# THE MAILLARD REACTION IN MICROBIOLOGICAL ASSAY

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This is to certify that the

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'The Maillard Reaction in Microbiclogical Assay'

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C. A. Hoffpauir
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# THE MAILLARD REACTION IN MICROBIOLOGICAL ASSAY

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# INTRODUCTION

In 1912 Maillard (1) showed that solutions of amino acids became brown when they were heated with reducing sugars. This is a reaction of great importance to the food industry and to many other industries which are concerned with materials of biological origin. The microbiological technique may be well suited to the study of the Maillard reaction since the preparation of bacterial media involves heating amino acids or simple proteins with a reducing sugar, usually glucose. Hill and Patton (2) have shown that a better growth response by Streptococcus faecalis occurred when graded levels of tryptophane were added to a tryptophane deficient medium if sucrose was used as the fermentable carbohydrate and heated with the medium than when glucose was so employed. A better growth response also occurred if glucose was autoclaved separately and added aseptically to the medium after both had cooled. In a later publication, Patton and Hill (3) demonstrated that the poorer growth response obtained when glucose was heated with the medium was due to the partial destruction of amino acids and vitamins of the B-complex group. The amount of destruction of amino acids in the process of heating with a reducing sugar was determined by subsequent microbiological assay of the test solution. A sucrose medium was used to prevent further browning. Significant destruction of L-tryptophane, D-L methionine, and L-lysine occurred. In addition, when L-tryptophane and D-glucose were heated in a solution buffered at pH 10 and in an unbuffered solution, greater browning, but less apparent loss

of L-trypotophane occurred in the buffered solution. The L-tryptophane was determined by microbiological assay. This suggests that in the reaction there occurs a partial destruction of tryptophane simultaneously with the production of a substance or substances which stimulate bacterial growth throughout the total period of growth. Orla-Jensen (4) demonstrated that activators necessary for the optimum growth of certain lactic-acid bacteria were produced by heating glucose with nitrogenous substances common to bacterial media Optimum growth was also produced when pyruvaldehyde, xylose, arabinose, or furfural was heated with the nitrogenous components of the medium. Acetaldehyde was shown to be without effect.

Hill, Patton, and Foreman (5) demonstrated by subsequent microbiological assay that when casein was heated with glucose and hydrolyzed enzymatically, only L-lysine, L-arginine, and L-tryptophane were appreciably inactivated.

In addition, McInroy, Murer, and Thiessen (6) autoclaved casein with glucose and found that it did not support the growth of weanling rats when employed as the sole source of protein in the ration. Thus, the work of these two groups illustrates the effect of the Maillard reaction on intact protein.

The results of these experiments do not seem to give a complete picture of the Maillard reaction as it occurs in bacterial media. The work presented here was undertaken in an attempt to determine the amino acids involved in the reaction, some of the products formed, and their effect on growth as a measure of the suitability of snythetic media in microbiological assay.

## EXPERIMENTAL METHODS

The organisms used in this study[1] were Streptococcus faecalis R(8043), Leuconostoc mesenteroides P-60 (8042), Lactobacillus arabinosus 17-5 (8014). The cultures were maintained as agar stabs in a modified Hunter (7) medium composed of 2% agar, 1% beef extract, 2% glucose, 1% peptone, 0.6% $Na_2HPO_4 \cdot 2H_2O$, pH 6.8 without adjustment. The medium was dispensed into tubes, plugged with cotton, and autoclaved for 15 minutes at 120° C. Duplicate or triplicate transfers were made every two or three weeks in this medium. One culture was always retained unused for subsequent transfer. The same medium, devoid of agar, was used for growing the inoculum. Usually, several transfers were made in the inoculum before use. In most cases, excellent growth was observed within ten hours.

Before use the inoculum medium containing the desired organism was centrifuged in a wind-shielded horizontal centrifuge for five or more minutes. The organisms were resuspended in 0.8% sterile saline and recentrifuged twice. The cells were then suspended in ten milliliters of sterile saline. One drop of this suspension from a sterile ten milliliter pipette was placed in each of the assay tubes. Care was taken so that the inoculum did not touch the sides of the tube.

The assay medium was that of Henderson and Snell (8). The composition is given in Table I.

1 The organisms were secured from the American Type Culture Collection. The numbers given in parentheses are those of the American Type Culture Collection.

TABLE I

COMPOSITION OF ASSAY MEDIUM

| Constituent | Amt/100 ml. of medium | | |
|---|---|---|---|
| glucose | 2.0 gm. | D-L alanine | 100 mgm. |
| sodium citrate | 2.0 | D-L aspartic acid | 100 |
| sodium acetate | 0.1 | L-glutamic acid | 100 |
| ammonium chloride | 0.3 | L-arginine hydrochloride | 20 |
| dipotassium phosphate | 0.5 | L-lysine dihydrochloride monohydrate | 20 |
| adenine sulfate[1] | 1.0 mgm. | L-histidine | 10 |
| guanine hydrochloride | 1.0 | D-L isoleucine | 20 |
| uracil | 1.0 | L-leucine | 10 |
| xanthine[2] | 1.0 | D-L methionine | 20 |
| magnesium sulfate heptahydrate | 80.0 | D-L phenylalanine | 20 |
| ferrous sulfate heptahydrate | 4.0 | L-proline | 10 |
| sodium chloride | 4.0 | D-L threonine | 20 |
| manganese sulfate heptahydrate | 16.0 | L-tyrosine | 10 |
| thiamin[3] | .100 | D-L valine | 20 |
| riboflavin | .100 | L-tryptophane | 10 |
| pyridoxal | .020 | L-cystine | 10 |
| calcium pantothenate | .100 | D-L serine | 20 |
| niacin | .100 | glycine | 10 |
| biotin[4] | .001 | water to make 100 ml. | |
| folic acid[5] | .001 | | |

1 Adenine sulfate, guanine hydrochloride, and uracil were dissolved in dilute hydrochloric acid so that one milliliter contained the amount needed for one hundred milliliters of medium and stored at 2-10° C.

2 Xanthine was dissolved in dilute ammonium hydroxide in a concentration such that one milliliter contained the amount needed for one hundred milliliters of media and stored at 2-10° C.

3 A mixture of the solid vitamins was made so that when 11.0 mgm was dissolved in dilute hydrochloric acid and made to twenty-five milliliters, one milliliter contained the amount needed for one hundred milliliters of medium. The solution was stored at 2-10° C. and kept for no longer than one week.

4 The biotin was received in five milliliter ampules which contained twenty-five mgm of biotin. This was diluted to twenty-five milliliters and stored at 2-10° C.

[5] Twenty milligrams of folic acid was dissolved in twenty milliliters of ethanol and stored at 6°C. A one to one hundred dilution of this was made just before use. One-tenth of a milliliter contained the amount needed for one hundred milliliters of medium.

[6] The amino acids were kept in a solution of a concentration such that twenty-five milliliters contained the amount necessary for one hundred milliliters of medium. The cystine and tyrosine were added first and dissolved in dilute hydrochloric acid and diluted before adding the other amino acids. Since most of the work presented here was done with graded levels of tryptophane, the tryptophane was dissolved and stored separately. The solutions were stored at 2-10°C.

The medium was usually diluted to 0.8 its final volume and pipetted automatically by the Cannon Automatic Dispenser (9). This dispenser operates on the principle of measuring a quantity of liquid by an interval of time since the volume of fluid which will flow through a given orifice will depend upon the length of time which it is allowed to flow if the temperature, viscosity, and pressure of the liquid remain constant. The device provides means by which the size of orifice, the pressure, and the duration of flow can be controlled to give any desired volume. Four milliliters of medium were pipetted into each tube. Other additions were made from pipettes.

Aluminum racks containing forty-eight tubes were used for incubation. During the early part of the work, stainless steel covers lined with gauzed-covered cotton wool were placed on the rack of tubes before autoclaving. Solutions from which additions were to be made to the medium after autoclaving were autoclaved at the same time as the medium. The conditions of autoclaving are stated with each experiment. The pipettes were autoclaved in an aluminum pipette can for fifteen or more minutes at 120°C. When additions were made to the medium after

autoclaving only one or two rows were uncovered at a time. The additions and inoculation were made rapidly to prevent contamination by spore-formers. A room as free from drafts as possible was washed down with a 1:1000 solution of mercuric chloride and was used when additions to the medium and inoculation were made.

During the later part of the work individual aluminum caps were used for the tubes. These proved to be very satisfactory. When sterile solutions of volatile compounds were to be added after autoclaving to the basal medium, they were sterilized by passage through a Jenkins filter. The Jenkins filter was used in connection with a filter flask, the latter containing a tube into which the tip of the filter could be placed. Pressure tubing and a calcium chloride tube filled with cotton wool were attached to the flask. The assembled apparatus was autoclaved for one and one-half hours or longer at 120°C. After filtration the tube was plugged with sterile cotton wool and taken from the flask.

During the early investigation the assay medium was incubated in an air incubator at 35-38°C. but it was found that the precision of repetitive assays was not as good as one should expect. Large water baths in which more exact temperature control and a shorter thermal adjustment could be made were built and subsequently used for incubation chambers.

The time at which growth response was determined is stated with each experiment. Growth response at specified times during the incubation period was determined by measuring the turbidity at 660 mmu in a Hellige photoelectric colorimeter using water as a blank. The optical density of uninoculated media was usually 0.008 at this wave length. At the end of

70-74 hours growth response was determined either by measuring turbidity, or in most cases by titration of the acid produced. An adaptation of the Gannon dispenser was used for titration. Electrometric titrations were made to pH 7.0 using calomel and quinhydrone half cells. The time of flow of 0.1 N sodium hydroxide was determined by a counter which was evaluated daily as to its volume equivalent.

## DATA AND RESULTS

The preliminary work consisted of determining the effect of the extent of autoclaving on the ability of various media containing glucose or an equimolar quantity of sucrose, added before or added after autoclaving, to support the growth of Strep. faecalis and L. arabinosus. The results are given in Table II. Six tubes were used for each condition under investigation.

Table II

EFFECT OF VARYING AUTOCLAVING PRESSURE AND CARBOHYDRATE USED ON THE GROWTH RESPONSE OF STREP. FAECALIS AND L. ARABINOSUS

| Carbohydrate | Time of Addition with respect to autoclaving | ‡ Pressure in lbs. | *Growth Response †Strep. faecalis | S.D. | †L. arabinosus | S.D. |
|---|---|---|---|---|---|---|
| glucose | before | 10 | 95 | 1 | 178 | 2 |
| glucose | after | 10 | 88 | 1 | 190 | 3 |
| sucrose | before | 10 | 96 | 1 | 187 | 2 |
| sucrose | after | 10 | 83 | 1 | 211 | 13 |
| glucose | before | 12½ | 112 | 3 | 206 | 6 |
| glucose | after | 12½ | 100 | 2 | 218 | 3 |
| sucrose | before | 12½ | 100 | 2 | 216 | 4 |
| sucrose | after | 12½ | 101 | 1 | 215 | 4 |
| glucose | before | 15 | 134 | 3 | 224 | 3 |
| glucose | after | 15 | 125 | 1 | 230 | 4 |
| sucrose | before | 15 | 124 | 2 | 237 | 2 |
| sucrose | after | 15 | 126 | 1 | 235 | 2 |

*Determined at 72 hours as counts of 0.1008 N NaOH; 224 counts = 10 ml.
†A washed saline suspension of a 16 hour culture was used for inoculum.
‡The time of autoclaving was 10 minutes.

Table II shows that growth response increased as the autoclaving pressure increased with both organisms used. However, Strep. faecalis and L. arabinosus differed in their reaction to the fermentable carbohydrate which was used. In general, the best growth response of Strep. faecalis

was obtained when glucose was added to the medium before autoclaving. The best growth response of L. arabinosus was obtained when sucrose was used as the fermentable carbohydrate or when glucose was added after autoclaving. A possible explanation for this would be the simultaneous destruction of essential nutrients and production of activators. It would seem that Strep. faecalis might respond to a smaller amount of the activator substances or substances than would L. arabinosus.

When the medium was taken from the autoclave as soon as the pressure had returned to atmospheric pressure, that is, after approximately fifteen minutes the results were of the same order as those given in Table II. If the medium was allowed to remain in the autoclave without disturbance until cool the growth response of Strep. faecalis was quite similar to that given in Table II. The growth response of L. arabinosus was somewhat different. The results obtained under such conditions are given in Table III. The time of autoclaving was ten minutes as before.

