. mesentericus	+	+*	-
B. mycoides	+	-	-
B. niger	+	+	-
B. panis	+	+	+
B. paraalvei	+	-	+
B. polymyxa	+	+	-*
B. prausnitzii	+	-	
B. robur	+	-	
B. rotans	-	-	+*
B. ruminatus	+	+	-
B. serositidis	-	-	+
B. silvaticus	+	+	+
B. simplex	-	-	+
B. sphaericus	-	-	+
B. subtilis	+	-*	?*
B. tumefaciens	+	-	-
B. tumescens	+	+*	-
B. vulgatus	+	+	+

DISCUSSION OF RESULTS

Interpretation of Graphical and Tabular Data

There are several ways in which the data in Table III and Figures 1-130 may be interpreted. In order to facilitate correlation of the extent of carbohydrate utilization with the pH produced in carbohydrate-peptone media, it is possible to separate the *Bacilli* into various groups. A grouping devised as the result of the activity of an organism in one carbohydrate medium does not necessarily hold for the other two carbohydrate media; therefore, the basis for these groups will be discussed under headings for each carbohydrate investigated. The practical value of these groups is undoubtedly open to question.

Glucose:

The grouping of the *Bacilli* on the basis of their activity in glucose-peptone medium has been devised first upon the extent of pH change from neutrality, and second, upon the extent of glucose utilization.

If the utilization values of the *Bacilli* for glucose are plotted on a scattergram (Fig. 131) visual inspection cannot determine a boundary which could be termed the dividing line between high and low utilization values. The distribution of the organisms plotted against utilization appears very nearly uniform. In order to establish some arbitrary boundary upon which to base a discussion of two degrees of glucose utilization (high and low) it was decided to establish a discussion boundary at that amount of glucose used by at least 50% of the strains of *Bacilli* investigated. Analysis of the glucose utilization data of Table III indicates that 50% of the strains utilize more than 6.2 mg.;

therefore, the arbitrary boundary upon which to discuss two degrees of glucose utilization and the pH produced within each degree is established at 6.2 mg. of glucose. A grouping of the Bacilli according to this plan may be found in Table VI.

The advantage to a grouping of this type may be illustrated in a comparison of Group 1 (pH more than 7.0; glucose utilization less than 6.2 mg.) and Group 3 (pH less than 7.0; glucose utilization less than 6.2 mg.). The pH difference between these two groups is difficult, if not impossible to explain, particularly since studies concerned with the nitrogen metabolism were outside the scope of this investigation.

A comparison of the pH values produced in peptone and glucose-peptone media (Table III and Figures 1-130) by any organism in Group 1 reveals that for all practical purposes, they are identical. A similar comparison for the organisms of Group 3 reveals a very broad difference in these values. The buffering capacity of the medium cannot account for these differences between the two groups, since many organisms in both groups were run concurrently on the same "batch" of media. They may be due to a fundamental difference in the metabolism of the organisms in the two groups.

TABLE VI

A Grouping of the Genus Bacillus According to the Extent of Glucose utilized and pH Produced in Glucose-peptone Media.

1. pH glucose-peptone more than 7.0; glucose utilized less than 6.2 mg.

B. brevis #604 A. T. C.

B. brevis U. S. D. A.

B. fusiformis A. T. C.

B. fusiformis Tex.

B. fusiformis #339 Tex.

B. fusiformis Wright Wis.

B. rotans A. T. C.

B. rotans Tex.

B. rotans Wis.

B. serositidis Neb.

Delong strain pectin fermenter

2. pH glucose-peptone less than 5.5; glucose utilized more than 6.2 mg.

B. adhaerens A. T. C.

B. agri U. S. D. A.

B. albolactis Tex.

B. albolactis V. P. I.

B. alvei #622 Lockheed Tex.

B. cereus #1 A. T. C.

B. cereus Neb.

B. cereus Clark Tex.

B. cereus Non-motile Tex.

B. cereus Stock Tex.

B. cereus #244 Tex.

B. cereus A. T. C. Wis.

B. cereus Wis.

B. graveolens #612 Tex.

B. graveolens U. S. D. A.

B. graveolens Wright Wis.

B. lactis #618 Tex.

B. lacticola Tex.

B. lacticola #876 Tex.

B. laterosporus V. P. I.

B. mycoides #410 Tex.

B. mycoides #1 V. P. I.

B. robur #946 Tex.

B. silvaticus #937 Tex.

B. subtilis "L" Neb.

B. subtilis #600 Neb.

B. tumefaciens Tex.

B. tumescens Tex.

TABLE VI (Continued)

3. pH glucose-peptone less than 5.5; glucose utilized less than 6.2 mg.

B. alvei A. T. C.

B. alvei Tex.

B. anthracis Smooth Tex.

B. anthracis Rall. Tex.

B. anthracis Tex.

B. anthracis (Wis.) Tex.

B. anthracis Wis.

B. cereus #2 A. T. C.

B. circulans A. T. C.

B. globigii A. T. C.

B. macerans U. S. D. A.

B. megatherium #1 A. T. C.

B. megatherium Neb.

B. megatherium #240 Tex.

B. megatherium Tex.

B. megatherium A. T. C. Wis.

B. megatherium #2 A. T. C.

B. megatherium U. S. D. A.

B. megatherium Wis.

B. mesentericus Neb.

B. mesentericus A. T. C.

B. mycoides Neb.

B. mycoides A-2 Tex.

B. mycoides A-6 Tex.

B. mycoides Beaded Tex.

B. mycoides A. T. C. #80 Tex.

B. mycoides #23 Tex.

B. mycoides #65 Tex.

B. mycoides #405 Tex.

B. mycoides #417 Tex.

B. mycoides #420 Tex.

B. mycoides #421 Tex.

B. mycoides #422 Tex.

B. mycoides #425 Tex.

B. mycoides #426 Tex.

B. mycoides #427 Tex.

B. mycoides #430 Tex.

B. mycoides Left-Hand Sarles Wis.

B. mycoides Wright Wis.

B. paraalvei Tex.

B. prausnitzii Neb.

B. ruminatus #952 Tex.

B. tumescens #995 Tex.

TABLE VI (Continued)

4. pH glucose-peptone 5.5-6.5; glucose utilized more than 6.2 mg.

B. aterrimus #355 Wis.

B. aterrimus U. S. D. A.

B. aterrimus Pesek Wis.

B. aterrimus R. C. Wis.

B. cereus Smith-Thom Tex.

B. cereus Tex.

B. coherens #840 Tex.

B. danicus A. T. C.

B. danicus U. S. D. A.

B. globigii #356 Wis.

B. globigii (probably Vogel) Wis.

B. graveolens Neb.

B. lautus V. P. I.

B. mesentericus A. T. C. Wis.

B. mesentericus Frost Wis.

B. mesentericus Tex.

B. niger A. T. C.

B. niger #228 Wis.

Borderline cases:

B. simplex #335 Tex.

B. simplex Tex.

B. niger "S" Wis.

B. niger "Old" Wis.

B. niger U. S. D. A.

B. panis A. T. C.

B. subtilis Marburg- Conn N. Y.

B. subtilis Marburg Neb.

B. subtilis Marburg Tex.

B. subtilis Marburg Wis.

B. subtilis #231 A. T. C.

B. subtilis #231 Tex.

B. subtilis Tex.

B. subtilis Koch-Novy Tex.

B. subtilis (Milk) V. P. I.

B. subtilis Gram-neg Tex.

B. vulgatus A. T. C.

B. vulgatus Neb.

B. vulgatus U. S. D. A.

5. pH glucose-peptone less than 5.5 within 2 days--more than 5.5 in 7 days; glucose utilized more than 6.2 mg.

B. mesentericus U. S. D. A. (used 9.76 mg.)

B. polymyxa Tex. (used 22.62 mg.)

B. polymyxa V. P. I. (used 21.86 mg.)

TABLE VI (Continued)

The following organisms do not belong to any of the above groups. They are discussed in the text.

B. firmus U. S. D. A.

B. flexus V. P. I.

B. freudenreichii A. T. C.

B. sphaericus #348 Tex.

B. simplex Tex.

B. simplex #335 Tex.

Figures 29 and 30, representing two strains of *B. brevis*, illustrates pH curves for peptone and glucose-peptone media and glucose utilization values typical of all the organisms within Group 1. Analysis of the pH curves for peptone broth indicates that the organism has utilized the carbon of the peptone molecule as an energy source. Nitrogen has accumulated in the medium, probably as ammonia, in a quantity sufficient to neutralize any acidic products of respiration and an alkaline pH reaction has resulted. For all practical purposes the pH produced in glucose-peptone is identical with the pH produced by the organism in peptone alone. It appears, therefore, that although these *Bacilli* have utilized glucose, they have also attacked the peptone molecule in such a manner that basic products, such as ammonia, accumulate to neutralize any acidic products formed from the carbohydrate. Not all products of carbohydrate dissimilation are acidic in character. They may be neutral in reaction; however, regardless of the nature of these products, there must have been basic substances formed, or liberated, from the peptone molecule in order for the pH to be alkaline. It appears that in this case the "sparing action" of carbohydrate for peptone is not exercised.

The pH values produced in peptone broth by the organisms of Group 3 necessitate dividing this group into two parts. Figures 75 and 76 represent typical peptone-broth pH curves for all strains of *B. mycoides* within Group 3. Figures 5 and 6 (*B. alvei* strains) represent typical peptone broth pH curves for all other organisms in this group. The fundamental difference between these two divisions is that the pH produced by the *B. mycoides* strains in peptone broth are alkaline throughout the entire incubation period. All other organisms within Group 3 produce an acidic pH in peptone broth after 24 hours incubation, which then reverts to an alkaline pH upon further incubation.

The activity of the *B. mycoides* strains in peptone broth parallels the activity of the organisms of Group 1 in this medium. It appears that all other organisms in Group 3 utilize the peptone molecule concurrently as an energy and growth source in the early stages of incubation, since basic substances do not accumulate to neutralize any acidic products formed. In the later stages of incubation, basic substances do accumulate and the pH becomes strongly alkaline.

All the organisms within Group 3 produce a pH below 5.5 in glucose-peptone medium. Like the organisms of Group 1, they utilize less than 6.2 mg. of glucose. Apparently when glucose is present the organisms of Group 3 use it as an energy source, and do not attack the peptone present except as nitrogenous substance is needed for growth; thus, basic nitrogen does not accumulate to react with the acidic products of respiration to yield an alkaline pH value. It would appear, therefore, that "sparing action" of carbohydrate for peptone has taken place in this case.

It is interesting to point out that curves representing the pH values produced by many *Bacilli* of Group 3 in glucose-peptone medium do not indicate a change from the pH produced at the end of 24 hours of incubation even though the incubation period extends for 7 days. This might be indicative of the effect of a low pH upon the metabolism of the organisms concerned.

The *Bacilli* included in Groups 2, 4, and 5 utilize from 6.2 to 25 mg. of glucose. They are divided into the respective groups according to the pH they produce in glucose-peptone medium. The pH resulting from the growth of the organisms of Group 2 is less than 5.5. The pH produced by the organisms of Group 4 is in the range of 5.5-6.5. The organisms of Group 5 produce a pH of less than 5.5 during the first two days of incubation but this pH becomes more alkaline before the end of the seven day incubation period.

It is impossible to explain this wide divergence of pH values produced by three groups of organisms which use comparable, or in many cases, identical quantities of glucose without definite knowledge of the organism's nitrogen metabolism and the type of end-products resulting from their carbohydrate dissimilation.

In part this would be accounted for if some of the end-products of respiration were neutral. This observation applies particularly to the Bacilli of Group 4.

The organisms of Group 4 are strong producers of alkali in peptone broth alone. It might be that these Bacilli attack peptone in such a manner that alkali is released to react with part of the acidic products formed by respiration.

There is practically no change in the pH produced by the organisms of Group 2 after the first 24 hours of incubation, during which time the initial pH is lowered to 5.5 or less. As with the Bacilli of Group 3, this absence of pH fluctuation is probably a result of the effect of a low pH upon the bacterial cell, although the exact mechanism of the effect is unknown.

Apparently the organisms of Group 2 are less resistant to low pH than those of Group 5, since the pH of the latter approaches neutrality by the end of the seven day incubation period. It is interesting to point out further, that the two *B. polymyxa* strains of Group 5 utilize 21 and 22 mg. of glucose out of the 25 mg. present.

The division of the Bacilli into the above five groups leaves 8 of the 130 strains employed in these investigations unaccounted for. The data for one of these (<u>B. vulgatus</u> Tex.) is incomplete. <u>B. firmus</u> U. S. D. A., <u>B. flexus</u> V. P. I., <u>B. freudenreichii</u> A. T. C., <u>B. panis</u> U. S. D. A., <u>B. simplex</u> Tex. and <u>B. simplex</u> #335 Tex. each used less than 6.2 mg. glucose and produced a pH of 6.0 or above. <u>B. sphaericus</u> #348 Tex. used less than 6.2 mg. of glucose and produced a pH which grew progressively more acidic to pH 5.6 after seven days incubation.

The activity of these organisms is difficult to explain. In peptone alone they do not produce alkali to the extent of many of the <u>Bacilli</u> in Groups 1-5. The pH they produce in glucose-peptone is decidedly lower than the pH produced in peptone alone. It is, then, advisable to observe the pH produced by these organisms in peptone and glucose-peptone media concurrently for a period of seven days, as recommended by Coffee (4) before attaching taxonomic value to the pH they produce in glucose-peptone. The pH of glucose-peptone may not drop below 6.6; however, according to the results of this investigation, these 5 strains of <u>Bacilli</u> should produce a pH at least 0.6 pH units more alkaline in peptone alone than they produce in glucose-peptone medium.

The results of the glucose utilization determination compared with the pH produced in glucose-peptone and peptone alone by members of the genus <u>Bacillus</u> employed confirm the following statements:

1. A pH of less than 6.0 produced by an actively growing culture of the genus <u>Bacillus</u> is indicative of positive glucose utilization by that organism. This pH is not indicative of the extent of glucose utilization.