TABLE III

THE EFFECT ON BACTERIAL MEDIUM OF VARIOUS AUTOCLAVING PRESSURES FOLLOWED BY A PROLONGED COOLING PERIOD

| Carbohydrate | Time of addition in respect to autoclaving | Pressure in lbs. | * Growth Response | | | |
|---|---|---|---|---|---|---|
| | | | †Strep. faecalis | S.D. | †L. arabinosus | S.D. |
| glucose | before | 10 | 106 | 1 | 297 | 6 |
| glucose | after | 10 | 92 | 4 | 265 | 6 |
| sucrose | before | 10 | 101 | 2 | 274 | 1 |
| sucrose | after | 10 | 92 | 3 | 251 | 8 |
| glucose | before | 12½ | 111 | 1 | 292 | 4 |
| glucose | after | 12½ | 85 | 3 | 266 | 8 |
| sucrose | before | 12½ | 96 | 2 | 266 | 11 |
| sucrose | after | 12½ | 91 | 2 | 251 | 5 |
| glucose | before | 15 | 119 | 6 | 248 | 4 |
| glucose | after | 15 | 120 | 2 | 212 | 6 |
| sucrose | before | 15 | 117 | 3 | 211 | 1 |
| sucrose | after | 15 | 109 | 2 | 224 | 9 |

* Determined as counts of 0.1027 N NaOH; 224 counts = 10 ml.
† A washed saline suspension of a 16 hour culture was used for inoculum.

The growth response of Strep. faecalis was greatest when glucose was autoclaved with the medium at the lower pressure. At 15 pounds pressure no appreciable difference is noted. Here L. arabinosus also showed the greatest growth response when glucose was autoclaved with the medium. The longer heating period may have produced enough of an activator or activators to produce this effect.

Considerable time was spent attempting to set up a standard curve for lysine using sucrose in the medium. For many experiments, no appreciable production of acid was noted. Sucrose and glucose respectively were added in triplicate before and after autoclaving and the results were noted in Table IV.

TABLE IV

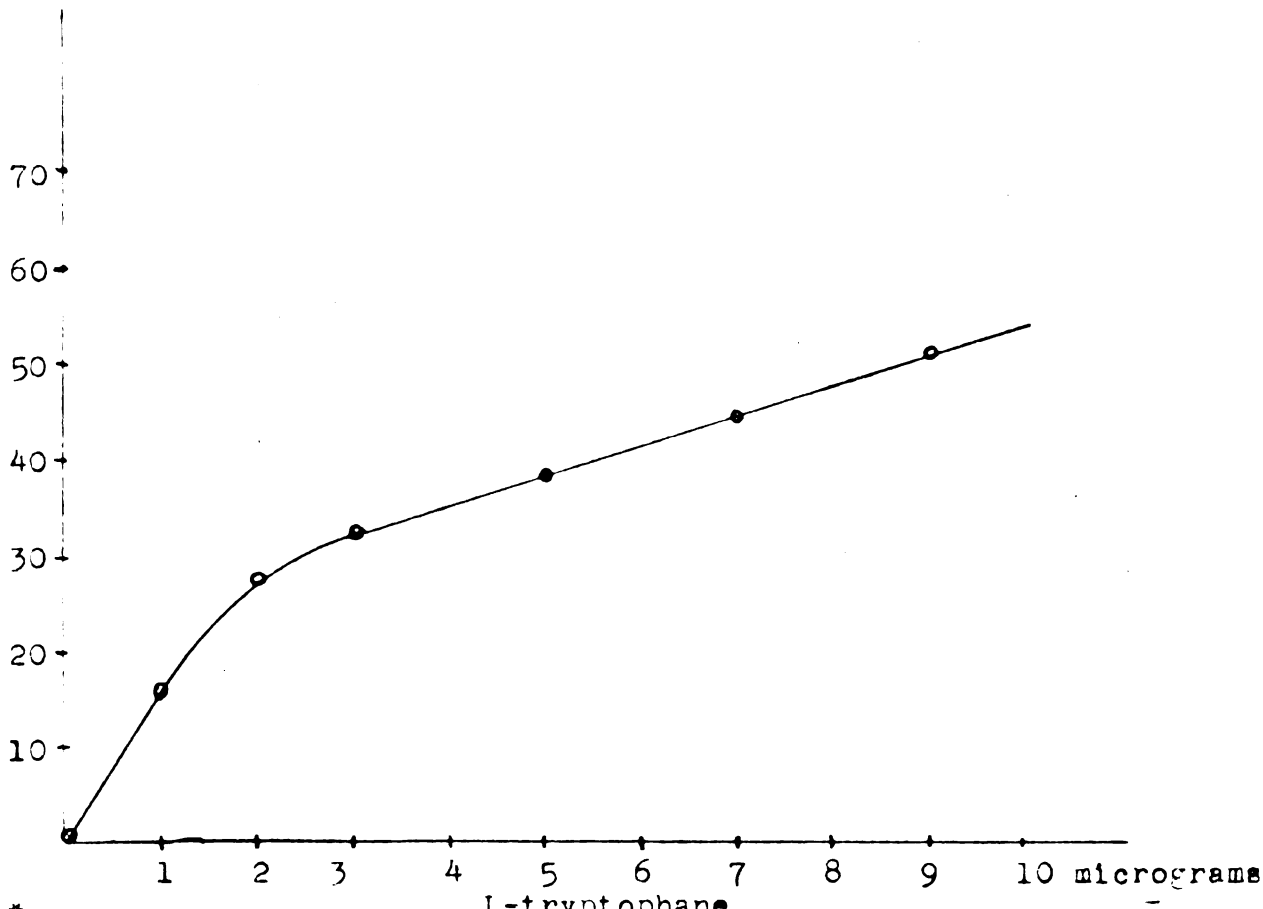
EFFECT OF THE MAILLARD REACTION ON LEUC. MESENTEROIDES

| | Growth Response of Leuc. Mesenteroides at 24 hrs. | 48 hrs. | 72 hrs. |
|---|---|---|---|
| 1. sucrose added to medium before autoclaving at 5 minutes at 120°C. | .007 ± .0007 | .008 ± .002 | 0 ± .00 |
| 2. sucrose added to medium after autoclaving | .010 ± .0007 | .014 ± .002 | 0 ± .00 |
| 3. glucose added to medium before autoclaving | .019 ± .007 | .332 ± .058 | 75.7 ± 2.92 |
| 4. glucose added to medium after autoclaving | .010 ± .000 | .320 ± .046 | 69.7 ± 3.54 |

At 24 and 48 hours the growth was determined in terms of density at 660 mu.
At 72 hours growth was determined as counts of 0.0998 N NaOH where 139 counts = 10 ml.
It is evident that the Maillard reaction produced something which was necessary for the growth of Leuc. Mesen. on what had previously been considered a complete medium.

FIGURE 1

STANDARD GROWTH RESPONSE CURVE FOR STREP. FAECALIS*




*Determined as counts of 0.0998N. NaOH; 197 counts = 10 ml. Inoculation was made with a washed saline suspension of a 16 hr. culture.

As discussed in the introduction, L-tryptophane is believed to be partially destroyed when heated with glucose. An attempt was made to determine the extent of tryptophane destruction when only L-tryptophane and D-glucose were heated together in various concentrations and for various periods of time. Accordingly 40 mg of L-tryptophane and 2.0 g. of D-glucose in 50 milliliters of water were adjusted to pH 7.0 and autoclaved for one hour at 120° C.

From this solution were made dilutions of 1:100; 1:150; 1:200, representing initially 8, 6, and 4 micrograms (mu g.) of L-tryptophane per milliliter. It was felt at this time that the concomitant addition of D-glucose (approximately 400 to 200 mu g.) would have insignificant effects upon the growth response. Subsequent microbiological assay in quadruplicate of these solutions was carried out using the usual Henderson and Snell medium with sucrose as the fermentable carbohydrate to prevent further browning. The medium (4 ml/tube) and the diluted solutions were autoclaved for fifteen minutes at 120° C. The results are presented in Table V and Figure 1.

TABLE V

ASSAY OF TRYPTOPHANE - GLUCOSE MIXTURE

| Mu. g. of tryptophane | Mu. g. of tryptophane from test solution | *Growth Response at 72 hrs. of †Strep. faecalis | S.D. |
|---|---|---|---|
| 0 | | 0 | 0 |
| 1 | | 16 | 2 |
| 2 | | 28 | 1 |
| 3 | | 33 | 1 |
| 5 | | 38 | 1 |
| 7 | | 44 | 0 |
| 9 | | 51 | 3 |
| | 4 | 46 | 2 |
| | 6 | 67 | 1 |
| | 8 | 67 | 0 |

* Determined as counts of 0.0998 N NaOH; 197 counts = 10 ml.
† A washed saline suspension of a 16 hour culture was used as inoculum.

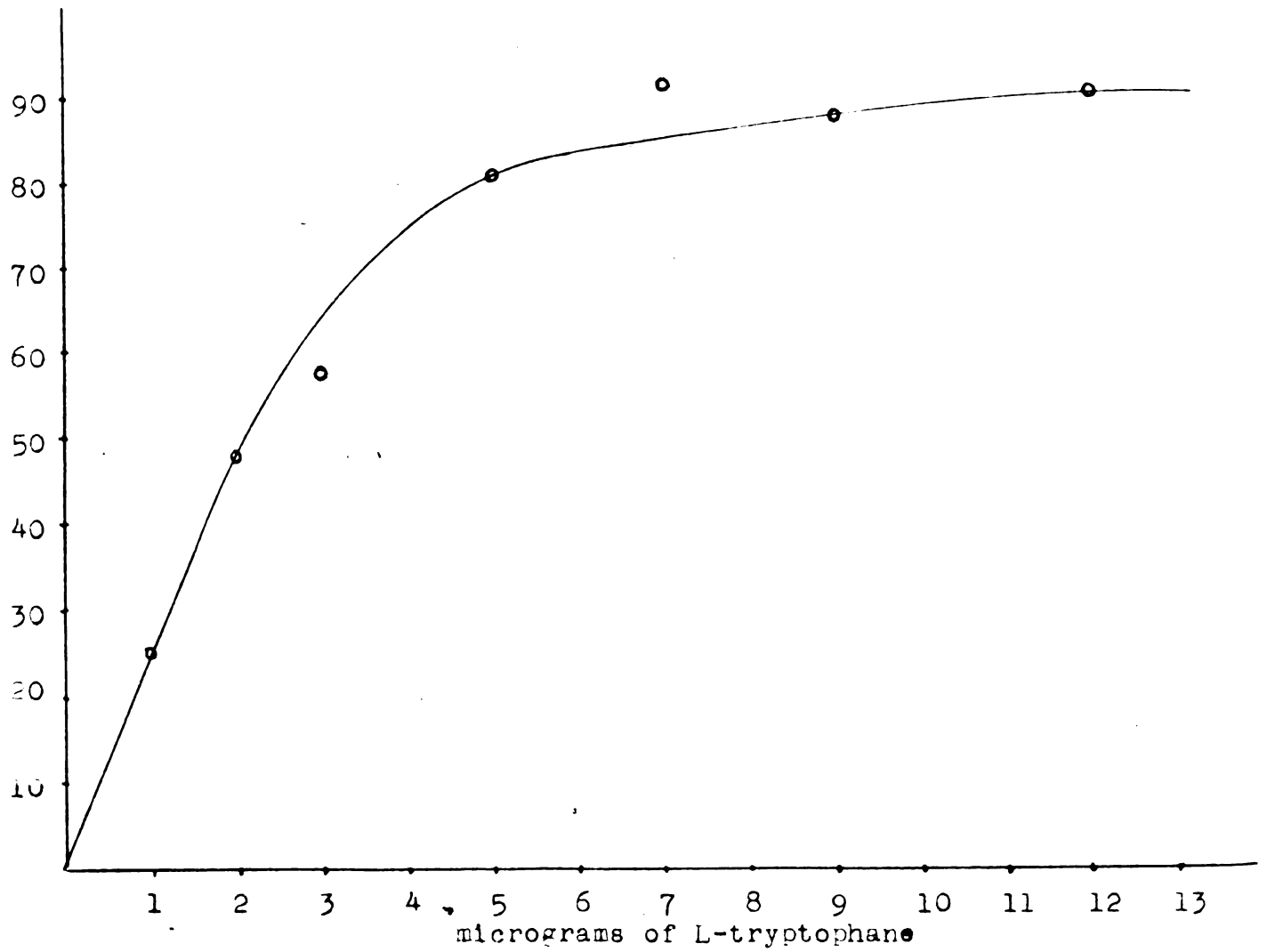
These data indicate that the test organism did not respond to the sample in the same manner as it did to the pure standard and are evidence against the validity of the assay.