2. No species of Bacillus tested produce a pH of less than 6.0 in peptone medium alone. Those <u>Bacilli</u> capable of lowering the pH of peptone medium to 6.0 produce pH values of less than

5.5 in glucose-peptone medium. It would not, therefore, be necessary to check the pH of peptone and glucose-peptone media concurrently before designating taxonomic significance to a pH value of less than 6.0 produced in glucose-peptone medium by any of these Bacilli.

3. A few Bacilli tested produce a pH in the range of 6.0-7.0 in glucose-peptone medium and at the same time utilize glucose. In these cases it is necessary to check the pH of peptone medium concurrently before assigning taxonomic significance to any value within this range. In this investigation, peptone medium has been at least 0.6 pH units more alkaline than glucose-peptone medium inoculated with the same Bacillus and observed for a seven day incubation period.

4. Some Bacilli utilize less than 5 mg. glucose, which is not detectable by pH measurement of glucose-peptone medium even when compared to the pH of peptone medium run concurrently. The Bacilli of Group 1 fall within this category.

5. These pH observations are in almost complete agreement with those of Coffee (4). The results obtained by Coffee were duplicated by the present author, in many cases to the recording of exact pH readings, although a two year period intervened. Minor differences do occur. These differences have been pointed out in Table IV.

The pH and glucose utilization values obtained from the strains of each species tested in this investigation are in excellent agreement with each other. The most notable discrepancy is demonstrated by the failure of B. alvei #622 Tex. to use glucose, while the other three strains employed used 1-7 mg. of this carbohydrate. There is also slight difference in the extent of glucose utilization by the various strains of B. globigii, B. megatherium, B. mycoides, and B. subtilis; the pH produced by all these strains is, however, less than 5.5 The strains of B. mesentericus tested vary in both the pH they produce and in the amount of glucose they utilize. In actual values the extent of this variation is very small.

l-Arabinose:

The Bacilli do not produce the wide diversity of pH values in l-arabinose-peptone medium which they produce in glucose-peptone medium (Figures 1-130). Neither do they utilize l-arabinose to the extent that they utilize glucose (Figure 131). The data concerned with the pH and utilization of l-arabinose (Table III, Figures 1-130) does not, therefore, justify the use of the grouping (Table VI) devised to compare pH and utilization values of glucose.

In order to summarize the l-arabinose utilization data of Table III, the amount of l-arabinose that each Bacillus utilized in this experiment has been plotted in Figure 131. From this scattergram it will be noted that 67 strains of Bacilli use less than 3 mg. of l-arabinose. The remainder of the Bacilli tested utilize 3-25 mg. of l-arabinose.

Table III and Figures 1-130 indicate that regardless of the amount of l-arabinose utilized 69 strains of Bacilli may produce essentially the same pH in l-arabinose-peptone medium that they produce in peptone medium alone, in the range below pH 6.8, remain neutral, or be converted to an alkaline pH value. The remainder of the Bacilli produce pH values in l-arabinose-peptone which are slightly lower than those they produce in peptone alone. These Bacilli yield pH values in l-arabinose-peptone that are at least 0.2 pH units more acidic, in the range below 6.8, than the pH they produced in peptone alone. The extent of l-arabinose utilization does not appear to be the determining factor in the production of a low pH.

Figure 131

Scattergram of Carbohydrate Utilized by the genus *Bacillus*

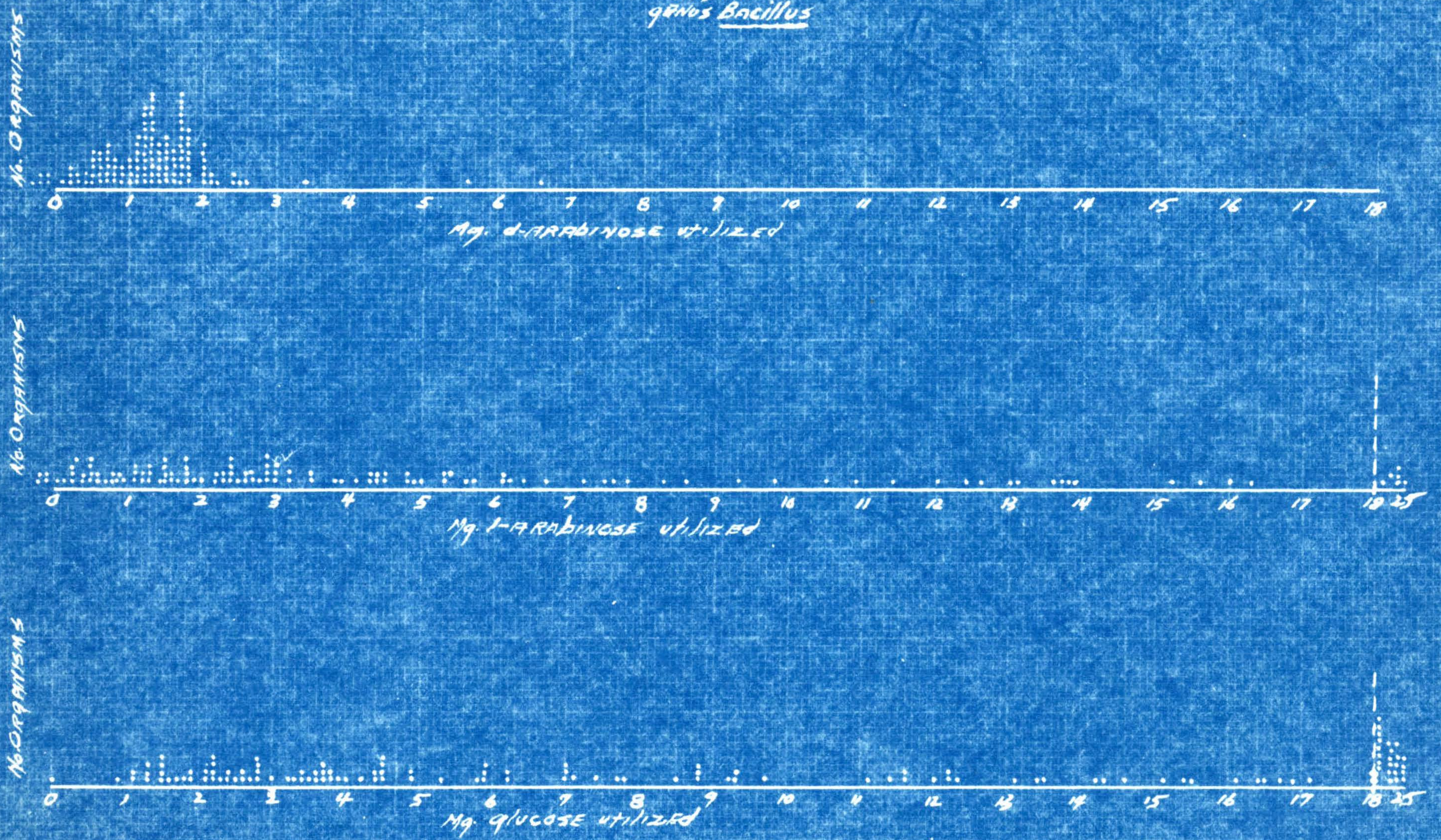


TABLE VII

A Summary of the Action of the Bacilli in l-arabinose-peptone Medium: pH Compared to pH Produced in Peptone Medium

1. pH produced in l-arabinose-peptone medium is 0.2 pH units more acidic, in the range below 6.8, than that produced by the same Bacillus in peptone medium.

A) Less than 3 mg. of l-arabinose utilized.

B. anthracis Tex.	B. laterosporus V. P. I.
B. anthracis Smooth Tex.	B. macerans U.S.D.A.
B. cereus #2 A.T.C.	B. megatherium #240 Tex.
B. circulans A.T.C.	B. mesentericus U.S.D.A.
B. firmus U.S.D.A.	B. mycoides #65 Tex.
B. flexus V.P.I.	B. panis A.T.C.
B. globigii A.T.C.	B. panis U.S.D.A.

B) More than 3 mg. of l-arabinose utilized.

B. adhaerans A.T.C.	B. cohaerens #840 Wis.
B. agri U.S.D.A.	B. danicus U.S.D.A.
B. aterrimus #353 Wis.	B. danicus #1 A.T.C.
B. aterrimus U.S.D.A.	B. graveolens U.S.D.A.
B. aterrimus Pesek Wis.	B. graveolens Neb.
B. aterrimus R.C. Wis.	B. lautus V.P.I.
B. cereus Smith-Thom Tex.	B. megatherium U.S.D.A.
B. cereus Wis.	B. megatherium #1 A.T.C.

TABLE VII (CON'T)

B) More than 3 mg. l-arabinose utilized. (con't)

B. megatherium #2 A.T.C.

B. megatherium Tex.

B. mesentericus Neb.

B. mesentericus Tex.

B. mesentericus A.T.C. Wis.

B. mesentericus Frost Wis.

B. niger A.T.C.

B. niger U.S.D.A.

B. niger #228 Wis.

B. niger "Old" Wis.

B. niger "S" Wis.

B. polymyxa Tex.

B. polymyxa V.P.I.

B. ruminatus #952 Tex.

B. silvaticus #957 Tex.

B. subtilis "L" Neb.

B. tumescens Tex.

B. tumescens #995 Tex.

B. vulgatus A.T.C.

B. vulgatus U.S.D.A.

B. vulgatus Neb.

2. pH produced in l-arabinose may be essentially the same as that produced by the same Bacillus in peptone medium, in the range below pH 6.8, remain neutral, or be converted to an alkaline pH value.

A) Less than 3 mg. of l-arabinose utilized.

B. albolactis Tex.

B. albolactis V.P.I.

B. alvei A.T.C.

B. alvei Tex.

B. alvei #622 Tex.

B. alvei #622 Lockheed Tex.

B. anthracis Wis.

B. anthracis Tex.

B. anthracis (Wis.) Tex.

B. anthracis Rall. Tex.

B. brevis #604 A.T.C.

B. brevis U.S.D.A.

B. cereus #1 A.T.C.

B. cereus Stock Tex.

B. cereus Non-motile Tex.

B. cereus #244 Tex.

TABLE VII (CONT'D)

A) Less than 3 mg of l-arabinose utilized. (cont'd)

B. cereus Clark Tex.	B. mycoides #426 Tex.
B. cereus A.T.C. Wis.	B. mycoides #427 Tex.
B. freundenreichii A.T.C.	B. mycoides #430 Tex.
B. fusiformis A.T.C.	B. mycoides Neb.
B. fusiformis Tex.	B. mycoides Left-hand Sarles Wis.
B. fusiformis Wright Tex.	B. paraalvei Tex.
B. fusiformis #339 Tex.	B. prausnitzii Neb.
B. graveolens Wright Wis.	B. robur #946 Tex.
B. lactis #618 Tex.	B. rotans A.T.C.
B. megatherium Wis.	B. rotans Tex.
B. megatherium A.T.C. Wis.	B. rotans (probably Wis.)
B. megatherium Neb.	B. serositidis Neb.
B. mycoides #405 Tex.	B. simplex Tex.
B. mycoides #410 Tex.	B. simplex. #335 Tex.
B. mycoides #417 Tex.	B. subtilis #600 Neb.
B. mycoides #420 Tex.	B. tumefaciens Tex.
B. mycoides #421 Tex.	Delong strain pectin fermenter
B. mycoides #422 Tex.	
B. mycoides #425 Tex.	

TABLE VII (CONT'D)

B) More than 3 mg. of l-arabinose utilized.

B. globigii (probably Vogel) Wis.

B. lacticola Tex.

B. lacticola #876 Tex.

B. mycoides #1 V.P.I.

B. sphaericus #348 Tex.

B. subtilis #231 A.T.C.

B. subtilis Tex.

B. subtilis Koch-Novy Tex.

B. subtilis Marburg Tex.

B. subtilis Gram-neg. Tex.

B. subtilis #231 Tex.

B. subtilis Marburg N.Y.

B. subtilis Marburg Wis.

B. subtilis Marburg Neb.

B. subtilis (Milk) V.P.I.

B. vulgatus Tex.

Table VII summarizes the action of the Bacilli in l-arabinose-peptone and compares the pH produced in this medium with the pH the same organism produces in peptone medium alone.

It is difficult, if not impossible, to state whether or not l-arabinose "spares" the peptone of the medium. In the case of those Bacilli which utilize more than 3 mg. of this carbohydrate and do not produce pH values at least 0.2 pH units more acidic, in the range below 6.8, than those produced in peptone alone, it seems apparent that a "sparing action" has not been effected; or, the organisms produce neutral, instead of acidic, products from respiration. On the other hand, those Bacilli which utilize l-arabinose and produce pH values lower than those produced in peptone medium alone appear to utilize l-arabinose in preference to the peptone of the medium as a source of food for respiration, as is evidenced by the lower pH produced in this medium.

d-Arabinose:

The data concerned with the pH produced from d-arabinose-peptone medium and the utilization of d-arabinose does not substantiate a grouping of any type. The pH produced by many of the Bacilli tested in this medium is for all practical purposes identical with the pH produced by them in peptone medium alone (Table III, Figures 1-130). A summary of the pH values produced by these Bacilli, together with the exceptions, may be found in Table V.

TABLE VIII

A Summary of the Metabolic Activity of the Genus Bacillus in d-Arabinose Media.

Strain of Bacillus tested	mg. d-arabinose utilized	taxonomic value of pH produced
B. albolactis Tex.	3.37	+ (at 24 hrs.)
B. albolactis V.P.I.	2.55	+ (at 24 hrs.)
B. alvei Tex.	2.37	-
B. alvei #622 Tex.	2.14	-
B. anthracis Smooth Tex.	2.04	+
B. brevis U.S.D.A.	2.04	-
B. cereus Wis.	2.07	+
B. danicus U.S.D.A.	2.20	+ (at 24 hrs.)
B. macerans U.S.D.A.	5.63	+
B. mesentericus Tex.	2.44	-
B. subtilis #600 Neb.	6.60	-

The pH values listed above are recorded as positive (+) if the pH produced in d-arabinose, in the range below pH 6.8, differs at least 0.2 pH units from that which the same organism produced in peptone medium alone.

The scattergram (Figure 131) denoting the degree of d-arabinose utilization by the strains of Bacilli tested indicates that only three strains used more than 3 mg.: 8 strains used 2-3 mg.; and all other strains tested used 2 mg. or less than 2 mg. Table VIII lists the Bacilli which use more than 2 mg. of d-arabinose along with the author's opinion of the taxonomic value of the pH produced in d-arabinose-peptone medium. It will be noted from comparing the pH produced in d-arabinose-peptone medium (Table III, Figures 1-130) with the degree of d-arabinose utilization and the pH produced in peptone medium alone that the pH produced in d-arabinose-peptone medium does not indicate d-arabinose utilization for all of these Bacilli; therefore, if the pH produced in d-arabinose-peptone medium does not differ by at least 0.2 pH units, in the range below 6.8, from the pH produced by the same organism in peptone medium alone, the pH value is not considered to be taxonomically significant from the standpoint of indicating d-arabinose utilization. It is, therefore, necessary to test the pH of peptone alone concurrently with d-arabinose medium.

General Discussion:

The results of the pH and carbohydrate utilization determinations are recorded in Table III and are given graphically in Figures 1-130.

Table IV presents an interpretation of the pH values recorded in Table III together with comparable data from the investigations of Coffee (4), and the reactions from other literature listed in Bergey's Manual (1).

Table V presents a summary of the data in Tables III and IV and in Figures 1-130. In this table the reactions of the various strains of Bacilli used in this investigation are summarized into a reaction apparently characteristic of the species represented. Only those reactions which appear to have taxonomic value are listed in this table.

As a result of the extent of glucose utilization and a comparison of the pH produced in glucose-peptone medium and peptone medium alone, it has been possible to separate the Bacilli used in this investigation into the 5 groups listed in Table VI.

Table VII summarizes the action of the Bacilli in l-arabinose-peptone and compares the pH produced in this medium with the pH the same organism produces in peptone medium alone.

Table VIII lists those Bacilli which utilize more than 2 mg. of d-arabinose together with the author's opinion of the taxonomic value of the pH these Bacilli produce in d-arabinose-peptone medium when compared to the pH produced by the same organism in peptone medium alone.

In order to interpret the data concerned with the pH produced in d- and l- arabinose-peptone media so that it may have taxonomic significance, it is compared with the pH produced by a Bacillus in peptone medium alone and the extent of utilization of the two carbohydrates (Table III, Figures 1-130). Such a comparison of these data indicates that those Bacilli which lower the pH of the carbohydrate-peptone media at least 0.2 pH units more acidic, in the range below 6.8, than the pH of the peptone medium utilize from 0.48 mg. (p. 18 & 19) to 25 mg. of carbohydrate. Evidence for the consistency and reproducibility of the pH values produced by the Bacilli employed in this investigation, may be found by comparing these values with those obtained by Coffee (4).

A difference of 0.2 pH units produced by a Bacillus culture in carbohydrate-peptone and peptone media, is not large. The interpretation of such a difference as indicating the positive utilization of d- and l-arabinose is, however, according to the results of this investigation, justifiable. This method of interpreting these results enables a maximum number of carbohydrate-using organisms to be detected by pH measurement. There is no possibility of misinterpreting these results using this criterion since non-carbohydrate users did not produce pH values in carbohydrate-peptone medium which were 0.2 pH units more acidic, in the range below 6.8, than the pH they produced in peptone alone.

Table III indicates very conclusively that although an acidic pH in d- or l-arabinose-peptone can be indicative of carbohydrate utilization, it cannot be interpreted in any manner to indicate the extent of utilization. An excellent example for this observation is cited in Figures 15, 64, 65, 111 (l-arabinose); 3, 4, 53 (d-arabinose). It is also important to point out that an alkaline pH does not necessarily mean that large amounts of carbohydrate have not been utilized (Figure 120 for d-arabinose; Figures 121, 117 for l-arabinose).

In this investigation the pH produced in glucose-peptone medium by 40 species of the genus Bacillus was determined. Four of the species investigated did not produce reactions in this medium which agree with those listed by Bergey's Manual (1) for these species. Probably because of inadequate data, this manual does not list reactions for six species tested in glucose-peptone medium in this investigation.

Bergey's Manual (1) lists reactions for only twelve of the 40 species of Bacilli tested in d- and l-arabinose peptone media. This manual makes no distinction between d- and l- forms of arabinose. Reactions produced in this medium are merely listed under "Arabinose".

This lack of distinction is probably due to an absence of data for d- and l- forms of arabinose. This may account for discrepancy between 7 reactions for species used in the present investigation when compared to the "Arabinose" reactions listed by Bergey (1).

The results of this investigation are in excellent agreement with those of Coffee (4). Minor differences do occur in the extent of low pH values produced by some of the Bacilli. These differences are not great enough to interfere with the method of interpretation employed in this investigation, or that of Coffee, and may be due to differences in experimental procedures, or to experimental error.

Roberts (34) report that glucose is utilized by B. rotans without acid production is confirmed. Koser and Saunders (19) report that B. megatherium does not utilize d- or l-arabinose. The present results are in opposition to this report; however, other results listed by these authors have been confirmed, as have the results of Merrill (28), Wedum (45), and Wedum and Walker (46).

Debord (6) reported that the presence of glucose in peptone medium increased the rate of production of amino nitrogen in cultures of B. subtilis. He infers that a more alkaline reaction is produced by B. subtilis under these conditions. Coffee (4) has obtained results suggestive of greater alkali production from peptone in the presence of d- or l-arabinose by certain Bacilli. Sufficient evidence to support or refute these results was not obtained in this investigation.

SUMMARY

The present investigation has been concerned with the ability of 130 strains, representing 41 species, of the genus Bacillus to utilize glucose and d- or l- arabinose as a source of food for respiration. Carbohydrate utilization was determined by direct titration of the amount of carbohydrate remaining after incubation.

The pH of the carbohydrate-peptone media employed was determined potentiometrically using a glass electrode and was compared with the pH produced by the same Bacillus in peptone medium alone.

The amount of glucose utilized by a particular Bacillus and the pH it produced in glucose-peptone medium was taken as a basis upon which to separate the strains and species of Bacilli tested into the following five groups:

1. Glucose utilization less than 6.2 mg.: pH glucose-peptone 7.0 or above.

   Typical examples: B. brevis, B. fusiformis, B. rotans, and B. serositidis.

2. Glucose utilization more than 6.2 mg.: pH glucose-peptone less than 5.5.

   Typical examples: B. adhaerens, B. agri, B. albolactis, B. cereus, B. graveolens, B. lactis, B. lacticola, B. laterosporus, B. robur, B. silvaticus, and B. tumefaciens.

3. Glucose utilization less than 6.2 mg.: pH glucose-peptone less than 5.5.

   Typical examples: B. alvei, B. anthracis, B. circulans, B. macerans, B. megatherium, B. mycoides, B. paraalvei, B. prausnitzii, and B. ruminatus.

4. Glucose utilization more than 6.2 mg.: pH glucose-peptone 5.5 - 6.5.

   Typical examples: B. atterrimus, B. cohaerens, B. danicus, B. globigii, B. lautus, B. mesentericus, B. niger, B. panis, B. subtilis, and B. vulgatus.

5. Glucose utilization more than 6.2 mg.: pH glucose-peptone less than 5.5 within 2 days - more than 5.5 in 7 days.

   Typical example: B. polymyxa.

The following species did not apparently belong to any of the above groups: B. firmus, B. flexus, B. freudenreichii, and B. simplex, because each used less than 6.2 mg. of glucose and produced a pH of 6.0 or above. B. sphaericus used less than 6.2 mg. of glucose and produced a pH which grew progressively more acidic to pH 5.6 after seven days incubation.

Results obtained from investigations of the action of the Bacilli in d- and l- arabinose-peptone media do not permit the use of similar groupings for those media.

The results obtained from the carbohydrate utilization studies, and the pH produced in carbohydrate-peptone medium and peptone medium alone further indicate that the following statements are justified:

1. A pH of less than 6.0 produced by an actively growing culture of the genus Bacillus is indicative of positive glucose utilization by that organism. This pH is not indicative of the extent of glucose utilization.
2. No species of Bacillus tested produce a pH of less than 6.0 in peptone medium alone. Those Bacilli capable of lowering

the pH of peptone medium to 6.0 produce pH values of less than 5.5 in glucose-peptone medium. It would not, therefore, be necessary to check the pH of peptone and glucose-peptone media concurrently before designating taxonomic significance to a pH value of less than 6.0 produced in glucose-peptone medium by any of these Bacilli.

3. A few Bacilli tested produce a pH in the range 6.0 - 7.0 in glucose-peptone medium and at the same time utilize glucose. In these cases it is necessary to check the pH of peptone medium concurrently before assigning taxonomic significance to any value within this range. In this investigation peptone medium has been at least 0.6 pH units more alkaline than glucose-peptone medium inoculated with the same Bacillus and observed for a seven day incubation period.

4. Some Bacilli utilize less than 6.2 mg. of glucose which is not detectable by pH measurement of glucose-peptone medium even when compared to the pH of peptone medium run concurrently. The pH of these media either remains neutral or becomes alkaline during the entire incubation period.

5. These pH observations are in almost complete agreement with those of Coffee (4). The results obtained by Coffee were duplicated by the present author, in many instances to the recording of exact pH readings, although a two year period intervened.

6. Sixty-seven (67) strains of the Bacilli tested for their ability to utilize l-arabinose utilize less than 3 mg. of this carbohydrate. The remainder utilized 3-25 mg. of this carbohydrate.

7. Regardless of the amount of l-arabinose utilized sixty-nine (69) strains of Bacilli, representing 21 species, produce the same pH in l-arabinose-peptone medium that they produce in peptone medium alone. The remainder, representing 20 species, produce pH values in l-arabinose-peptone which are at least 0.2 pH units more acidic, in the range below 6.8, than the pH they produced in peptone medium alone.

8. Eleven strains of Bacilli, representing 8 species, utilized more than 2 mg. of d-arabinose. The remainder used less than this amount.

9. Thirty-three strains of Bacilli representing 19 species, produce pH values in d-arabinose-peptone which are at least 0.2 pH units lower, in the range below 6.8 than the pH they produce in peptone medium alone. The remainder, 98 strains, representing 22 species, produce identical pH values in both d-arabinose-peptone and peptone media.

10. Practically all of the Bacilli tested were capable of using glucose and d- or l- arabinose to some extent in their metabolism. An acidic pH value can in no manner be indicative of the extent of this utilization. The order of decreasing utilizability of these carbohydrates is glucose, l-arabinose and d-arabinose.

11. The strains of the genus Bacillus used in this investigation show diversity in the extent of their ability to utilize the d- and l- forms of arabinose. It is therefore recommended that Bergey's Manual (1) change its designation of arabinose

media from "Arabinose" to d- Arabinose and l-Arabinose in order to avoid conflicting reports as to the ability of an organism to utilize a particular carbohydrate.

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AN ABSTRACT OF THE PHYSIOLOGY OF THE GENUS *BACILLUS*

A Study of the Metabolism of Certain Members of the Genus *Bacillus* in Relation to Their Ability to Ferment Glucose, d- and l-Arabinose.

by

Samuel R. Bozeman

A Thesis Submitted to the Graduate Committee in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in

BACTERIOLOGY

Approved:

In Charge of Major Work

Head of Department

Dean of Applied Science

Virginia Polytechnic Institute

Department of Biology

1945

AN ABSTRACT OF THE PHYSIOLOGY OF THE GENUS *BACILLUS*

It is routine bacteriological procedure to consider an acidic pH reading, taken on a carbohydrate-peptone medium in which an organism is growing, as evidence that the bacterium in question utilizes the carbohydrate. Numerous investigators (2-5, 6-8, 11, 12, 16-19, 20, 22, 23) have demonstrated that although used routinely by bacteriologists, acid-indicators are inadequate to determine the utilization of carbohydrate from a carbohydrate-peptone medium by many organisms. This failure may often be due to a lack of "sparing action" of carbohydrate for the peptone of the medium. This sometimes results in an alkaline or neutral pH reaction even though carbohydrate has been utilized.

A lack of "sparing action" of carbohydrate for peptone has been demonstrated in quantitive studies which compared the pH produced in a carbohydrate-peptone medium with the utilizability of the carbohydrate for the following organisms: *B. rotans* (20), the genus *Mycobacterium* (13), the tubercle bacillus (9), the hydrocarbon bacteria (10), the *Brucella* (14, 15, 24) and certain miscellaneous organisms (22).

It has been shown by acid indicator techniques (16, 18, 19), that a lack of "sparing action" by species of the genus *Bacillus* makes it difficult and sometimes impossible to determine the utilization of various carbohydrates as measured by a lowering of the pH. The resulting pH of the medium, where there is no "sparing action," is dependent not only on acid produced from carbohydrate dissimilation, but also upon concurrent acid and ammonia produced from peptone decomposition. The change in pH, therefore, may result from factors other than carbohydrate utilization.

The object of the present investigation was to determine quantitatively the extent of glucose and d- and l- arabinose utilization in order to separate it from the other two factors influencing the pH change in the medium. The pH of the carbohydrate media employed was determined potentiametrically, in order that the pH produced could be compared with the extent of utilization of these carbohydrates. Likewise, the pH produced by the same Bacilli in peptone alone was determined so that it could be compared with the pH produced in the carbohydrate-peptone media.

The methods for differentiating the aerobic spore formers of the genus Bacillus have been devised from inadequate and often unreliable data (1). The fundamental observations derived from this investigation concerned with the metabolism of the genus Bacillus may be of value in the necessary rearrangement of the present taxonomic procedure. Such studies might also lead to other interesting conclusions regarding the metabolism of the Bacilli in pure laboratory culture and also in the mixed cultures of their natural habitat.