Thompson and Kirby (10) have reported a method for the assay of lysine in a urine which contained material toxic to the test organism. For this method a standard curve was employed to determine whether or not the values obtained in the assay represented a linear dose-response curve. The technique consisted of adding 6 and 12 mu. g. of L-lysine respectively to 1, 2, and 3 milliliters of urine in the assay medium. The growth response to the urine, and the growth response to each addition was found. By dividing the growth response of the urine by the average growth response to each addition and multiplying by the increase of each addition of lysine over the previous the concentration of lysine in the urine was determined. It was suggested that this might be a general method for assaying substances in the presence of toxic materials.

In order to test this approach in the presence of possible stimulatory substances 1, 2, and 3 mu. g. (initial concentration) of L-tryptophane from the sterile L-tryptophane-D-glucose solutions were added aseptically to tubes containing respectively, 0, 1, and 2 mu. g. of L-tryptophane in the otherwise complete Henderson and Snell medium. The standard response curve is given in Figure 2. The results of the assay appear in Table VI.

FIGURE 2

STANDARD GROWTH RESPONSE CURVE FOR STREP. FAECALIS*




* Determined as counts of 0.0998N NaOh; 212 counts = 10 ml.
Inoculation was made with a washed saline suspension of a 16 hour cul

TABLE VI

DETERMINATION OF TRYPTOPHANE CONTENT OF TRYPTOPHANE-GLUCOSE SOLUTION BY THE METHOD OF THOMPSON AND KIRBY

| mu g. L-tryptophane | mu g. L-tryptophane of test solution | *Growth Response of † Strep. faecalis | S.D. |
|---|---|---|---|
| 0 | 0 | 0 | 0 |
| 0 | 1 | 32 | 1 |
| 0 | 2 | 53 | 1 |
| 0 | 3 | 66 | 1 |
| 1 | 1 | 56 | 1 |
| 1 | 2 | 61 | 2 |
| 1 | 3 | 79 | 2 |
| 2 | 1 | 79 | 2 |
| 2 | 2 | 74 | 1 |
| 2 | 3 | 89 | 2 |

*Determined after 72 hours incubation as counts of 0.0998 N NaOH 212 counts = 10 ml.

†A washed saline suspension of a 16 hour culture was used as inoculum.

Calculations were made according to the method of Thompson and Kirby. Values above 75 counts were not used since this was considered the limit of the linear dose-response portion of the standard curve. The calculations follow:

Response due to 1 mu.g. of L-tryptophane of test solution = 32-0 = 32

Response due to 1 mu. g. of L-tryptophane = 56-32 = 24

Response due to 1 mu. g. of L-tryptophane = 79-56 = 23

Average response due to 1 mu. g. of L-tryptophane = $\frac{24 + 23}{2}$ = 23.5

Tryptophane content of test solution = 1 x $\frac{32}{23.5}$ = 1.36 mu.g.

Response due to 2 mu g. of L-tryptophane of test solution = 53-0 = 53

Response due to 1 mu g. of L-tryptophane = 61-53 = 8

Response due to 1 mu g. of L-tryptophane = 74-61 = 13

Average response due to 1 mu g. of L-tryptophane = $\frac{8 + 13}{2}$ = 10.5

Tryptophane content of test solution = 1 x $\frac{53}{10.5}$ = 5.05 mu g.

These calculations illustrate that in most cases the growth response is not in proportion to the probable tryptophane content of the reaction mixture.

Another method which can be used for calculating the dose response in an assay is the five point method of Emmens (11). A common point, often an extrapolated zero point is used in the calculation. Calculations are made in this manner:

To simplify calculations T-G is used to mean the L-tryptophane D-glucose test solution. The number used as a prefix indicates the tryptophane content in micrograms which was originally present.

For the standards (s)

| Response to: | | |
|---|---|---|
| 1 T-G | 1 T-G plus 1 mu g. of L-tryptophane | 1 T-G plus 2 mu g. of L-tryptophane |
| 32 | 56 | 79 |

For the unknown (u)

| 1 T-G | 2 T-G | 3 T-G |
|---|---|---|
| 32 | 53 | 66 |

$L_s = \bar{Y}_o + \bar{Y}_{s_2} - 2\,\bar{Y}_{s_1} = 32 + 79 - 2(56) = -1$

$L_u = \bar{Y}_o + \bar{Y}_{u_2} - 2\,\bar{Y}_{u_1} = 32 + 66 - 2(53) = -8$

$b_s = \frac{(\bar{Y}_{s_2} - \bar{Y}_o)}{2} + \frac{(6\,L_u - L_s)}{70} = \frac{79-32}{2} + \frac{6(-8)-(-1)}{70} = 22.8$

$b_u = \frac{(\bar{Y}_{u_2} - \bar{Y}_o)}{2} + \frac{(6\,L_s - L_u)}{70} = \frac{(66-32)}{2} + \frac{6(-1)-(-8)}{70} = 17.0$

FIGURE 3

GROWTH RESPONSE OF L. ARABINOSUS AT 48 HOURS*

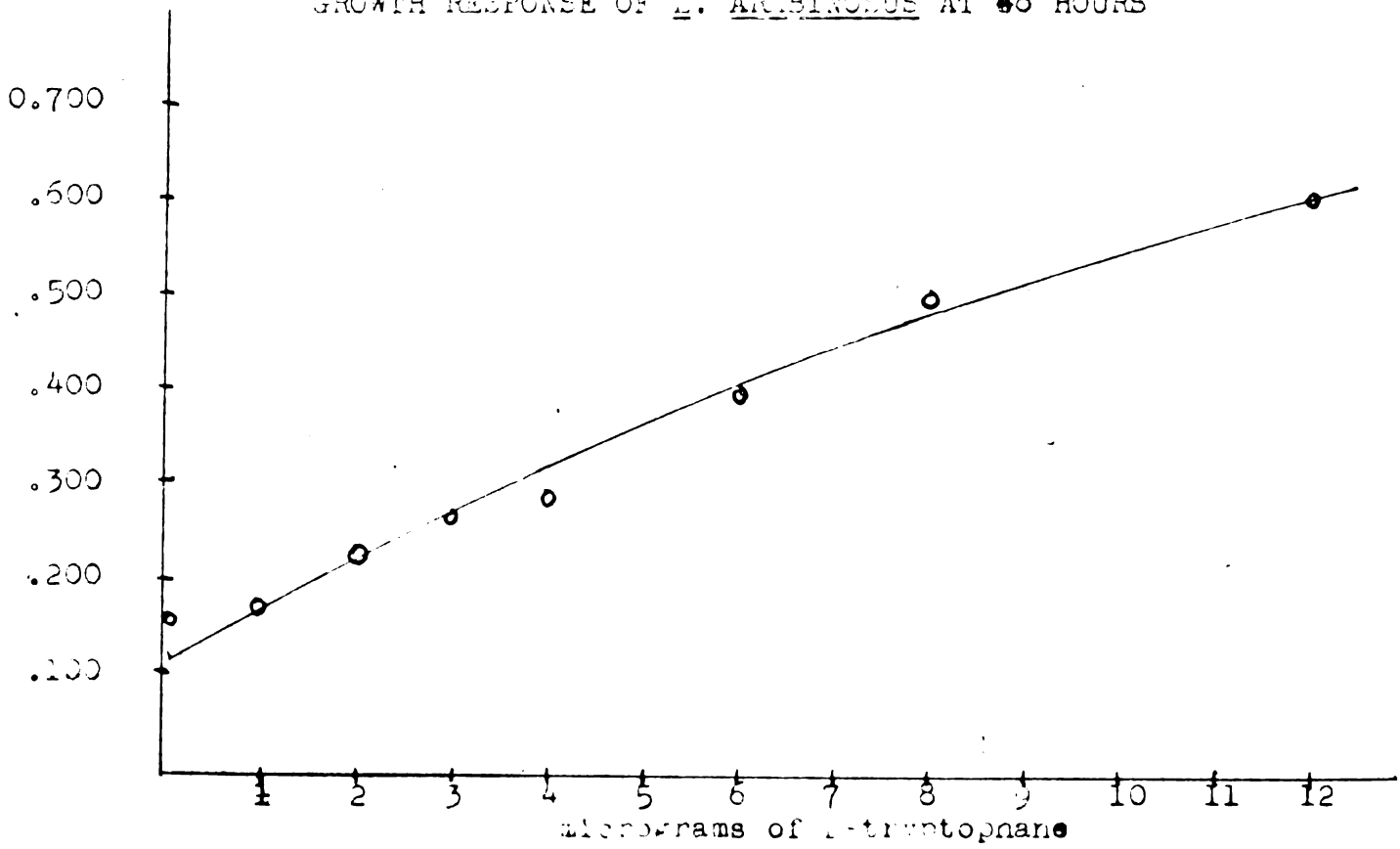
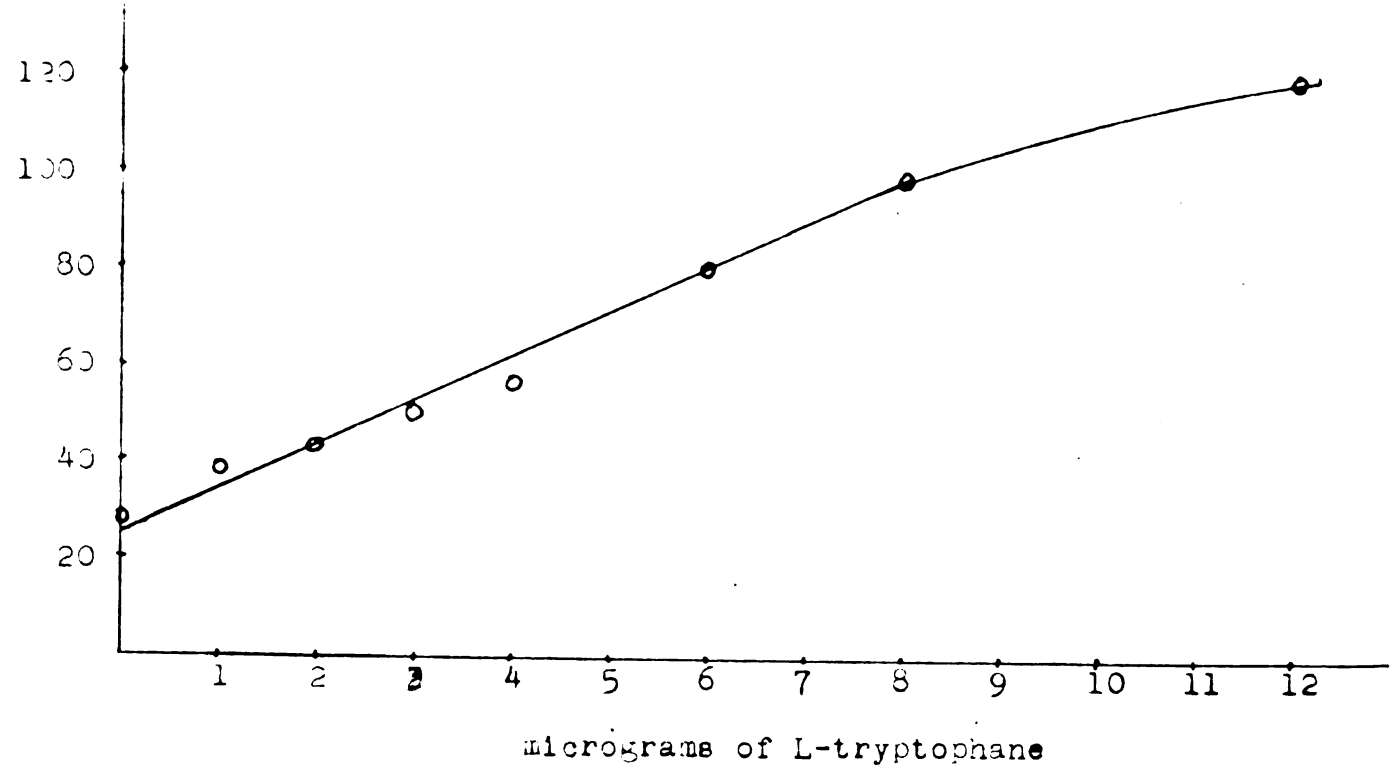



FIGURE 4

GROWTH RESPONSE OF L. ARABINOSUS AT 72 HOURS*




* Determined as turbidity at 660mμ mu at 48 hours, and as counts of 0.0998N NaOH; 128 counts= 10 ml.

Inoculation: washed saline suspension of

$$R = \frac{bu}{bs} = \frac{17.0}{22.8} = 0.74 \text{ mu g.}$$

This value is undoubtedly a truer indication of the amount of tryptophane actually present.

$L_s$ and $L_u$ measure the departure of the slopes from linearity

$b_s$ and $b_u$ are respectively the slopes of the lines of the standard and unknown.

$\bar{Y}$ = mean of values for a particular response.

R = amount of the substance which is present.