THE INVESTIGATION

One hundred and thirty strains of the genus Bacillus, representing 41 species, were used in these studies. Determination of the pH resulting from the growth of an organism in peptone alone and in peptone containing glucose, d- arabinose, or l- arabinose was made after 1, 2, 3, and 7 days of incubation. A Beckman potentiometer equipped with a glass electrode was used for taking the pH measurements. At the same time pH readings were made, the amount of carbohydrate remaining in a tube after each incubation period was determined quantitatively according to the method of Stiles, Peterson and Fred (21).

Fig. 1

B. adhaerens

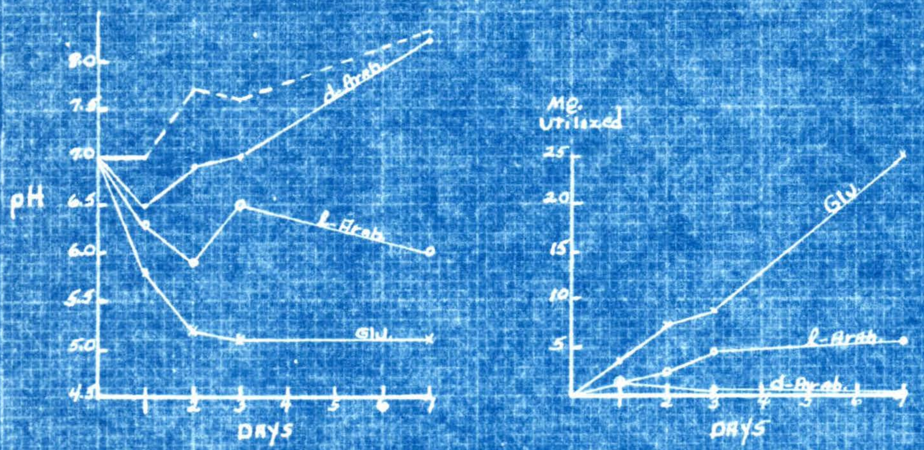


Fig. 2

B. agri

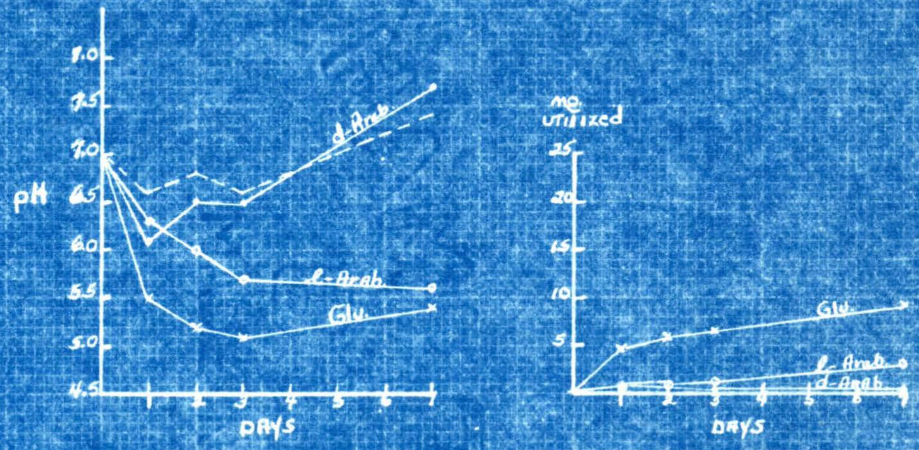


Fig. 3

B. albolactis

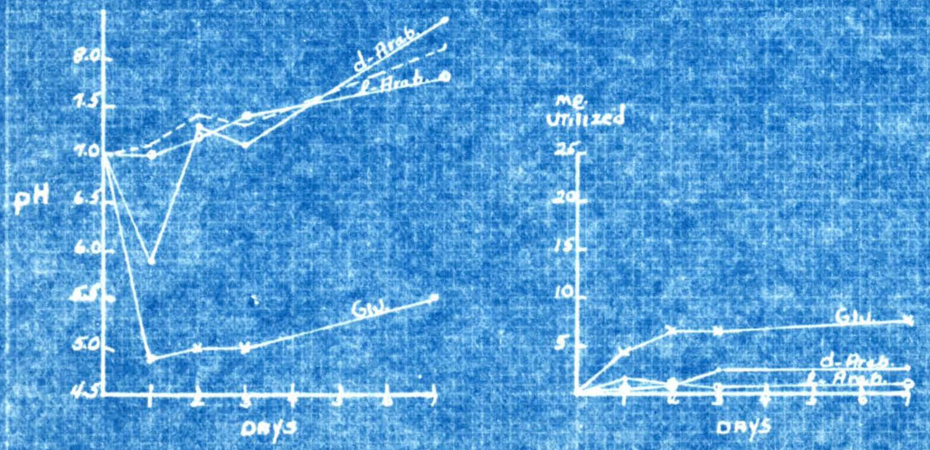
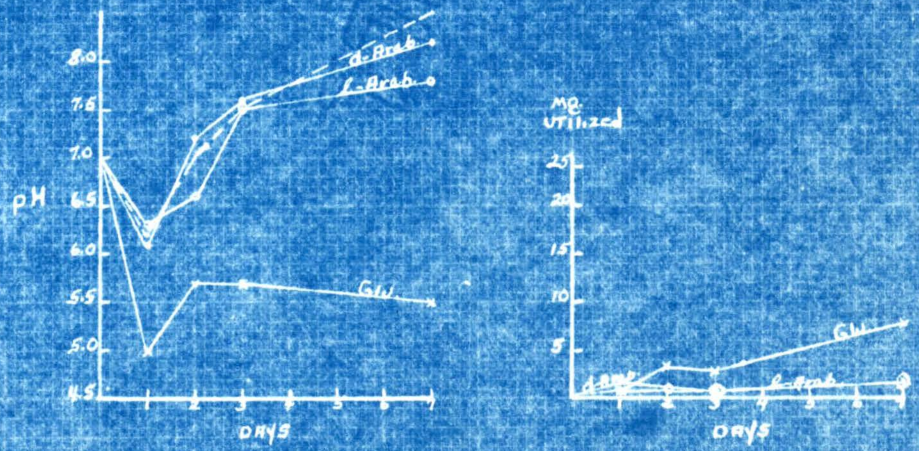


Fig. 4

B. alvei



--- peptone

Fig. 5

B. anthracis

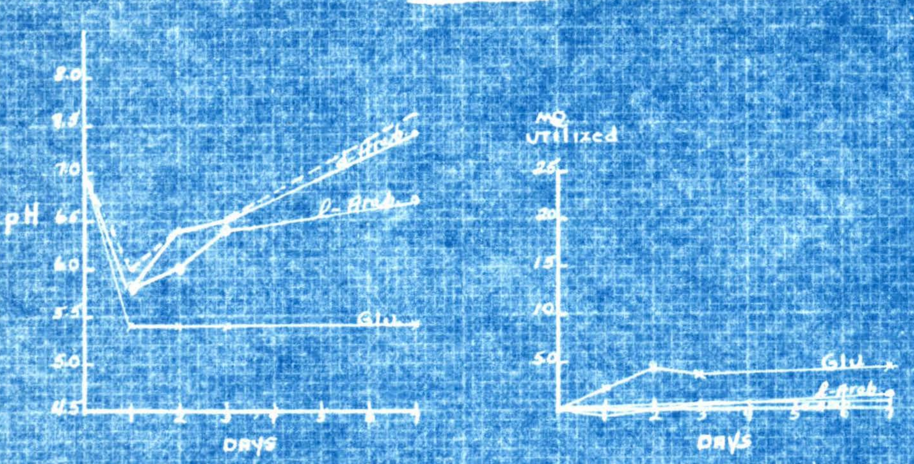


Fig. 6

B. aterrimus

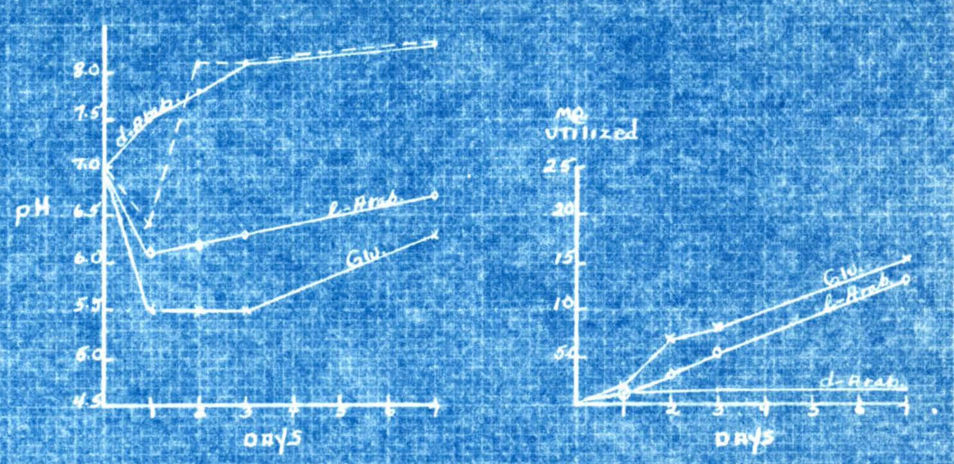


Fig. 7

B. brevis

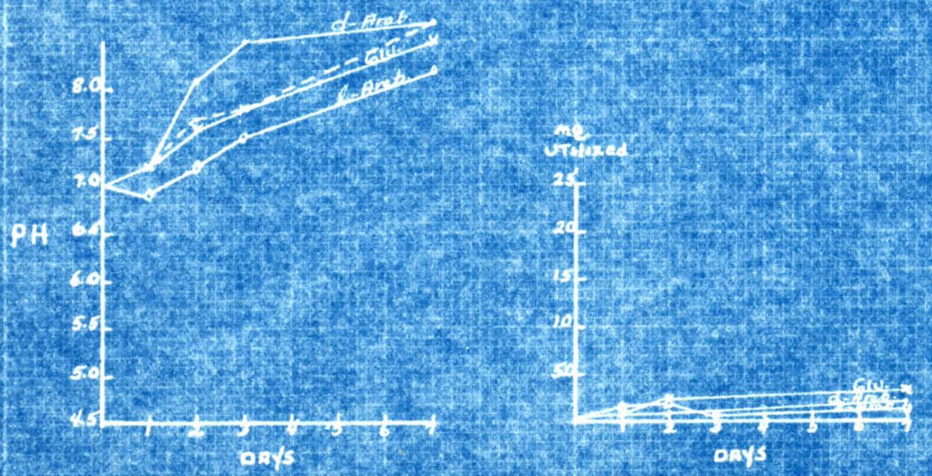
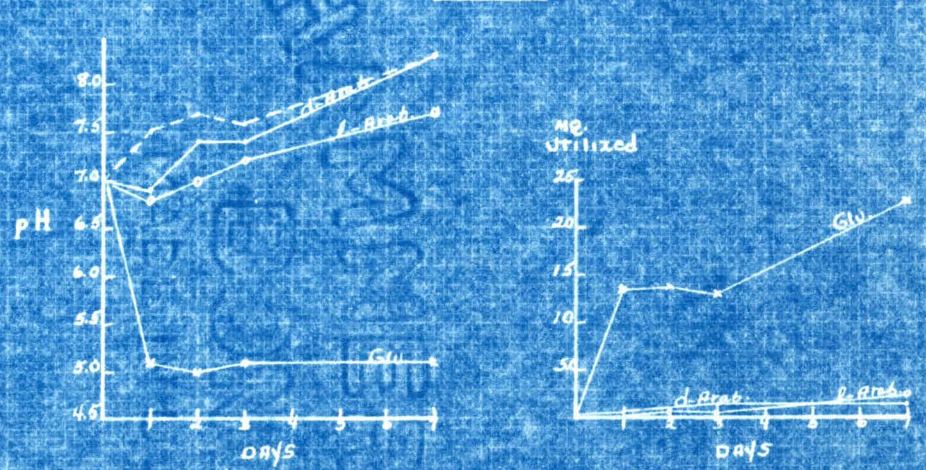