This experiment was repeated using L. arabinosus as the test organism. The medium was that of Henderson and Snell deficient in tryptophane. The medium, a tryptophane solution, and the T-G solutions were autoclaved for five minutes at 120° C. Additions of tryptophane and T-G were made aseptically after autoclaving. The results appear in Table VII, and the standard dose response curves are given in Figures 3 and 4.

TABLE VII

TRYPTOPHANE ASSAY OF TEST SOLUTION USING L. ARABINOSUS

| mu g. tryptophane | T-G | *Growth Response of †L. arabinosus at 24 hours | at 48 hours | at 72 hours |
|---|---|---|---|---|
| 0 | 0 | 0.075 | 0.157 | 30 |
| 0 | 1 | .155 | .198 | 53 |
| 0 | 2 | .194 | .251 | 56 |
| 0 | 3 | .217 | .277 | 56 |
| 1 | 1 | .200 | .247 | 56 |
| 1 | 2 | .244 | .303 | 64 |
| 1 | 3 | .247 | .324 | 70 |
| 2 | 1 | .226 | .289 | 65 |
| 2 | 2 | .272 | .349 | 73 |
| 2 | 3 | .300 | .376 | 78 |

*Measured as turbidity at 660 m mu, using water as a blank for 24 hour and 48 hour period and as counts of 0.0998 N NaOH; 128 counts = 10 ml. for 72 hour period.
†A washed saline suspension of a 16 hour culture was used for inoculum.

Calculations were made according to the method of Thompson and Kirby (10).

For 24 hour period of growth:

Response due to 1 T-G = 0.155 - 0.075 = 0.080

Response due to 1 mu g. of tryptophane = 0.200-0.155 = 0.045

Response due to 1 mu g. of tryptophane = 0.226-0.200 = 0.026

Average response due to 1 mu g. of tryptophane = $\frac{0.045 + 0.026}{2}$ = 0.0355

Tryptophane content of 1 T-G = $\frac{0.080}{0.0355}$ = 2.25 mu g.

Response due to 2 T-G = 0.194-0.075 = 0.119

Response due to 1 mu g. of tryptophane = 0.244-0.194 = 0.050

Response due to 1 mu g. of tryptophane = 0.272-0.244 = 0.028

Average response to 1 mu g. of tryptophane = $\frac{0.050 + 0.028}{2}$ = 0.039

Tryptophane content of 2 T-G = $\frac{0.119}{0.039}$ = 3.06 mu g.

Response due to 3 T-G = 0.217-0.075 = 0.142

Response due to 1 mu g. of tryptophane = 0.247-0.217 = 0.030

Response due to 1 mu g. of tryptophane = 0.300-0.247 = 0.053

Average response to 1 mu g. of tryptophane - $\frac{0.030+0.053}{2}$ - 0.0415

Tryptophane content of 3 T-G = $\frac{0.142}{0.0415}$ = 3.43 mu g.

For 48 hour period of growth:

Response due to 1 T-G = 0.198-0.157 = 0.041

Response due to 1 mu g. of tryptophane = 0.247-0.198 = 0.049

Response due to 1 mu g. of tryptophane = 0.289-0.247 = 0.045

Average response to 1 mu g. of tryptophane = $\frac{0.049 + 0.045}{2}$ = 0.047

Tryptophane content of 1 T-G = $\frac{0.041}{0.047}$ = 0.87 mu g.

Response due to 2 T-G = 0.251-0.157 = 0.094

Response due to 1 mu g. of tryptophane = 0.303-0.251 = 0.052

Response due to 1 mu g. of tryptophane = 0.349-0.303 = 0.046

Average response to 1 mu g. of tryptophane = $\frac{0.052 + 0.046}{2}$ = 0.049

Tryptophane content of 2 T-G = $\frac{0.094}{0.049}$ = 1.92 mu g.

Response due to 3 T-G = 0.277-0.157 = 0.120

Response due to 1 mu g. of tryptophane = 0.324-0.277 = 0.047

Response due to 1 mu g. of tryptophane = 0.376-0.324 = 0.052

Average response to 1 mu g. of tryptophane = $\frac{0.047 + 0.052}{2}$ = 0.0495

Tryptophane content of 3 T-G = $\frac{0.120}{0.0495}$ = 2.41 mu g.

For the 72 hour period of growth:

Response due to 1 T-G = 53-30 = 23

Response due to 1 mu g. of tryptophane = 56-53 = 3

Response due to 1 mu g. of tryptophane = 65-56 = 9

Average response due to 1 mu g. of tryptophane = $\frac{3 + 9}{2}$ = 6.0

Tryptophane content of 1 T-G = $\frac{23}{6}$ = 3.83 mu g.

Response due to 2 T-G = 56-30 = 26

Response due to 1 mu g. of tryptophane 64-56 = 8

Response due to 1 mu g. of tryptophane = 73-64 = 9

Average response to 1 mu g. of tryptophane = $\frac{8 + 9}{2}$ = 8.5

Tryptophane content of 2 T-G = $\frac{26}{8.5}$ = 3.06 mu g.

Response due to 3 T-G = 56-30 = 26

Response due to 1 mu g. of tryptophane = 70-56 = 14

Response due to 1 mu g. of tryptophane = 78-70 = 8

Average response due to 1 mu g. of tryptophane = $\frac{1448}{2}$ = 11.0

Tryptophane content of 3 T-G = $\frac{26}{11.0}$ = 2.36 mu g.

These results show that a very marked stimulation occurred at 24 hours. By the end of the 48 hour growth period the stimulation had disappeared to a considerable extent. The results at 72 hours seem higher than expected. This resulted because the dose response to each added mu g. of tryptophane was small.

In the next experiment reaction mixtures of 40 mg. L-tryptophane and 2.0 g. of D-glucose and 400 mg L-tryptophane and 2.0 g. of D-glucose in 50 milliliters of water were adjusted to a pH 7.0 and autoclaved for four hours at 120° C. In addition 400 mg. of L-tryptophane and 2.0 g. of D-glucose in 50 milliliters of water were adjusted to pH 7.0 and autoclaved for one hour at 120° C. Dilutions of the solutions were made so that one milliliter represented 1, 2, or 3 mu. g. of tryptophane originally present. The solutions were assayed for tryptophane content. In this case all additions to the medium were made before autoclaving at 120° C. for five minutes. The results appear in Table VIII.

TABLE VIII

COMPARISON OF VARIOUS TEST SOLUTIONS

| T-G | Test Solution | *Growth Response of Strep. faecalis† | | |
|---|---|---|---|---|
| | | 24 hours | 48 hours | 72 hours |
| 1 | 40 mg. L-tryptophane | 0.070 | 0.087 | 37 |
| 2 | 2.0 g. D-glucose | 0.091 | 0.123 | 58 |
| 3 | heated for 4 hrs. | 0.111 | 0.150 | 74 |
| 1 | 400 mg. L-tryptophane | 0.063 | 0.093 | 38 |
| 2 | 2.0 g. D-glucose | 0.093 | 0.128 | 64 |
| 3 | heated for 1 hour | 0.126 | 0.160 | 81 |
| 1 | 400 mg. L-tryptophane | 0.065 | 0.091 | 36 |
| 2 | 2.0 g. D-glucose | 0.096 | 0.128 | 58 |
| 3 | heated for 4 hours | 0.128 | 0.157 | 78 |

*Determined as turbidity at 24 and 48 hours at 660 m mu. using water as a blank and at 72 hours as counts of 0.0980 N NaOH; 262 counts = 10 ml.

†A washed saline suspension of a 16 hour culture was used as inoculum.

The best growth response was observed with the solution which contained 400 mg. of tryptophane and 2.0 g. of D-glucose and had been heated together for one hour despite the fact that the dilution was ten times greater. Therefore, this ratio and time of heating was chosen for further investigation.

The next experiment was undertaken to determine the effect of making the additions of the tryptophane and the test solution (T-G) before or after autoclaving. The results appear in Table IX and the standard growth response curves appear in Figures 5 and 6.

FIGURE 5

GROWTH RESPONSE OF STREP. FAECALIS AT 18 HOURS*

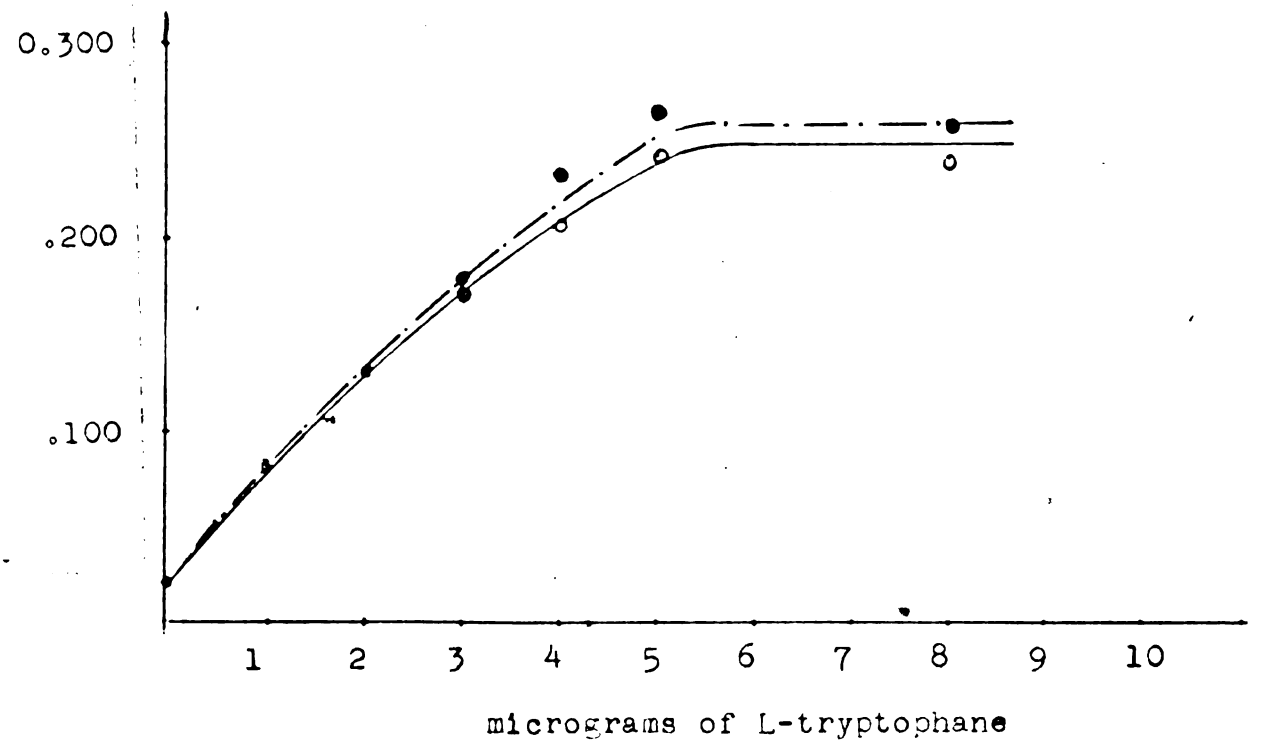
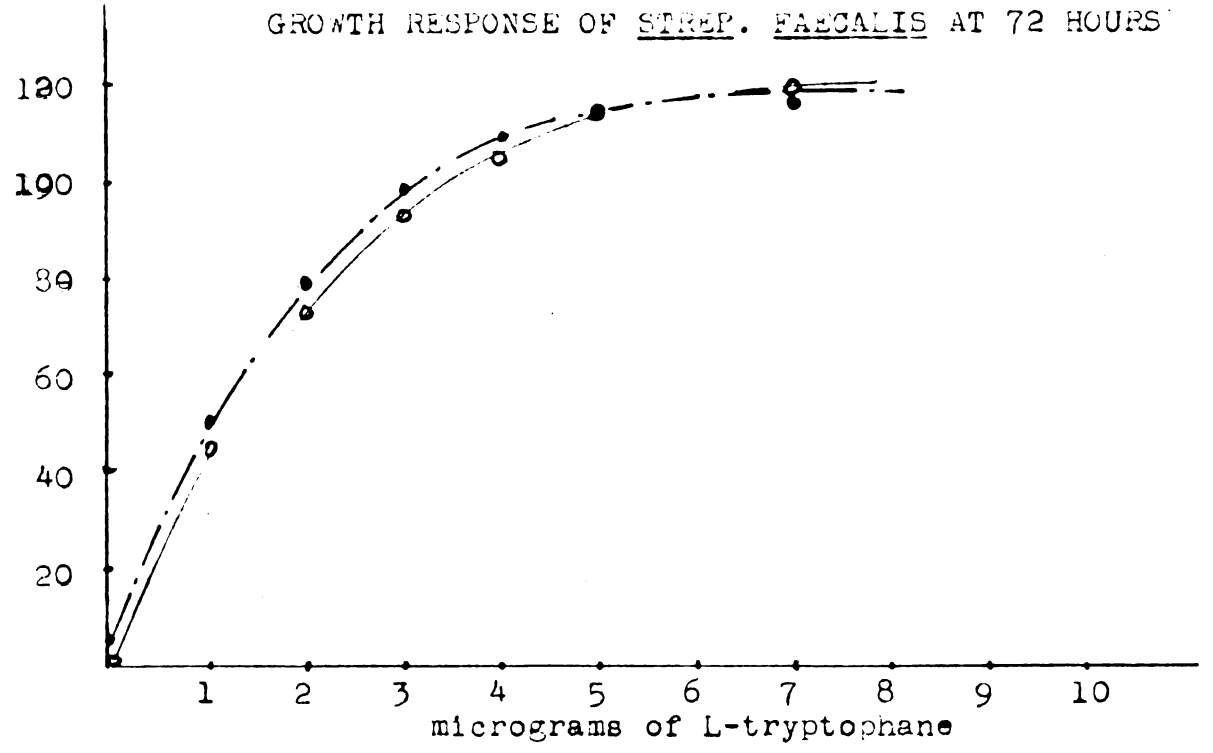



FIGURE 6

GROWTH RESPONSE OF STREP. FAECALIS AT 72 HOURS




——— tryptophane autoclaved with media

—·—·— tryptophane added after autoclaving

* Determined as turbidity at 660 m. mu at 18 hours, and as counts of 0.09 NaOH at 72 hours; 333 counts = 10 ml.

Inoculation was made with a washed saline suspension of a 16 hour cultu

TABLE IX

EFFECT OF MAKING ADDITIONS BEFORE OR AFTER AUTOCLAVING

| mu. g. of tryptophane | T-G | *Growth Response of Strep. faecalis† at 18 hours | S.D. | at 72 hours | S.D. |
|---|---|---|---|---|---|
| Additions Before Autoclaving | | | | | |
| 0 | 0 | 0.018 | 0.001 | 0 | 0 |
| 0 | 1 | 0.083 | .003 | 42 | 3 |
| 0 | 2 | .149 | .001 | 70 | 1 |
| 0 | 3 | .189 | .002 | 92 | 1 |
| 1 | 1 | .133 | .002 | 74 | 1 |
| 1 | 2 | .197 | .002 | 93 | 2 |
| 1 | 3 | .237 | .004 | 103 | 1 |
| 2 | 1 | .176 | .005 | 94 | 1 |
| 2 | 2 | .247 | .003 | 108 | 1 |
| 2 | 3 | .258 | .002 | 114 | 3 |
| Additions After Autoclaving | | | | | |
| 0 | 0 | 0.017 | .001 | 3 | 0 |
| 0 | 1 | 0.078 | .001 | 43 | 1 |
| 0 | 2 | .129 | .001 | 70 | 0 |
| 0 | 3 | .203 | .007 | 93 | 1 |
| 1 | 1 | .138 | .001 | 74 | 0 |
| 1 | 2 | .177 | .001 | 91 | 1 |
| 1 | 3 | .226 | .001 | 107 | 2 |
| 2 | 1 | .186 | .003 | 92 | 1 |
| 2 | 2 | .229 | .007 | 104 | 1 |
| 2 | 3 | .238 | .015 | 113 | 1 |

*Determined at 18 hours as turbidity at 660 m mu using water as a blank; at 72 hours as counts of 0.0992 N NaOH; 233 Counts = 10 ml.
†A washed saline suspension of a 16 hour culture was used as inoculum.

The amount of L-tryptophane present appears much greater than the actual content of the test solutions (T-G) both when additions were made before or after autoclaving. The amount of L-tryptophane present was calculated by the method of Thompson and Kirby (10) as follows:

At 18 hours when additions were made before autoclaving:

Response due to 1 T-G = 0.083-0.018 = 0.065
Response due to 1 mu g. of L-tryptophane = 0.133-0.083 = 0.050
Response due to 1 mu g. of L-tryptophane = 0.176-0.133 = 0.043

Average response to 1 mu g. of L-tryptophane = $\frac{0.050 + 0.043}{2}$ = 0.0465

Tryptophane content of 1 T-G = $\frac{0.065}{0.0465}$ = 1.40 mu g.

Response due to 2 T-G = 0.149-0.018 = 0.131

Response due to 1 mu g. of L-tryptophane = 0.197-0.149 = 0.048

Response due to 1 mu g. of L-tryptophane = 0.247-0.197 = 0.050

Average response to 1 mu g. of L-tryptophane = $\frac{0.048 + 0.050}{2}$ = 0.049

Tryptophane content of 2 T-G = $\frac{0.131}{0.049}$ = 4.06 mu g.

Response due to 3 T-G = 0.189-0.017 = 0.171

Response due to 1 mu. g. of L-tryptophane = 0.237-0.189 = 0.048

Response due to 1 mu g. of L-tryptophane = 0.258-0.237 = 0.021

Average response to 1 mu g. of L-tryptophane = $\frac{0.048 + 0.021}{2}$ = 0.0345

Tryptophane content of 3 T-G = $\frac{0.171}{0.0345}$ = 4.95 mu g.

At 18 hours when additions were made after autoclaving:

Response due to 1 T-G = 0.078-0.017 = 0.061

Response due to 1 mu g. of L-tryptophane = 0.138-0.078 = 0.060

Response due to 1 mu g. of L-tryptophane = 0.186-0.138 = 0.052

Average response to 1 mu g. of L-tryptophane = $\frac{0.060+0.052}{2}$ = 0.056

Tryptophane content of 1 T-G = $\frac{0.061}{0.056}$ = 1.09 mu g.

Response due to 2 T-G = 0.129 - 0.017 = 0.112

Response due to 1 mu g. of L-tryptophane = 0.177-0.129 = 0.058

Response due to 1 mu g. of L-tryptophane = 0.229-0.177 = 0.052

Average response to 1 mu g. of L-tryptophane = $\frac{0.058 - 0.052}{2}$ = 0.055

Tryptophane content of 2 T-G = $\frac{0.112}{0.055}$ = 2.04 mu g.

Response due to 3 T-G = 0.203-0.017 = 0.186

Response due to 1 mu g. of L-tryptophane = 0.226-0.203 = 0.023

Response due to 1 mu g. of L-tryptophane = 0.238-0.226 = 0.012

Average response to 1 mu. g. of L-tryptophane = $\frac{0.023 + 0.012}{2}$ = 0.018

Tryptophane content of 3 T-G = $\frac{0.186}{0.018}$ = 10.33 mu g.

At 72 hours when additions were made before autoclaving:

Response due to 1 T-G = 42-0 = 42

Response due to 1 mu g. of L-tryptophane = 74-42 = 32

Response due to 1 mu g. of L-tryptophane = 94-74 = 20

Average response to 1 mu g. of L-tryptophane = $\frac{32 + 20}{2}$ = 26.0

Tryptophane content of 1 T-G = $\frac{42}{26}$ = 1.62 mu g.

Response due to 2 T-G = 70-0 = 70

Response due to 1 mu g. of L-tryptophane = 93-70 = 23

Response due to 1 mu g. of L-tryptophane = 108-93 = 15

Average response to 1 mu g. of L-tryptophane = $\frac{23 + 15}{2}$ = 19.0

Tryptophane content of 2 T-G = $\frac{70}{19}$ = 3.68 mu g.

The growth response to 3 T-G plus tryptophane additions is above the linear portion of the curve, so valid calculations for 3 T-G cannot be made.

At 72 hours when additions were made after autoclaving:

Response due to 1 T-G = 43-3 = 40

Response due to 1 mu g. of L-tryptophane = 74-43 = 31

Response due to 1 mu g. of L-tryptophane = 92-74 = 18

Average response to 1 mu g. of L-tryptophane = $\frac{31 + 18}{2}$ = 24.5

Tryptophane content of 1 T-G = $\frac{40}{24.5}$ = 1.57 mu g.

Response due to 2 T-G = 70-3 = 67

Response due to 1 mu g. of L-tryptophane = 91-70 = 19

Response due to 1 mu g. of L-tryptophane = 104-91 = 13

Average response to 1 mu g. of L-tryptophane = $\frac{19 + 13}{2}$ = 16

Tryptophane content of 2 T-G = $\frac{67}{16}$ = 4.18 mu g.

Here again valid calculations can not be made on the series of tubes containing 3 T-G plus tryptophane additions since the growth response is above the linear portion of the standard curve.

The apparent tryptophane content of the L-tryptophane-D-glucose (T-G) test solution was considerably greater than the amount originally present. This is true when additions of L-tryptophane and the test solution are made either before or after autoclaving. The calculated tryptophane values are slightly less when these additions are made after autoclaving than when the additions are made before autoclaving. In general, the growth response to the addition of the second microgram of L-tryptophane to tubes which contain the test solution is not as great as the growth response to the first added microgram of L-tryptophane. The presence of a stimulatory factor or factors even in the

greatest dilution (1:8,000) makes an accurate assay of the test solution impossible with the synthetic medium of Henderson and Snell.

Calculations were also made according to the method of Emmens (11).

At the end of 24 hours:

For the standards (s):

| 1 T-G | 1 T-G plus 1 mu g of L-tryptophane | 1 T-G plus 2 mu g. of L-tryptophane |
|---|---|---|
| Addition made before autoclaving 0.078 | 0.138 | 0.186 |
| Addition made after autoclaving 0.083 | 0.133 | 0.176 |

For the unknown (u)

| 1 T-G | 2 T-G | 3 T-G |
|---|---|---|
| Additions made before autoclaving: 0.078 | 0.129 | 0.203 |
| Additions made after autoclaving: 0.083 | 0.149 | 0.189 |

Calculations based on additions made after autoclaving:

$L_s = 0.078 + 0.186 - 2(0.138) = 0.012$

$L_u = 0.078 + 0.203 - 2(0.129) = 0.043$

$b_s = \frac{0.186-0.078}{2} + \frac{6(.043)-(-0.012)}{70} = 0.0555$

$b_u = \frac{0.203-0.078}{2} + \frac{6(.-0.012) - 0.043}{70} = 0.0621$

$R = \frac{0.0621}{0.0555} = 1.12$ mu g.

Calculations for additions made before autoclaving:

$L_s = 0.083 + 0.186 - 2(0.133) = -0.007$

$L_u = 0.083 + .189 - 2(0.149) = -0.026$

$$b_s = \frac{0.176-0.083}{2} + \frac{6(-0.026) - (-0.007)}{70} = 0.0252$$

$$b_u = \frac{0.189-0.083}{2} + \frac{6(-0.027) - (-0.026)}{70} = 0.0507$$

$$R = \frac{b_u}{b_s} = \frac{0.0507}{0.0252} = 2.01 \text{ mu g.}$$

Calculations for 72 hour data:

For the standards (s):

| 1 T-G | 1 T-G plus 1 mu g. of L-tryptophane | 1 T-G plus 2 mu g. of L-tryptophane |
|---|---|---|
| Additions made before autoclaving: | | |
| 42 | 74 | 94 |
| Additions made after autoclaving: | | |
| 43 | 74 | 92 |

For the unknown (u):

| 1 T-G | 2 T-G | 3 T-G |
|---|---|---|
| Additions made before autoclaving: | | |
| 42 | 70 | 92 |
| Additions made after autoclaving: | | |
| 43 | 70 | 93 |

For additions made after autoclaving:

$$L_s = 43 + 92 - 2(74) = -13$$

$$L_u = 43 + 93 - 2(70) = -4$$

$$B_s = \frac{92 - 43}{2} + \frac{6(-4) - (-13)}{70} = 24.34$$

$$b_u = \frac{93 - 43}{2} + \frac{6(-13) - (-4)}{70} = 23.94$$

$$R = \frac{b_u}{b_s} = \frac{23.94}{24.34} = 0.98 \text{ mu g.}$$

For additions made before autoclaving:

$L_s = 42 + 94 - 2(74) = -12$

$L_u = 42 + 92 - 2(70) = -6$

$b_s = \frac{94-42}{2} - \frac{6(-6)-(-12)}{70} = 25.76$

$b_u = \frac{92-42}{2} - \frac{6(-12)-(-6)}{70} = 24.06$

$R = \frac{b_u}{b_s} = \frac{24.06}{25.76} = 0.93$ mu g.