Fig. 8

B. cereus



--- peptone

Fig. 9

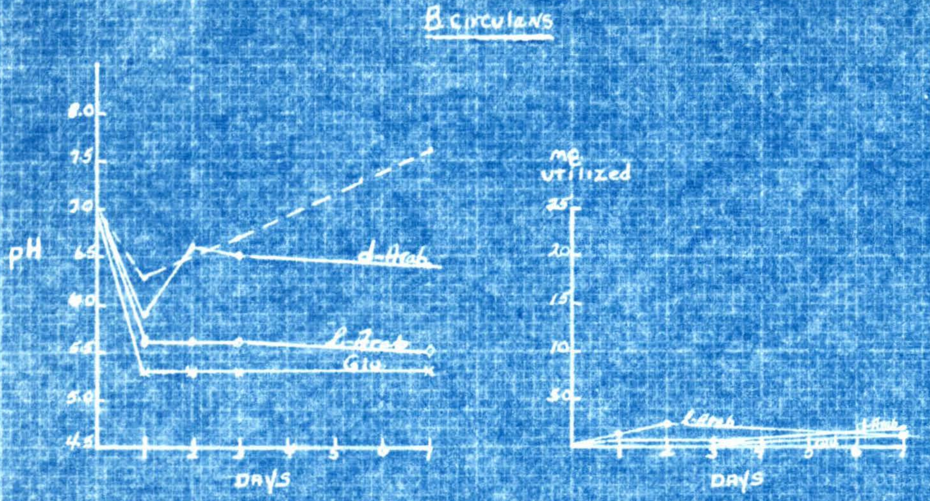


Fig. 10

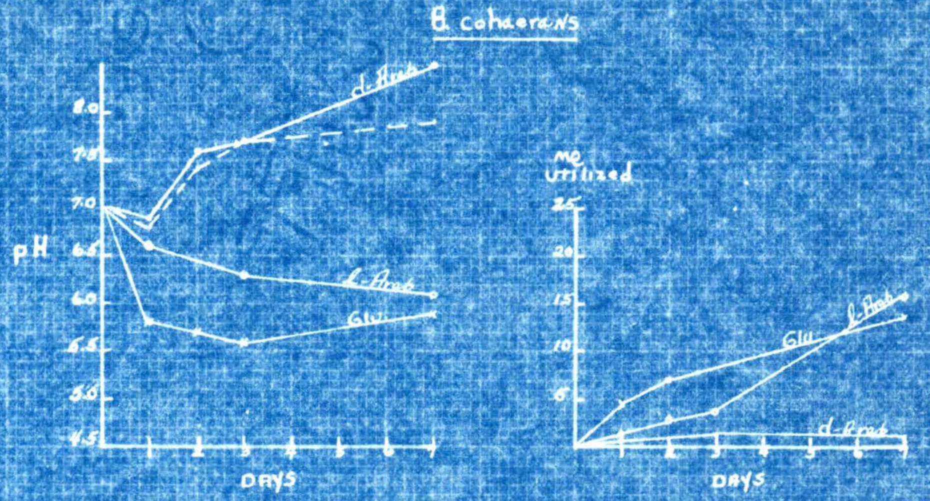


Fig. 11

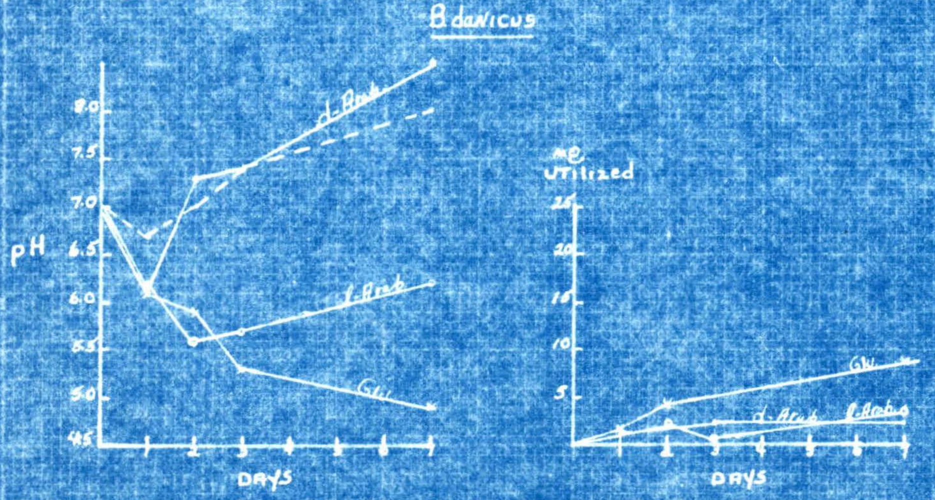
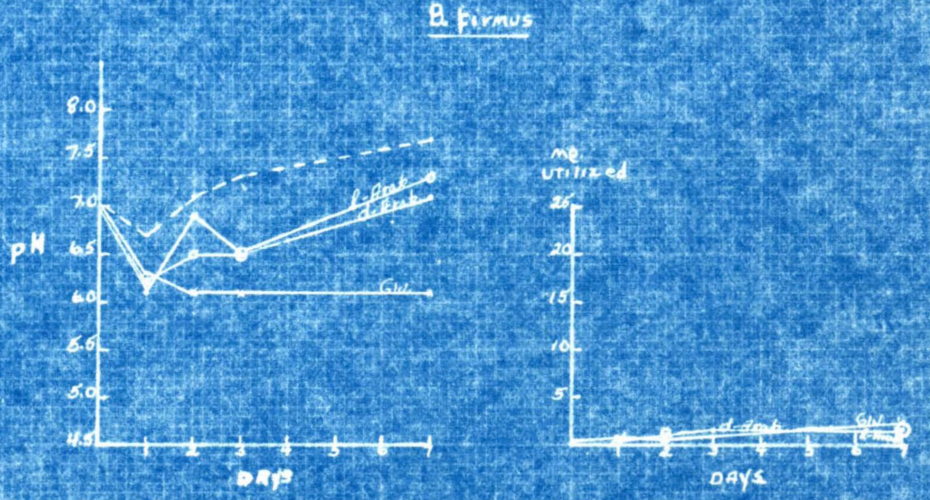


Fig. 12



---- peptone

Fig. 13

B. flexus

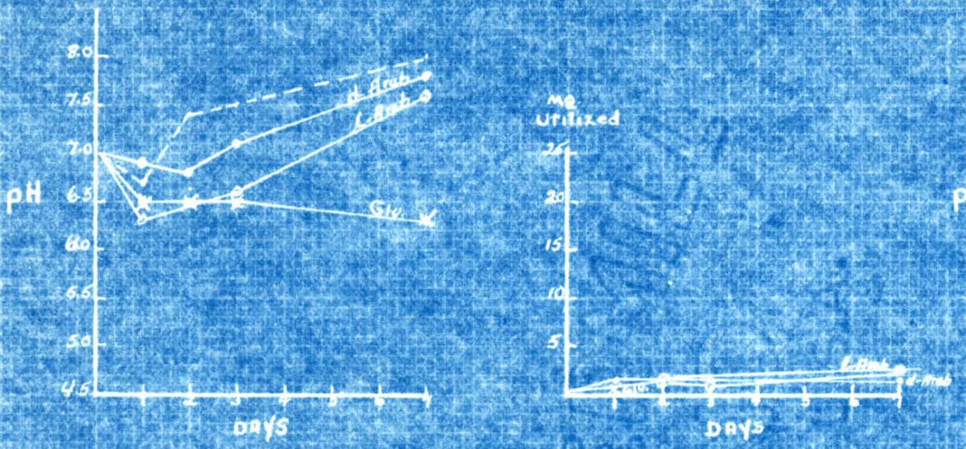


Fig. 14

B. freudenreichii

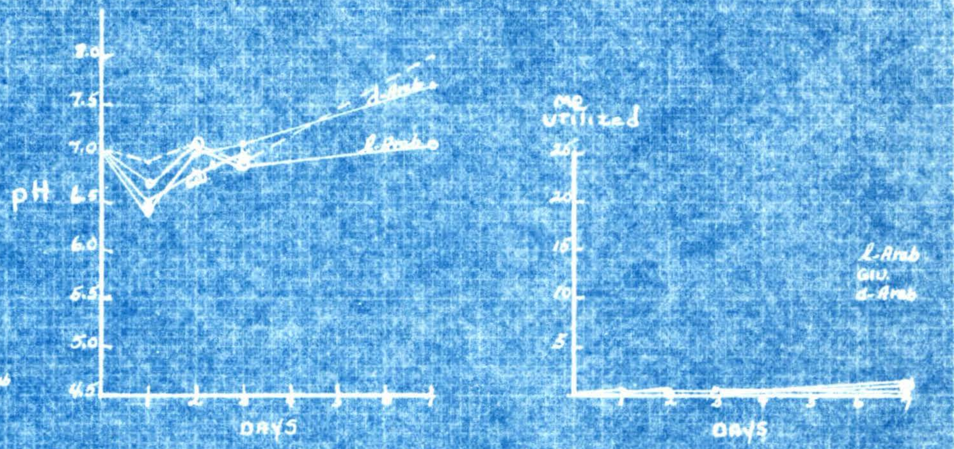


Fig. 15

B. fusiformis

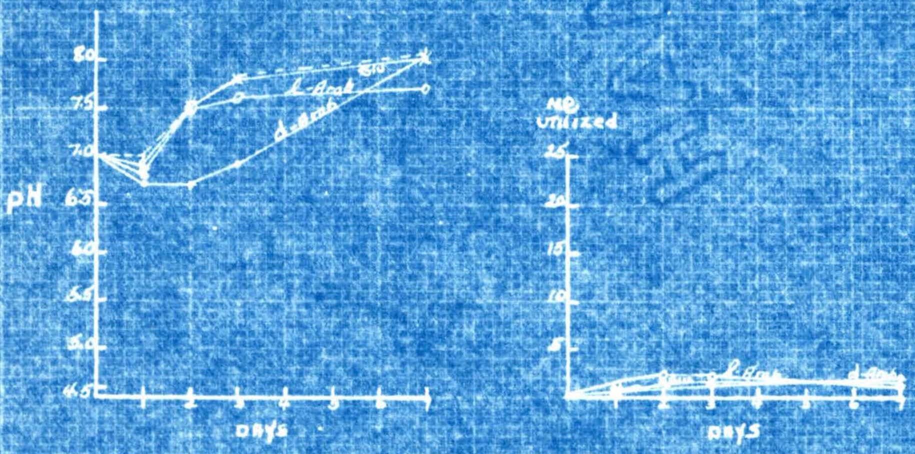
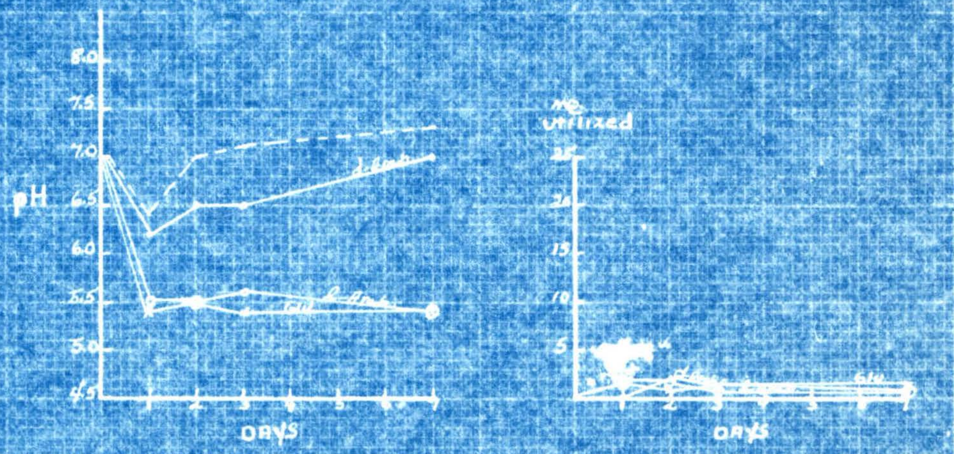