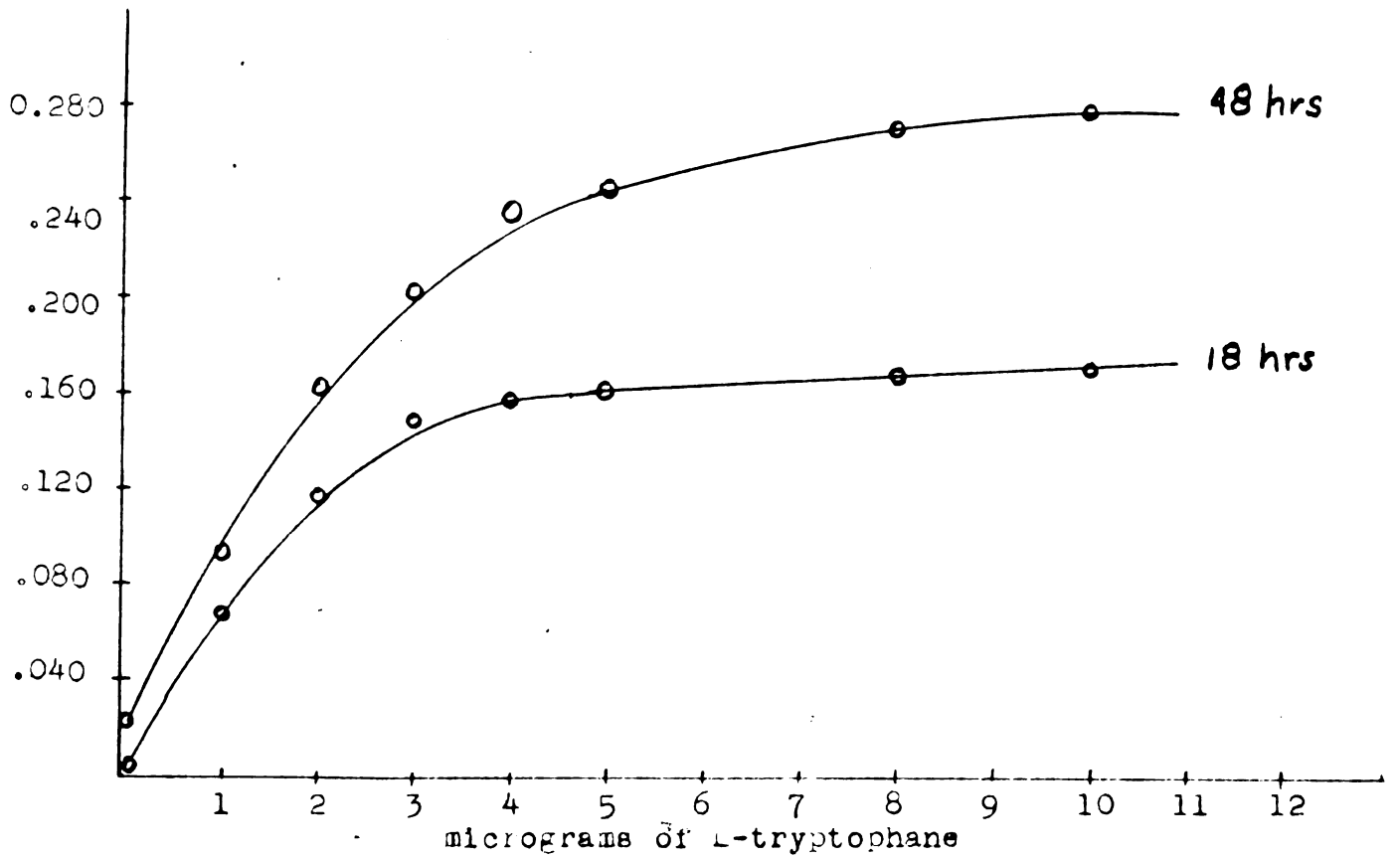
Again this method gives a lower value for the apparent tryptophane content. However, this value also seems to be much above the actual tryptophane content, especially when calculations are made from data collected during the early growth period. The conclusion was reached that a stimulatory factor which was concerned with the metabolism of tryptophane was produced by the Maillard reaction.

Orla-Jensen (4) stated that in the process of heating reducing sugars with nitrogenous substances, degradation products of the sugars were formed. Among these products were pyruvaldehyde, diacetyl, furfuraldehyde, and acetaldehyde. In the next experiment pyruvaldehyde was heated with L-tryptophane to determine what the effect of pyruvaldehyde would be on a subsequent microbiological assay of the test solution for tryptophane. Since pyruvaldehyde is a volatile substance it was easy to remove the pyruvaldehyde so that the pyruvaldehyde itself would have no effect on the assay.

Fifty milligrams of L-tryptophane were refluxed with ten milliliters of 0.005 M pyruvaldehyde for one hour. About five milliliters of the solution were distilled off; pyruvaldehyde was detected in the distillate.

FIGURE 7

STANDARD GROWTH RESPONSE CURVE FOR STREP. FAECALIS*




*

Determined as turbidity at 660 m. mu, using water as a blank.

Inoculation was made with a washed saline suspension of a 16 hour cult

The reaction product was assayed for tryptophane in quadruplicate, using the usual Henderson and Snell medium. The results appear in Table X, and the standard curve is given in Figure 7.

TABLE X

ASSAY OF TRYPTOPHANE WHICH HAD BEEN REFLUXED WITH PYRUVALDEHYDE

| mμ. g. of tryptophane | mμ. g. of tryptophane according to original content | *Growth Response of † Strep faecalis | | | |
|---|---|---|---|---|---|
| | | at 18 hours | S.D. | at 48 hours | S.D. |
| 0 | 0 | 0.017 | 0.001 | 0.025 | 0.002 |
| 0 | 1 | .057 | .001 | 0.066 | 0.004 |
| 0 | 2 | .083 | .005 | 0.113 | .004 |
| 0 | 3 | .114 | .003 | .150 | .001 |
| 1 | 1 | .096 | .004 | .121 | .003 |
| 1 | 2 | .127 | .004 | .170 | .003 |
| 1 | 3 | .141 | .003 | .197 | .004 |
| 2 | 1 | .139 | .002 | .166 | .004 |
| 2 | 2 | .143 | .002 | .196 | .004 |
| 2 | 3 | .152 | .001 | .220 | .001 |

*Determined as turbidity at 660 m mμ., using water as a blank.
†A washed saline suspension of a 16 hour culture was used as inoculum.

The apparent tryptophane content of the test solution as calculated from the first five series of tubes as the results are determined from the standard curve is 0.73 mμ g. The values for the other tubes fall above the linear dose-response portion of the standard curve. This is probably a good index of the amount of tryptophane which remained in the test solution. Evidently no stimulatory factor was produced by the reaction of pyruvaldehyde and L-tryptophane, and a significant portion of tryptophane was destroyed by the reaction.

The effect of various carbohydrate degradation products on the growth of Strep. faecalis was determined. The first of these tested was the pyruvate ion. One milliliter of a sodium pyruvate solution (0.5 mg.)

FIGURE 8

EFFECT OF SODIUM PYRUVATE IN COMPLETE MEDIUM ON THE GROWTH OF STREP FAECALIS*

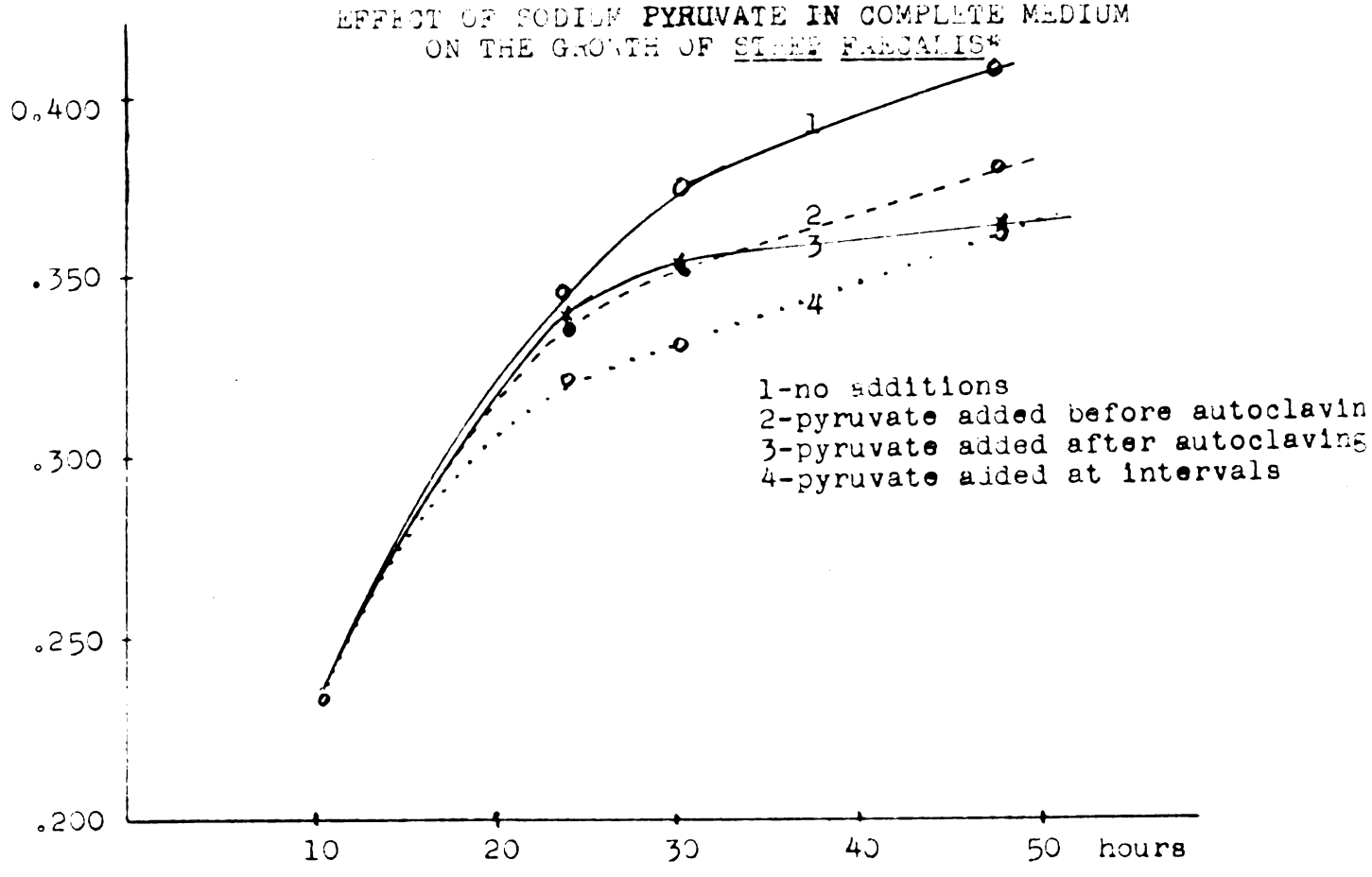
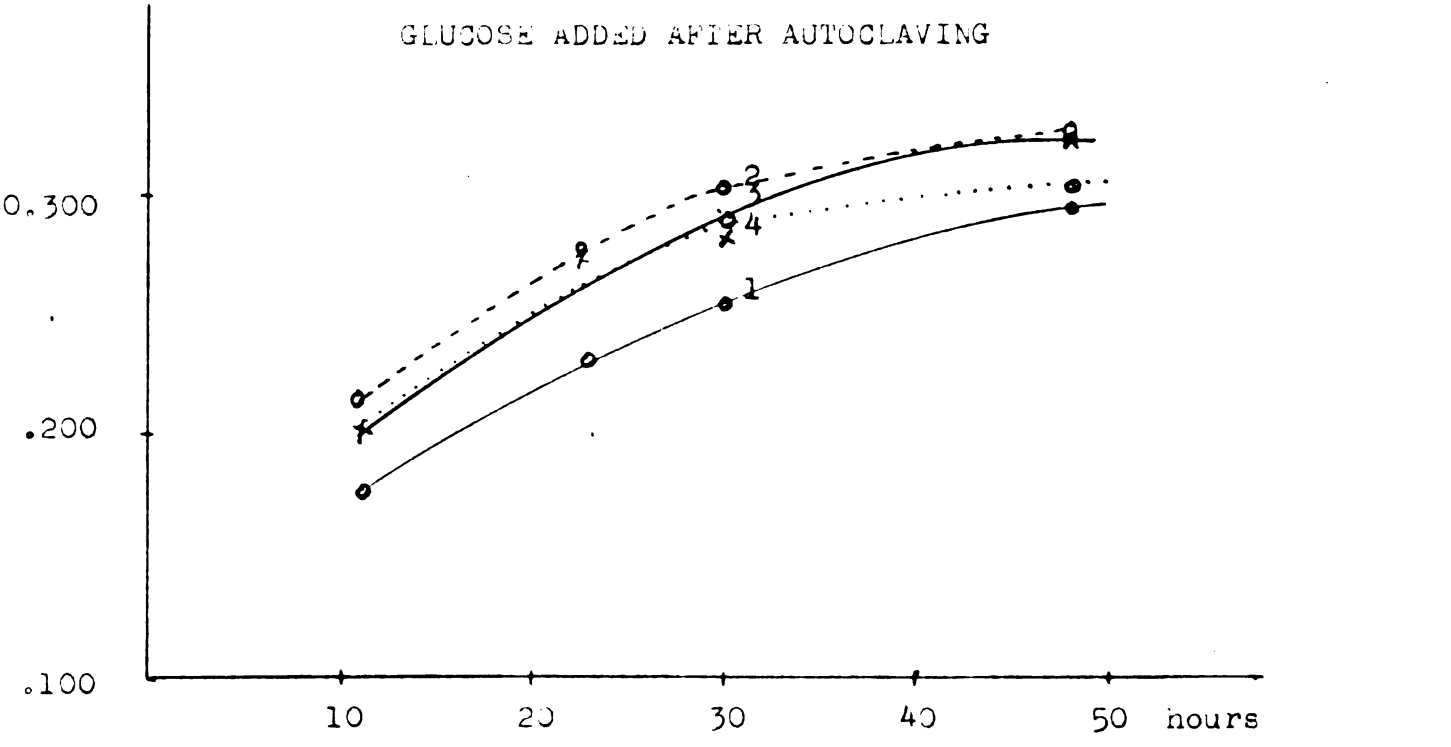



FIGURE 9

GLUCOSE ADDED AFTER AUTOCLAVING




*Determined as turbidity at660 m. mu, using water as a blank. Inoculation was made with a washed saline suspension of a 12 hr. cult

FIGURE 10

EFFECT OF SODIUM PYRUVATE IN LOW TRYPTOPHANE MEDIUM

ON THE GROWTH OF STREP. FAECALIS*

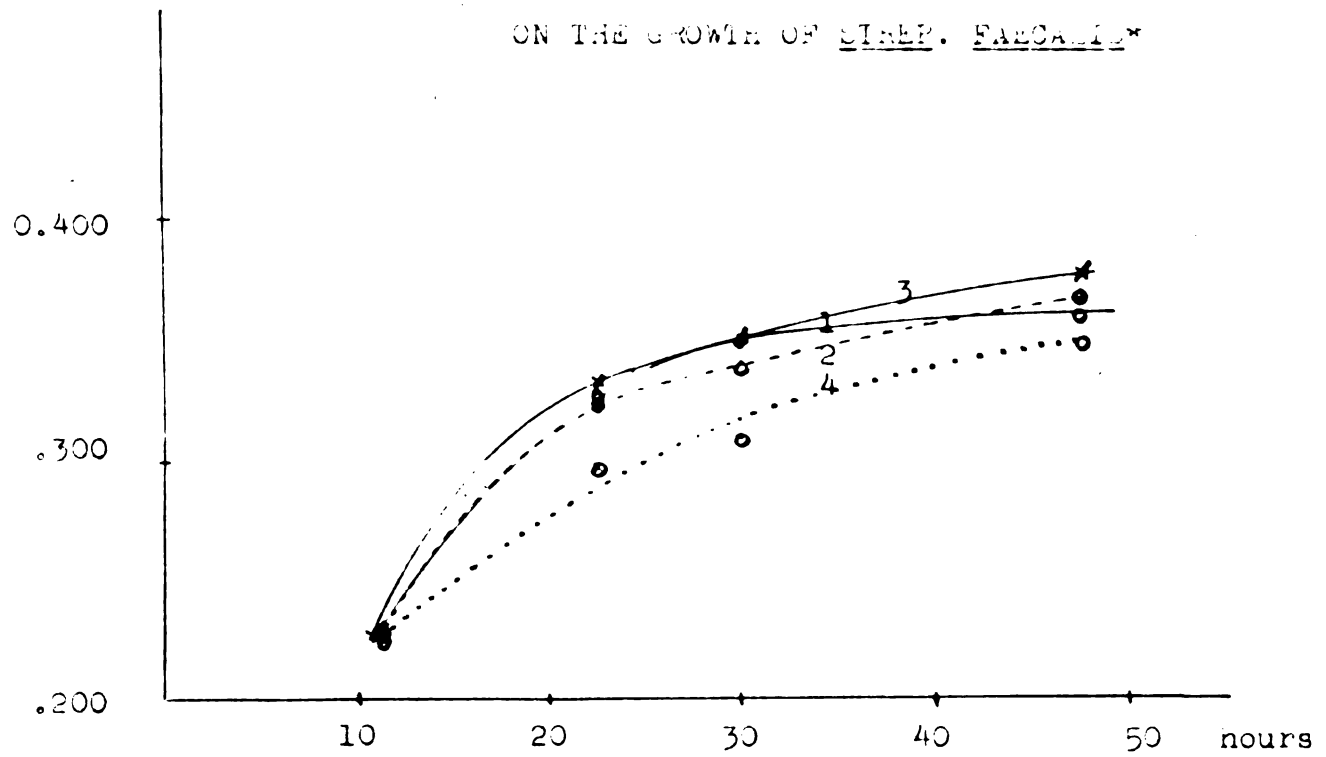
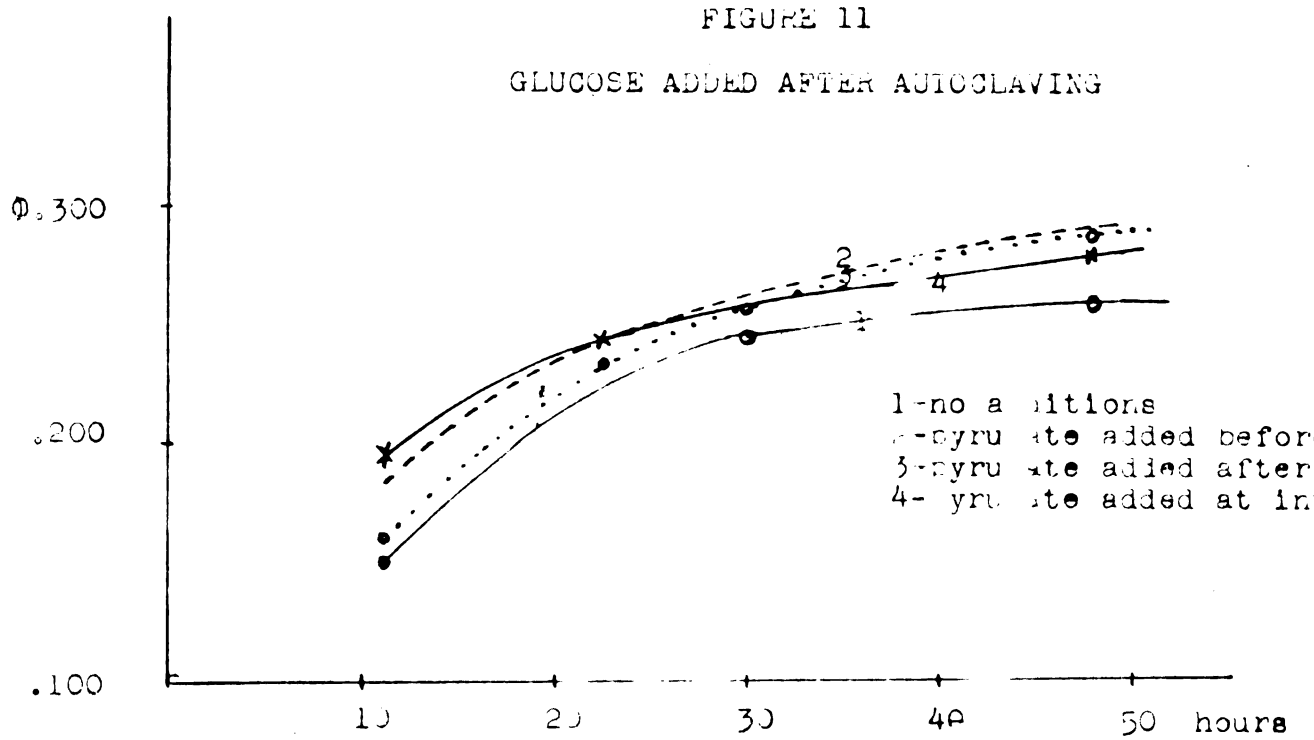



FIGURE 11

GLUCOSE ADDED AFTER AUTOCLAVING




*Determined as turbidity at 660 m. μ, using water as a blank. Inoculation was made with a washed saline suspension of a 12 hr. cultur

was added in quadruplicate to the complete Henderson and Snell medium before autoclaving and to another series aseptically after autoclaving. To a third series the sodium pyruvate was added in amounts of 0.1 mg. at 0, 2, 4, 8, and 12 hours after inoculation. This same procedure was followed using the Henderson and Snell medium with glucose added aseptically after autoclaving. The results are shown in Figures 8 and 9. A similar study was made in a low tryptophane (5 mm g. of L-tryptophane per tube) medium using exactly the same proceedure. The results are shown in Figures 10 and 11.

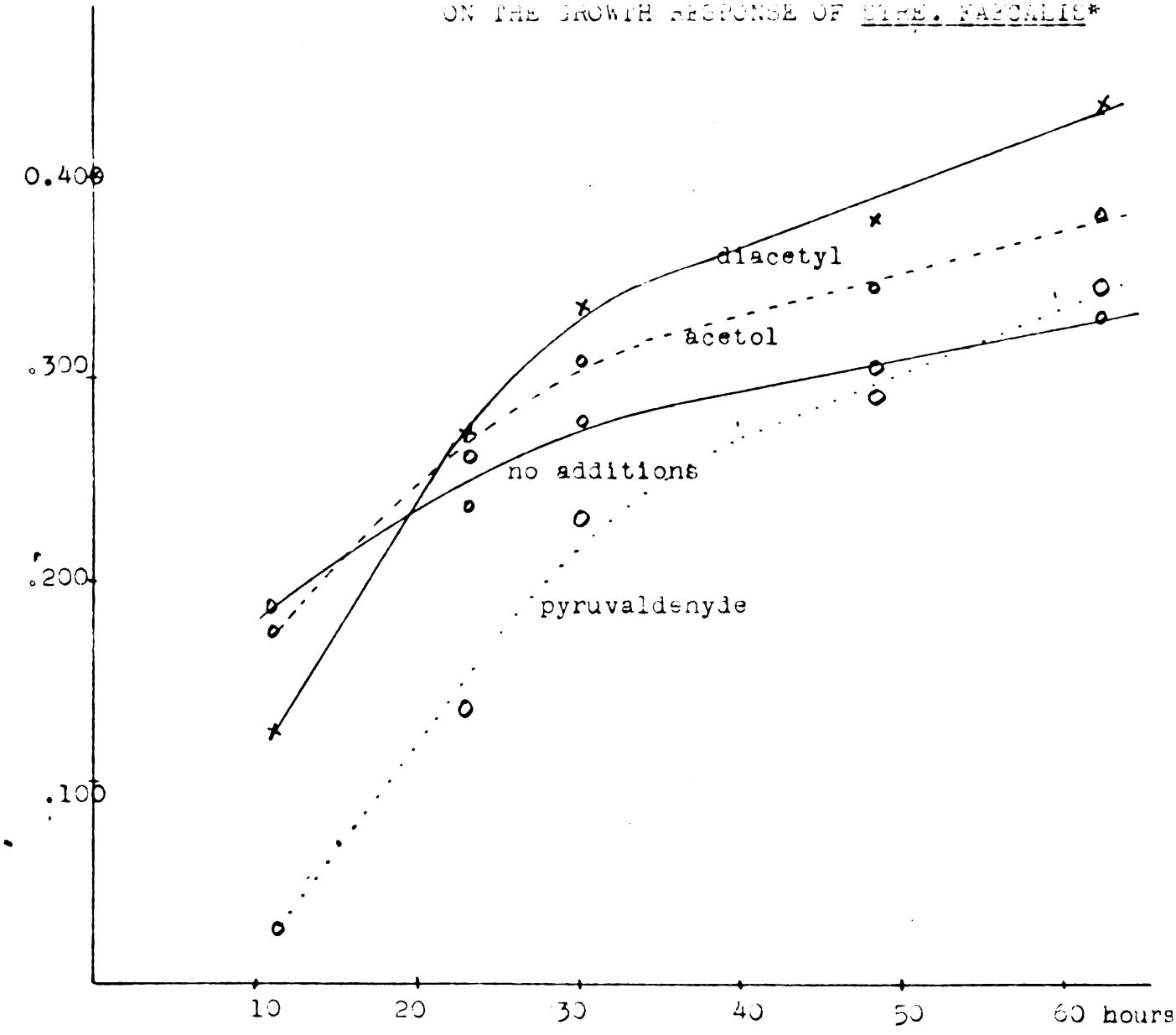
Other substances which were tested included acetaldehyde, pyruvaldehyde, acetol, and diacetyl. Solutions of each of these compounds were made so that one milliliter contained 0.5 mg. Since these are volatile compounds, their solutions were sterilized by passage through a Jenkins filter. One milliliter of the sterile solutions was added aseptically to the medium in quadruplicate after cooling. The results of the growth response of Strep. faecalis to added acetaldehyde in comparison to a series to which no additions had been made is found in Table XI. The results of the growth response of Strep. faecalis when acetol, diacetyl, and pyruvaldehyde were added to the medium of Henderson and Snell is given in Figure 12. Acetol produced moderate stimulation of growth, diacetyl produced marked stimulation, while pyruvaldehyde apparently inhibited growth in the early hours after inoculation. Goto (12) showed that acetol and diacetyl were formed in the decomposition of sugars by weak alkali.