Fig. 16

B. globigii



— peptone

Fig. 17

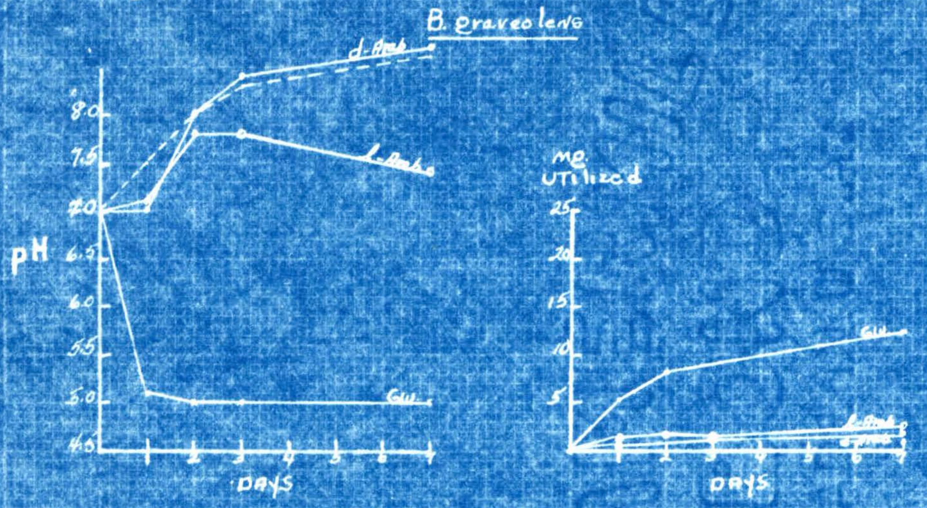


Fig. 18

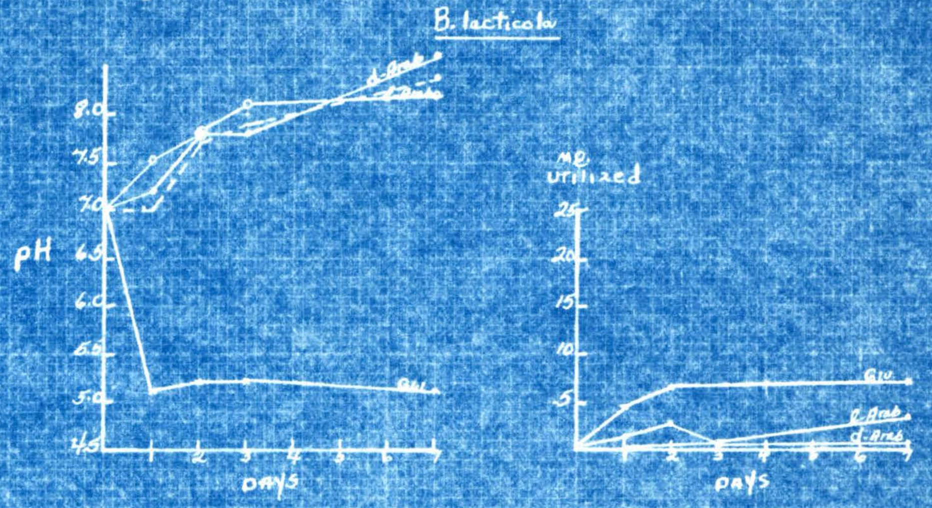


Fig. 19

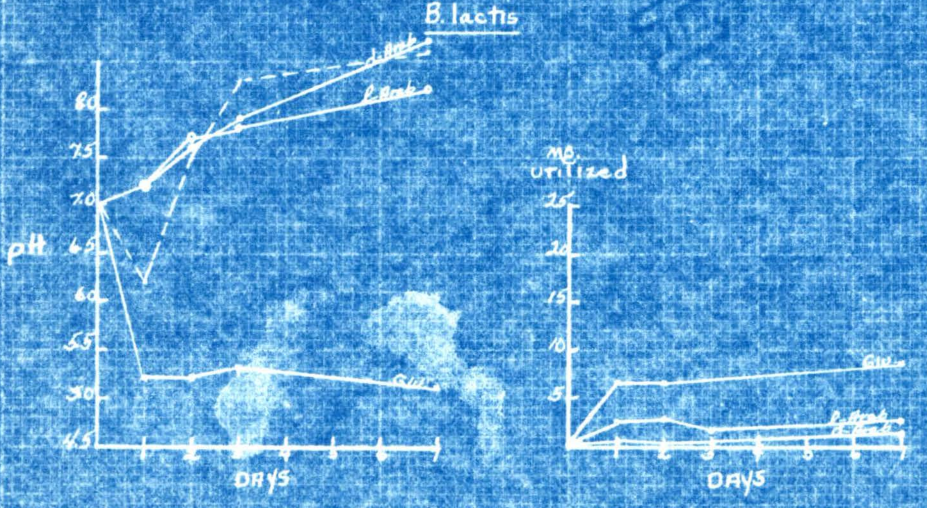
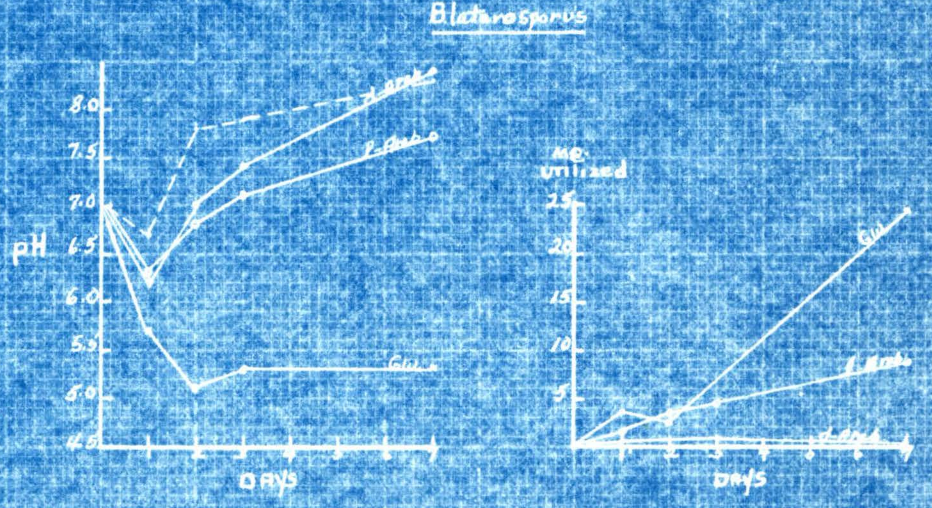


Fig. 20



--- peptone

Fig. 21

B. lautus

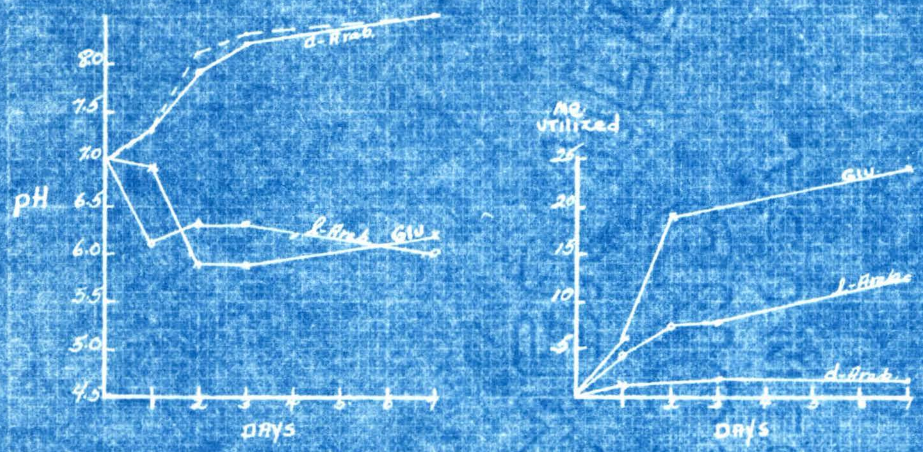


Fig. 22

B. macerans

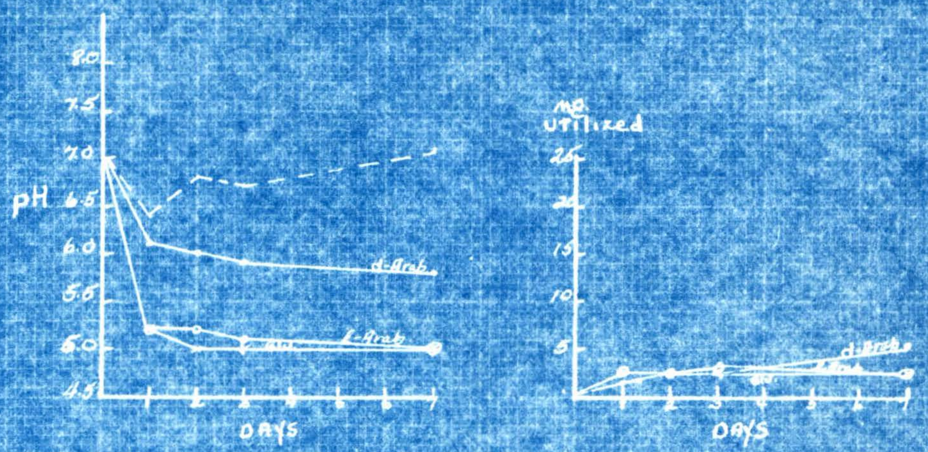


Fig. 23

B. megatherium

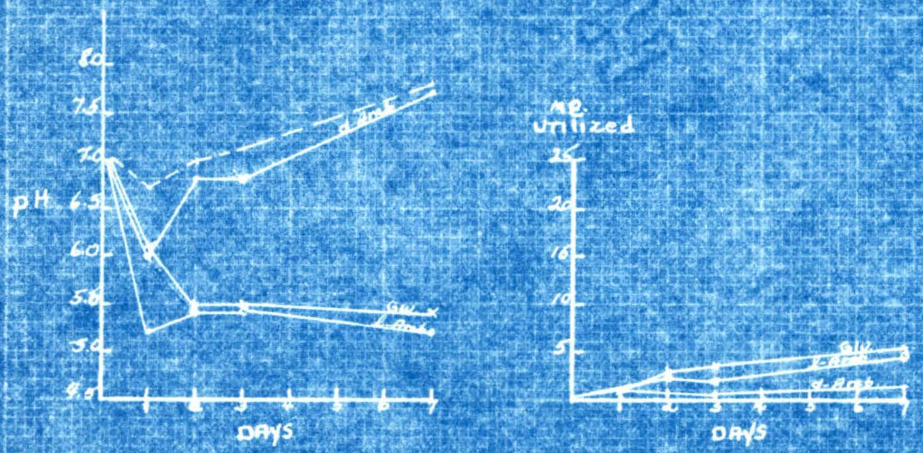
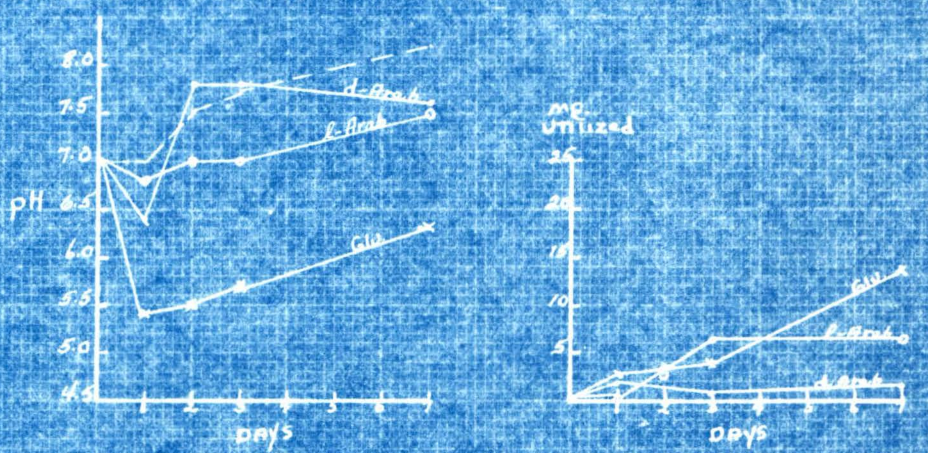


Fig. 24

B. mesentericus



--- peptone

Fig. 25

B. mycoides.

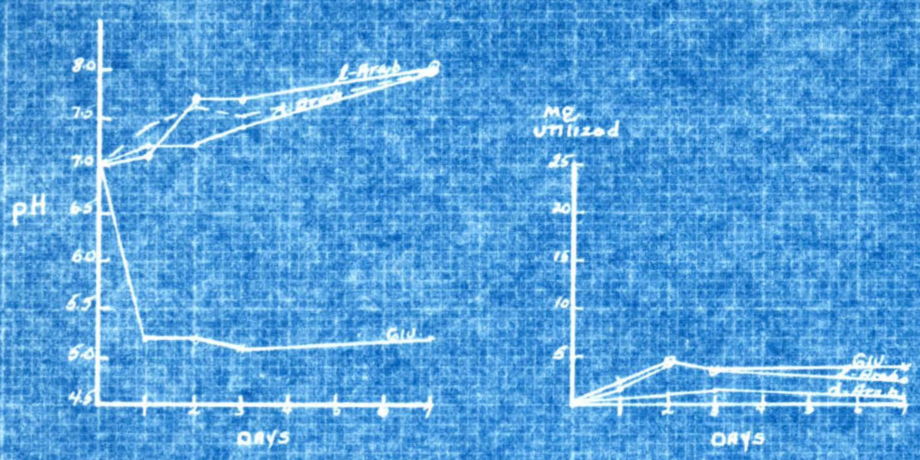


Fig. 26

B. niger

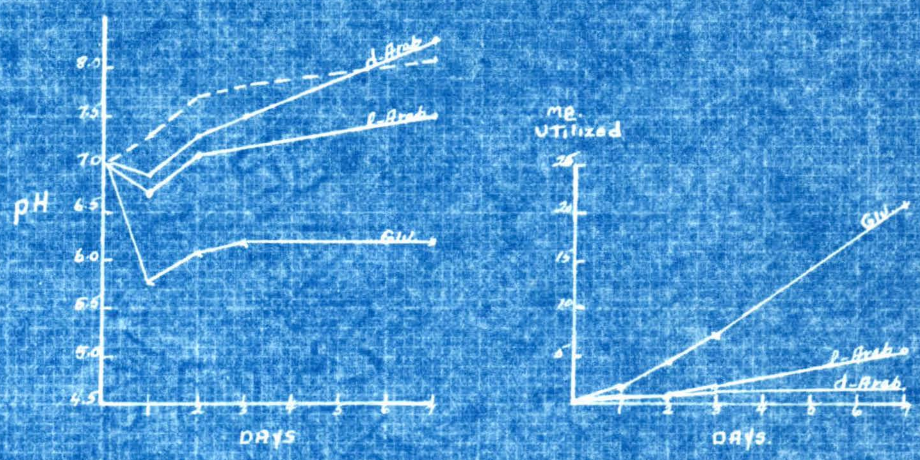


Fig. 27

B. panis

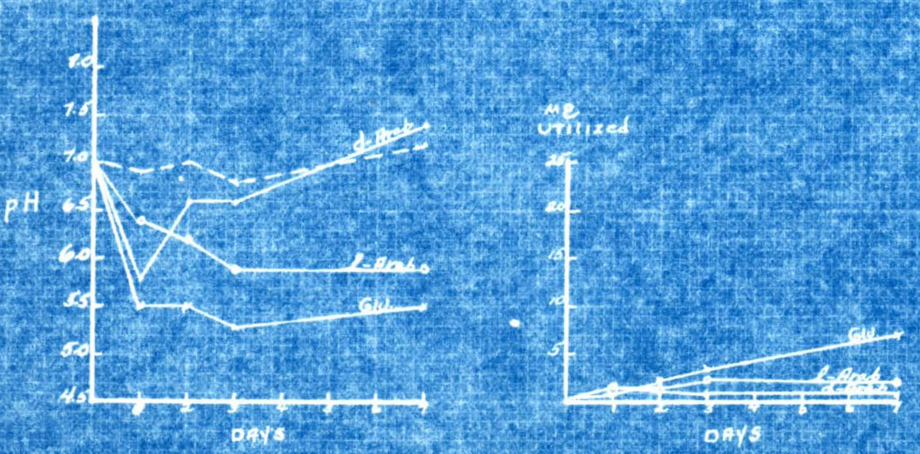
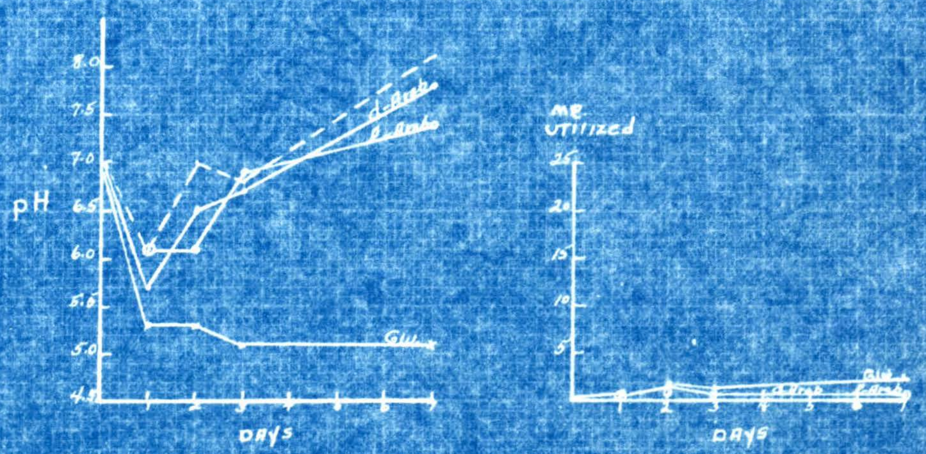