The other amino acids present in the Henderson and Snell medium were individually autoclaved for one hour with 2.0 g. of D-glucose.

FIGURE 12

EFFECT OF SOME CARBOHYDRATE DEGRADATION PRODUCTS

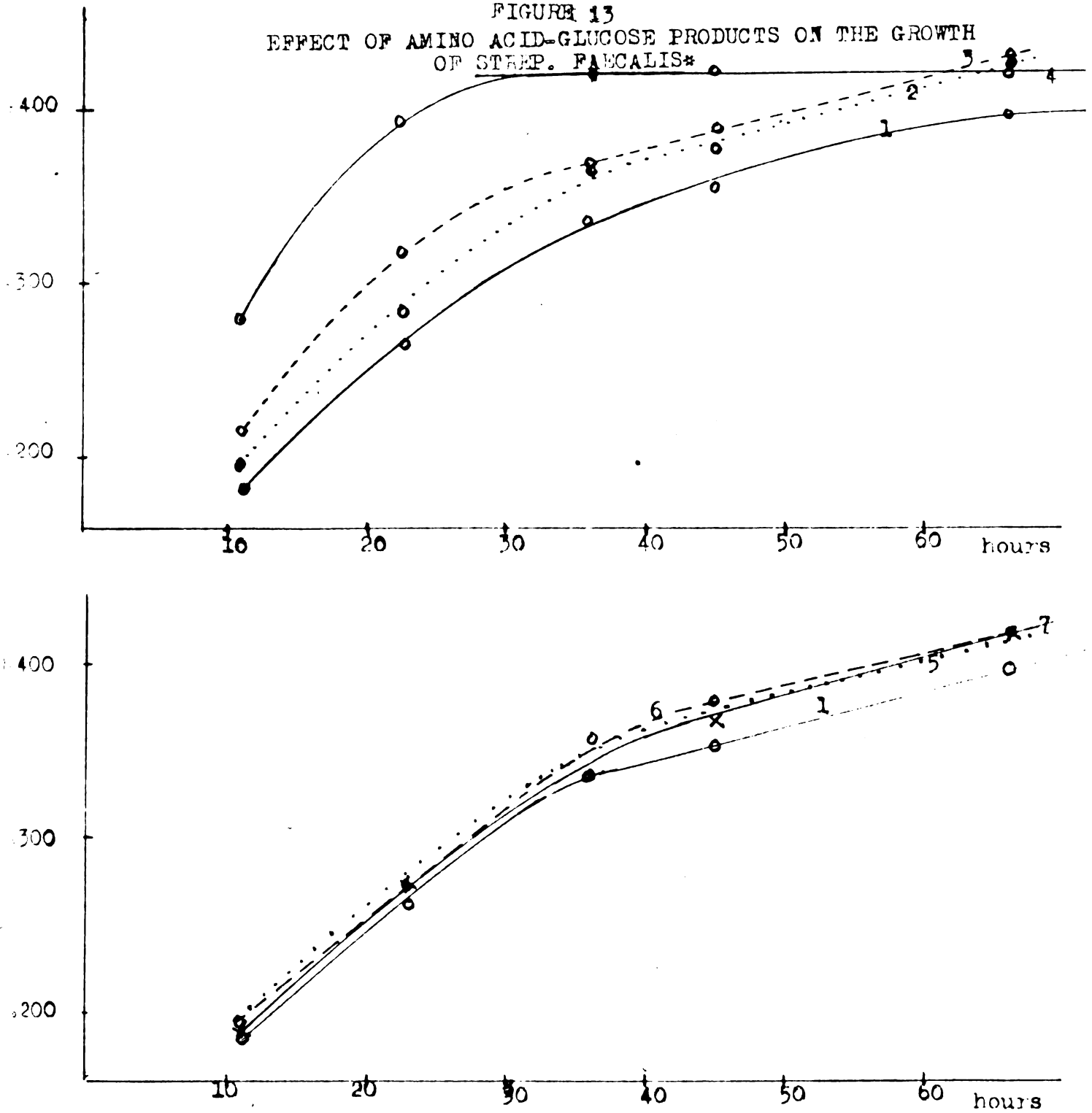
ON THE GROWTH RESPONSE OF STREP. FAECALIS*




*Determined as turbidity at 660 m. mu, using water as a blank. Inoculation was made with a washed saline suspension of a 6 hr. cult

FIGURE 13

EFFECT OF AMINO ACID-GLUCOSE PRODUCTS ON THE GROWTH OF STREP. FAECALIS*




*Inoculation was made with a 6 hour culture. Growth response was determined as turbidity at 660 m. mu.

1-no additions
2-product of cystine-glucose added
3-product of tryptophane-glucose added
4-product of arginine-glucose added
5-product of glycine-glucose added
6-product of serine-glucose added
7-product of methionine-glucose added

The same molar ratio of amino acid to glucose was used as in the previous experiments with L-tryptophane. Dilutions of the solutions were made such that one milliliter contained 0.5 mg. of D-glucose according to its original concentration. The effect of these products on the growth of Strep. faecalis was determined in quadruplicate. One milliliter of each of these solutions was added to a complete medium in quadruplicate before autoclaving for five minutes at 120° C. The results appear in Tables XI and XII. The results of the products of several amino acids are also given graphically in Figure 13.

Apparently each of the amino acids produced some stimulatory factor or factors in the process of heating with D-glucose. However, the effect is most marked in the case of L-arginine and L-tryptophine. Slightly less stimulation was produced by the test solutions of D-L methionine, D-L serine, and glycine.

TABLE XI

THE EFFECT OF OTHER AMINO ACID-GLUCOSE TEST SOLUTIONS OF THE GROWTH OF STREP. FAECALIS

| Amino Acid | *Growth Response of †Strep. faecalis at 11 hrs. | S. D. | 23 hrs. | S. D. | 36 hrs. | S. D. | 45 hrs. | S. D. | 66 hrs. | S. D. |
|---|---|---|---|---|---|---|---|---|---|---|
| No additions | 0.184 | 0.003 | 0.244 | 0.002 | 0.336 | 0.002 | 0.353 | 0.002 | 0.399 | 0.005 |
| L-histidine | .190 | .002 | .253 | .002 | .347 | .005 | .371 | .004 | .412 | .004 |
| L-tyrosine | .190 | .002 | .254 | .001 | .344 | .001 | .366 | .002 | .419 | .003 |
| L-leucine | .189 | .002 | .250 | .001 | .350 | .001 | .356 | .004 | .406 | .004 |
| D-L isoleucine | .191 | .002 | .249 | .003 | .349 | .003 | .358 | .004 | .408 | .002 |
| D-L methionine | .185 | .001 | .253 | .001 | .360 | .003 | .366 | .004 | .420 | .004 |
| L-cystine | .194 | .001 | .263 | .001 | .370 | .003 | .377 | .004 | .430 | .002 |
| D-L phenylalanine | .184 | .001 | .256 | .003 | .356 | .004 | .366 | .005 | .412 | .006 |
| L-proline | .186 | .002 | .265 | .002 | .355 | .006 | .368 | .006 | .408 | .005 |
| D-L threonine | .185 | .001 | .258 | .001 | .356 | .003 | .372 | .006 | .410 | .004 |
| D-L valine | .195 | .002 | .260 | .002 | .357 | .004 | .376 | .003 | .406 | .002 |
| D-L serine | .193 | .002 | .260 | .002 | .361 | .005 | .378 | .004 | .419 | .003 |
| glycine | .192 | .003 | .271 | .004 | .345 | .005 | .362 | .004 | .417 | .003 |
| L-arginine | .279 | .004 | .391 | .004 | .428 | .007 | .421 | .006 | .419 | .001 |
| L-tryptophane | .214 | .003 | .298 | .005 | .370 | .009 | .387 | .006 | .428 | .004 |
| acetaldehyde | .199 | .002 | .276 | .002 | .350 | .007 | .351 | .001 | .388 | .003 |

*Determined as turbidity at 660 m mu using water as a blank
†A washed saline suspension of a six hour culture was used as inoculum.

TABLE XII

EFFECT OF AMINO ACID-GLUCOSE TEST SOLUTIONS ON THE GROWTH OF STREP. FAECALIS

| Amino Acid | *Growth Response of †Strep. faecalis at 11 hrs. | S. D. | 23 hrs. | S. D. | 30 hrs. | S. D. | 48 hrs. | S. D. | 62 hrs. | S. D. |
|---|---|---|---|---|---|---|---|---|---|---|
| No additions | 0.182 | 0.002 | 0.224 | 0.002 | 0.282 | 0.001 | 0.292 | 0.003 | 0.331 | 0.005 |
| D-L alanine | .150 | .008 | .240 | .002 | .319 | .002 | .335 | .002 | .381 | .003 |
| D-L aspartic acid | .169 | .012 | .242 | .003 | .310 | .006 | .332 | .006 | .382 | .006 |
| L-glutamic acid | .188 | .003 | .233 | .004 | .294 | .004 | .320 | .003 | .372 | .004 |
| No additions | .233 | .008 | .340 | .007 | .348 | .002 | .357 | .003 | | |
| L-lysine | .249 | .005 | .345 | .005 | .359 | .004 | .366 | .004 | | |

## CONCLUSIONS

From the early part of the experimental work it was concluded that the best growth of Strep. faecalis was obtained when glucose was used as the fermentable carbohydrate and autoclaved with the medium. Less optimal growth was obtained when the glucose was autoclaved separately and added aseptically to the medium or when sucrose was used and autoclaved separately or with the medium. A better growth response was obtained when the medium was autoclaved at 15 pounds pressure than at 12½ or 10 pounds pressure. The above effects were observed when autoclaving was followed by a prolonged cooling period. The growth response of L. arabinosus was greatest when sucrose was used or when glucose was used and autoclaved separately. A similar effect of autoclaving pressures was observed in the growth response of L. arabinosus, except that when a prolonged cooling period followed the best growth response was obtained when 10 pounds pressure had been used for autoclaving and when glucose was used and heated with the medium. It was also observed that sucrose could not be substituted for glucose as the fermentable carbohydrate for Leuc. mesenteroides, and that early growth was obtained only when the glucose was autoclaved with the medium.

These observations suggested that heating a reducing sugar (glucose) with the nitrogenous components of the medium partially destroyed some of the essential nutrients and at the same time produced material which stimulated the growth of the organisms used. This material appeared necessary for the growth of Leuc. mesenteroides.

The attempt to determine the amount of destruction of L-tryptophane which occurred when it was heated with D-glucose was not successful since the process resulted in the formation of a stimulatory material which was active even in the highest dilution employed (1:8000). It would have been possible to determine the amount of L-tryptophane present either by the method of Thompson and Kirby or by the method of Emmens if this material had been active to the same degree at various levels of L-tryptophane in an assay medium. The fact that this was not possible suggests that the material formed was in some manner involved in the metabolism of tryptophane. This would explain the greater activity of these test solutions at lower tryptophane concentrations. The stimulatory effect could also be demonstrated on a complete medium.

When the amino acid L-arginine was heated with D-glucose and the product added to a complete medium significant stimulation of growth was observed. Stimulation to a lesser extent occurred when D-L-methionine, D-L serine, and glycine were tested in the same manner.

Certain carbohydrate degradation products were tested for their effect on the growth of Strep. faecalis. Acetaldehyde exerted a slightly stimulatory effect in the early period after inoculation, while pyruvaldehyde markedly inhibited growth in the early period. In either case no effect was noted after a longer growth period. When pyruvaldehyde was heated with L-tryptophane considerable destruction of L-tryptophane resulted as determined by subsequent microbiological assay.

Acetol produced a moderate and diacetyl a marked stimulation of growth, particularly in the final growth period. Sodium pyruvate produced moderate stimulation if it was added after autoclaving to media

low in tryptophane. Slight inhibition resulted when sodium pyruvate was added at intervals during the early growth period.

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