Fig. 28

B. paraalvei



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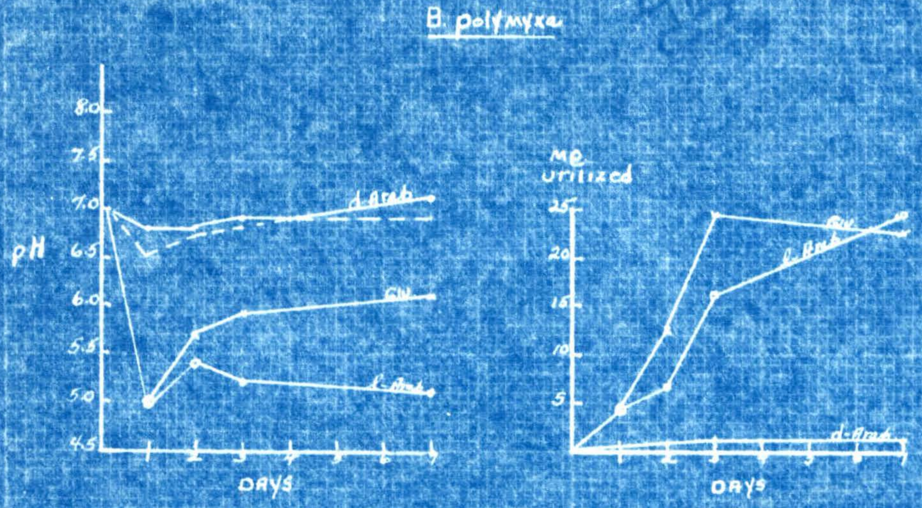


Fig. 30

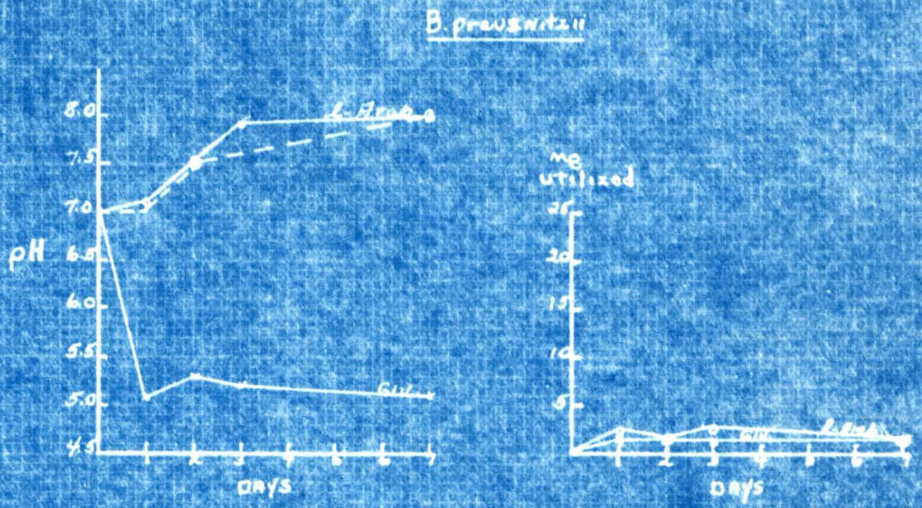


Fig. 31

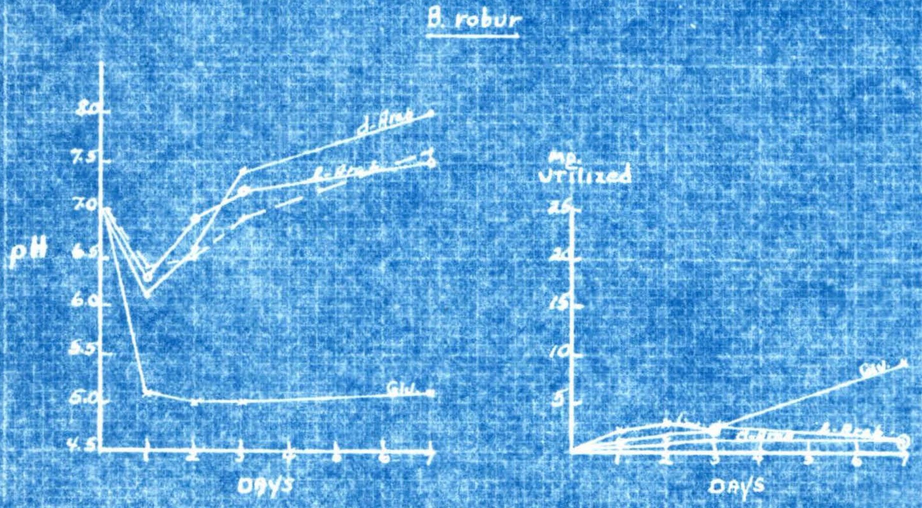
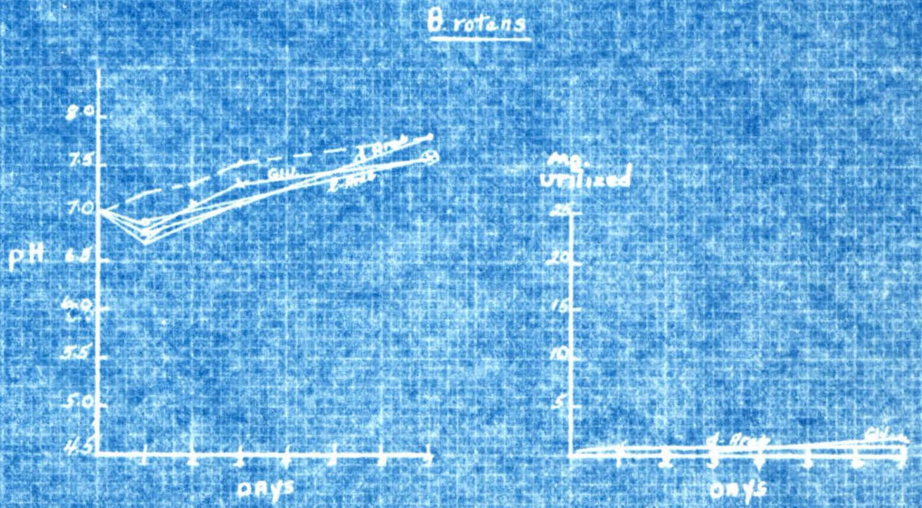


Fig. 32



--- peptone

Fig. 33

B. ruminatus

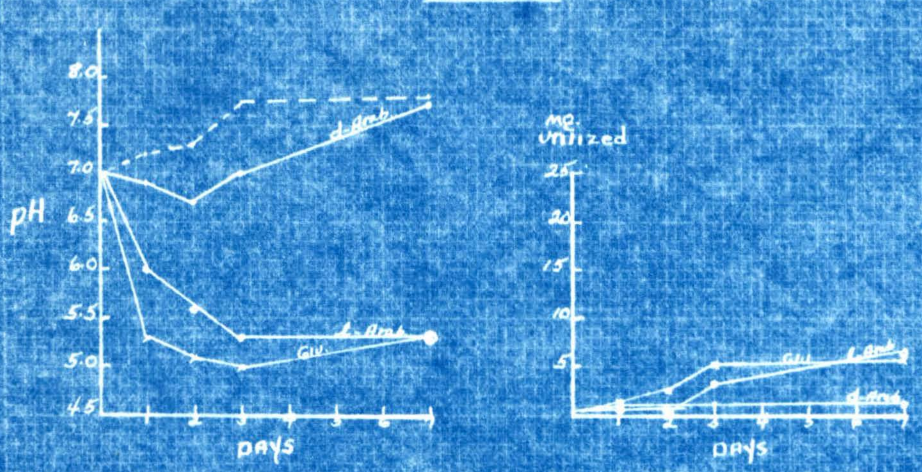


Fig. 34

B. serositidis

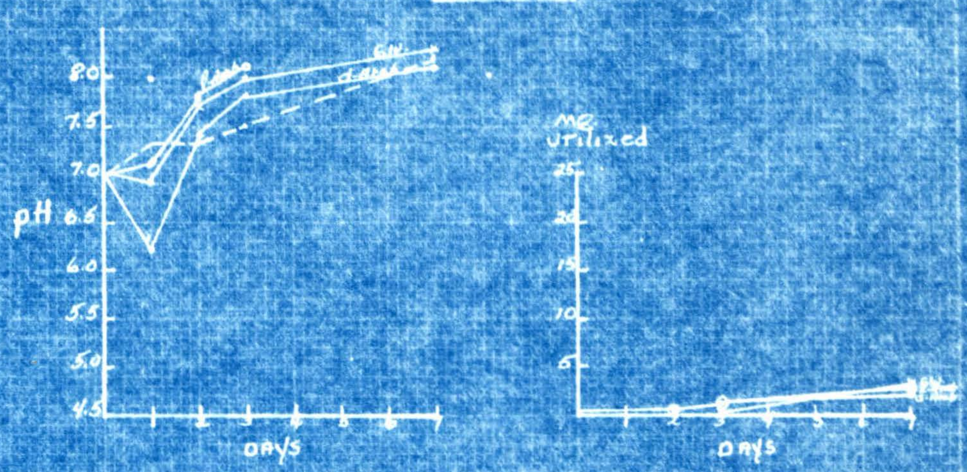


Fig. 35

B. silvaticus

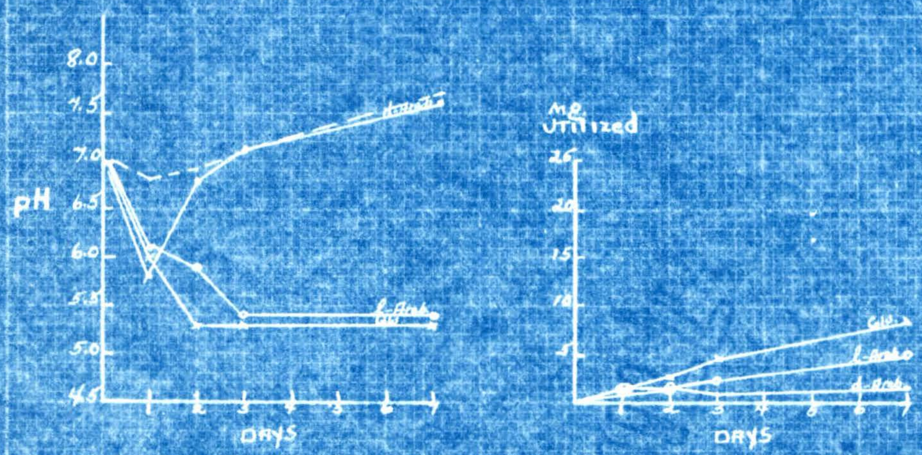
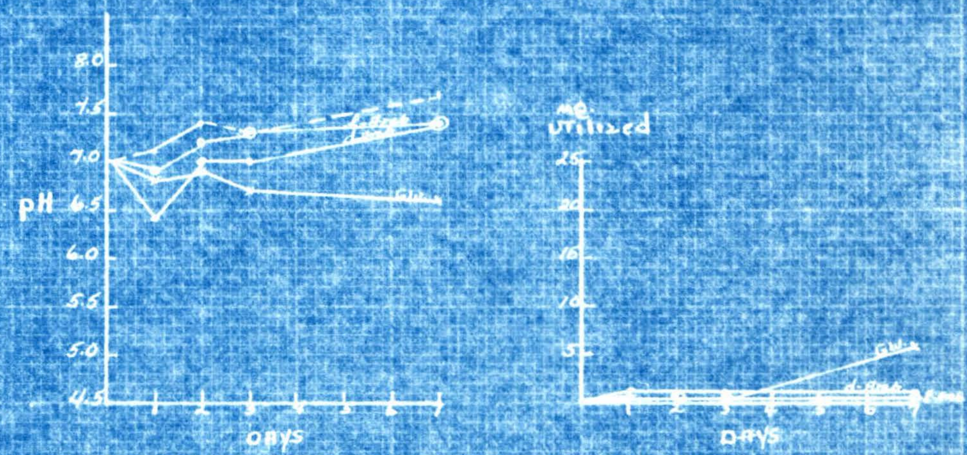


Fig. 36

B. simplex



--- peptone

Fig. 37

B. sphaericus

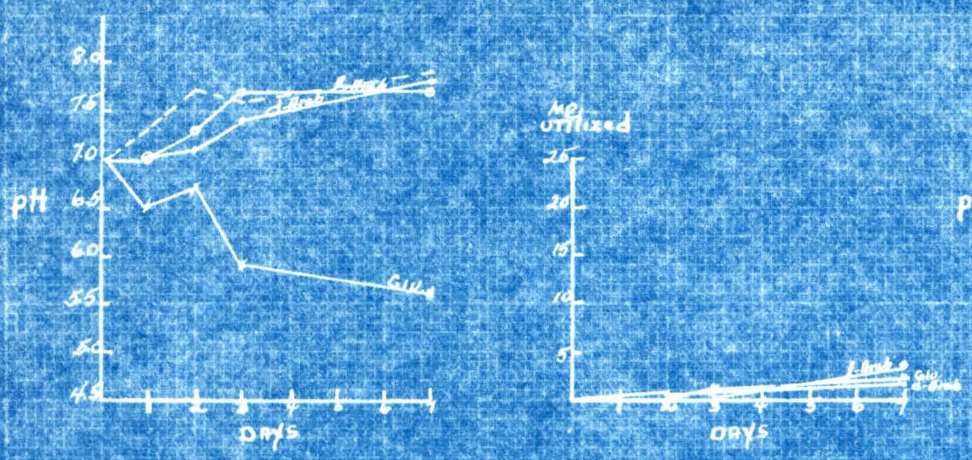


Fig. 38

B. subtilis

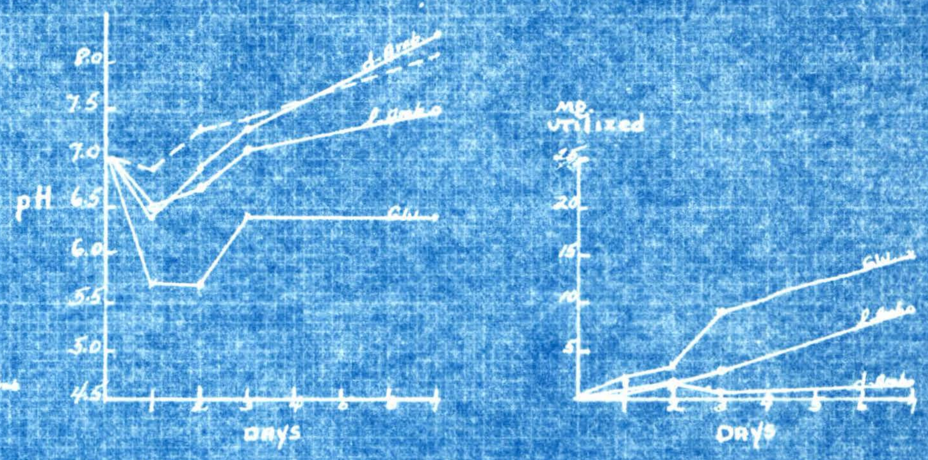


Fig. 39

B. tumefaciens

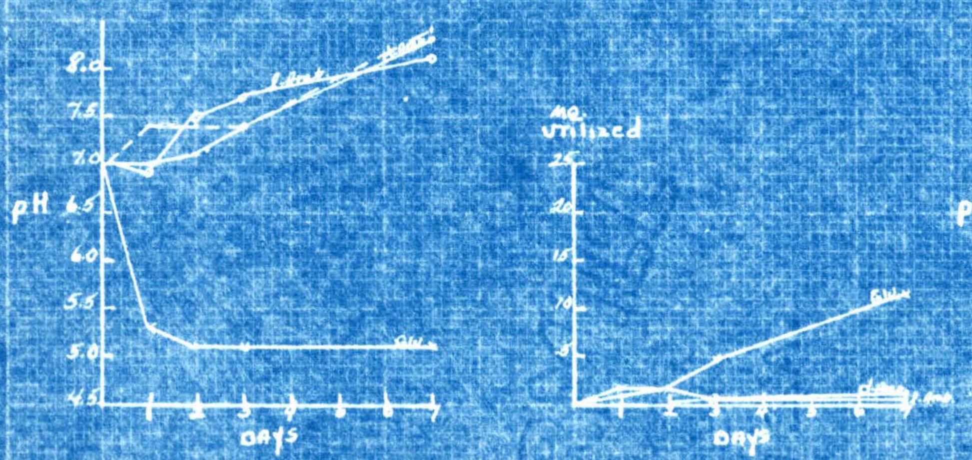
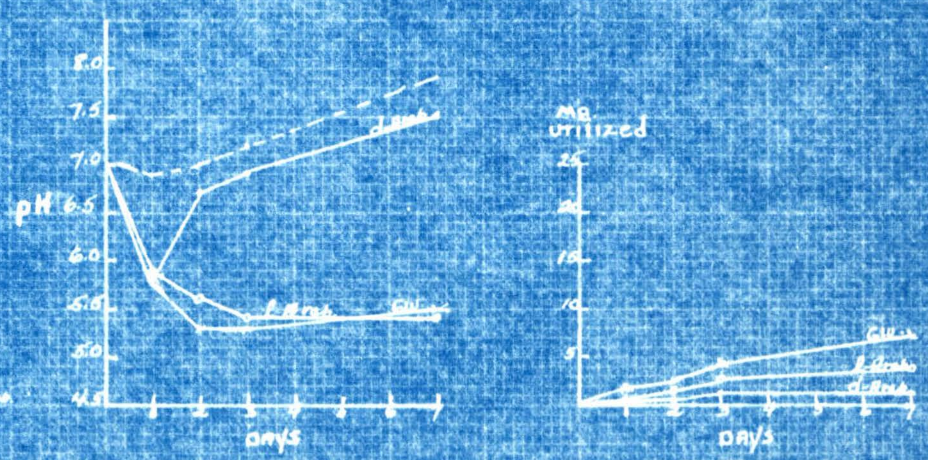


Fig. 40

B. tumescens



--- peptone

Fig 41

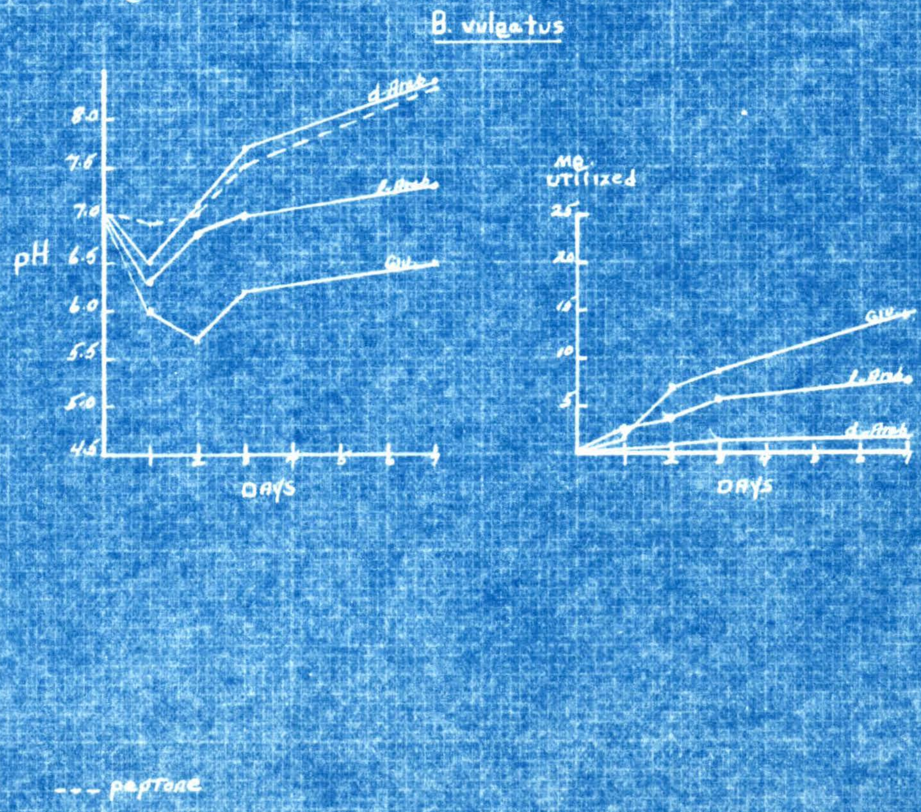
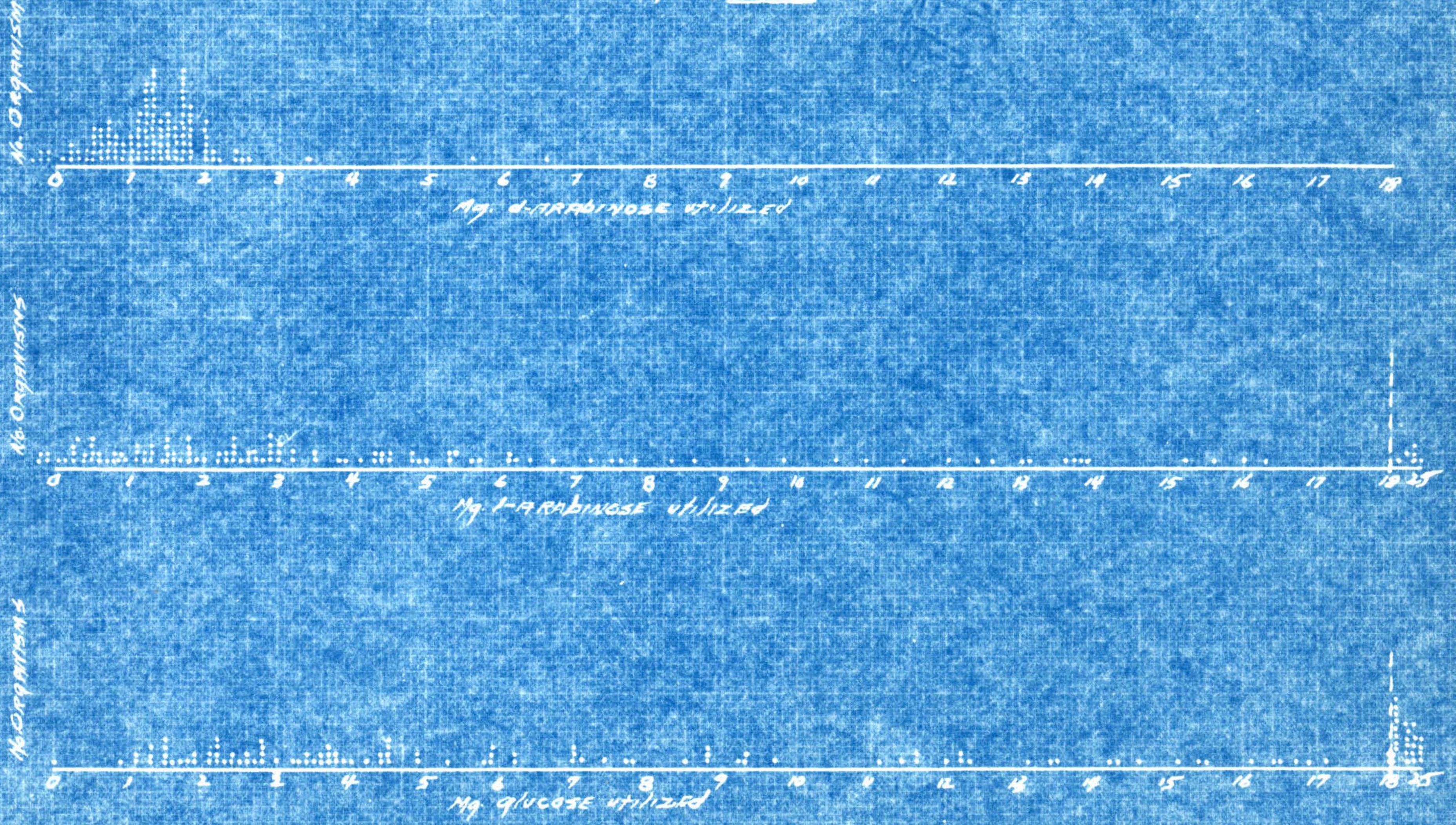

B. vulgatus
pH
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
d-Arab.
l-Arab.
Glu.
DAYS
Mg. utilized
25
20
15
10
5
Glu.
l-Arab.
d-Arab.
DAYS
--- peptone

Figure 131

Scattergram of Carbohydrate Utilized by the genus *Bacillus*



All carbohydrate media used were measured by automatic pipette to contain 25 mg. of a particular carbohydrate.

A summary of the results obtained have been plotted graphically in Figures 1-41. These graphs illustrate the species of Bacilli investigated and were selected to represent the action apparently characteristic of the strains of the various species under investigation.

Figure 42 represents the degree of utilization of carbohydrate by the various strains of Bacilli employed. A study of Figure 42 indicates that the utilization of glucose is such that it would be difficult, if not impossible to establish a boundary which could be called the dividing line between high and low glucose utilization. It has therefore been necessary to consider a purely arbitrary boundary at that amount of glucose utilized by at least 50 % of the strains investigated in order that glucose utilization might be more easily compared with the pH produced. According to this division it is possible when comparing pH with utilization to separate the Bacilli into six groups. The data for d- and l- arabinose do not permit the use of such groupings.

SUMMARY

The data obtained in these studies substantiate the following statements:

1. A pH of less than 6.0 produced by an actively growing culture of the genus Bacillus is indicative of positive glucose utilization by that organism. This pH is not indicative of the extent of glucose utilization.
2. No species of Bacillus tested produce a pH of less than 6.0 in peptone medium alone. Those Bacilli capable of lowering the pH of peptone medium to 6.0 produce pH values of less than 5.5 in glucose-peptone medium. It would not, therefore, be necessary to check the pH of peptone and glucose-

peptone media concurrently before designating taxonomic significance to a pH value of less than 6.0 produced in glucose-peptone medium by any of these Bacilli.

3. A few Bacilli tested produce a pH in the range 6.0 - 7.0 in glucose-peptone medium and at the same time utilize glucose. In these cases it is necessary to check the pH of peptone medium concurrently before assigning taxonomic significance to any value within this range. In this investigation peptone medium has been at least 0.6 pH units more alkaline than glucose-peptone medium inoculated with the same Bacillus and observed for a seven day incubation period.

4. Some Bacilli utilize less than 6.2 mg. of glucose which is not detectable by pH measurement of glucose-peptone medium even when compared to the pH of peptone medium run concurrently. The pH of these media either remains neutral or becomes alkaline during the entire incubation period.

5. These pH observations are in almost complete agreement with those of Coffee (4). The results obtained by Coffee were duplicated by the present author, in many instances to the recording of exact pH readings, although a two year period intervened.

6. Sixty-seven (67) strains of the Bacilli tested for their ability to utilize l-arabinose utilize less than 3mg. of this carbohydrate. The remainder utilized 3-25 mg. of this carbohydrate.

7. Regardless of the amount of l-arabinose utilized sixty-nine (69) strains of Bacilli, representing 21 species, produce the same pH in l-arabinose-peptone medium that they produce in peptone medium alone. The remainder, representing 20 species, produce pH values in l-arabinose-peptone which are at least 0.2 pH units more acidic, in the range below 6.8, than the pH they produced in peptone medium alone.

8. Eleven strains of Bacilli, representing 8 species, utilized more than 2 mg. of d-arabinose. The remainder used less than this amount.

9. Thirty-three strains of Bacilli representing 19 species, produce pH values in d-arabinose-peptone which are at least 0.2 pH units lower, in the range below 6.8 than the pH they produce in peptone medium alone. The remainder, 98 strains, representing 22 species, produce identical pH values in both d-arabinose-peptone and peptone media.

10. Practically all of the Bacilli tested were capable of using glucose and d- or l-arabinose to some extent in their metabolism. An acidic pH value can in no manner be indicative of the extent of this utilization. The order of decreasing utilizability of these carbohydrates is glucose, l-arabinose and d-arabinose.

11. The strains of the genus Bacillus used in this investigation show diversity in the extent of their ability to utilize the d- and l- forms of arabinose. It is therefore recommended that Bergey's Manual (1) change its designation of arabinose media from "arabinose" to d- Arabinose and l-Arabinose in order to avoid conflicting reports as to the ability of an organism to utilize carbohydrate.

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