



Factors affecting utilization of aspartic acid by *Leuconostoc Mesenteroides* P-60
by Richard S Clark

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
of Master of Science in Chemistry at Montana State College

Montana State University

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Abstract:

A study of the utilization by *Leuconostoc mesenteroides* P-60 of the factors affecting this utilization was made. It was shown that the D-isomer is utilized. An indication of enzymatic adaptation appeared. A difference in the type and amount of buffer affected the response to D-aspartic acid.... Varying amounts of alanine had no appreciable affect and D- and L-asparagine would not substitute for D- and L-aspartic acid.

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BY LEUCONOSTOC MESPENTEROIDES P-60

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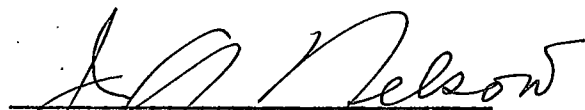
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ABSTRACT

A study of the utilization by Leuconostoc mesenteroides P-60 of the factors affecting this utilization was made. It was shown that the D-isomer is utilized. An indication of enzymatic adaptation appeared. A difference in the type and amount of buffer affected the response to D-aspartic acid. Varying amounts of alanine had no appreciable affect and D- and L-asparagine would not substitute for D- and L-aspartic acid.

INTRODUCTION

The D-isomers of amino acids have long been considered the synthetic or "unnatural" form. The possibility of their utilization by microorganisms has been recognized only within the past few years. The question of whether these forms occur as components of living tissue or only as extracellular metabolic products has been debated during the past few years and is still being debated.

It now seems apparent that a great many different microorganisms can utilize various D-isomers of the amino acids. The D-isomers of all but five or six amino acids have been reported as being utilized partially or completely by various microorganisms. The six reported as most generally utilized are D-alanine, D-valine, D-leucine, D-aspartic acid and D-glutamic acid. Of these, D-glutamic acid has been, perhaps, the most extensively studied and D-aspartic acid the least.

According to Rydon (1948) the only lactic acid producing organism found capable of utilizing D-aspartic acid is Lactobacillus delbrückii. Assays run in the laboratory of the Department of Chemistry Research, Montana State College Agricultural Experiment Station indicated the D-aspartic acid was substituting, at least partially, for L-aspartic acid with Leuconostoc mesenteroides P-60. This organism is of particular interest since it has been used as the principal assay organism for aspartic acid. Because of peculiarities which had been noted in its response to this amino acid further study seemed advisable.

HISTORICAL RESUME

Early investigators believed that the L-forms of amino acids were the only naturally occurring forms of amino acids. The D-forms were considered as synthetic compounds and metabolically inert. These concepts were based on Fischer and Abderhalden's (1905) report that only the peptide linkages built up from amino acids of the L-series were hydrolyzed by enzymes of the pancreas and intestine.

The first experiments with the utilization of D-amino acids were performed by Wohlgemuth (1905). He fed racemic tyrosine, leucine, aspartic acid and glutamic acid to rabbits. He reported that the corresponding D-amino acids were excreted in the urine. Dakin (1909) injected 8 grams of optically inactive phenylalanine into a cat and recovered 2 grams of inactive phenylalanine from the urine. Dakin (1910) also injected inactive tyrosine into a cat. He stated as a result of this experiment, "the naturally occurring laevo-acid was in every case more readily decomposed but the rates of decomposition of the d and l forms cannot be very widely different since when smaller doses of the inactive acid are given, no unchanged tyrosine may be found in the urine." Berg and Potgieter (1931) observed that rats fed on a tryptophan deficient diet responded as well to racemic tryptophan as to L-tryptophan. Whipple and Robschey-Robbins (1937) showed that D-histidine and D-tyrosine are utilized by an anemic dog for the regeneration of hemoglobin to the same extent as the L-series. Conrad and Berg (1937) noted that rats fed a histidine

deficient diet supplemented with D-histidine were able to maintain normal activity. When the tissues of these rats were examined they were shown to contain only L-histidine. The natural occurrence of a D-isomer of an amino acid was first demonstrated by Jacobs and Craig (1935) who obtained a methyl ester of D-proline as a cleavage product of ergotine. Smith and Timmis (1937) confirmed the findings of Jacobs and Craig. Ivanovics and Bruckner (1937) isolated D-glutamic acid from Bacillus mesentericus vulgatus. Heinsen (1936) reported the isolation of DL-arginine from a kidney autolysate. Kotake and Goto (1937) using rat kidney slices found an inversion of D-tryptophan into L-tryptophan. Hotchkiss and Dubos (1940) reported that nearly half of the amino acids in the acid hydrolysate of gramicidin and gramidinic acid occur as the D-isomer. Lipmann, Hotchkiss and Dubos (1941) and Christensen, Edwards and Piersma, (1941) isolated D-leucine from gramicidin. Bovarnick (1942) reported a strain of Bacillus subtilis that produces a polypeptide composed solely of D-glutamic acid. Hanby and Rydon (1946) showed that the capsular polypeptide of Bacillus anthracis contained large amounts of D-glutamic acid. Kogl and Erxleben (1939) claimed to have isolated the D-form of glutamic acid from hydrolysates of tumour tissue in amounts of up to 48 per cent of the total glutamic acid. They postulated that the growth controlling enzymes are deprived of their controlling ability when they have the D-form in their structure. The findings and theory of Kogl and Erxleben had been postulated in 1907 by Margaret A. Cleaves (1939). Arnow and Opsahl (1939), Schroeder (1939), White and White (1939), and others confirmed the findings of Kogl and Erxleben whereas Chibnall, Rees, Tristram, Williams and Boyland

(1939), Graff (1939), Chargaff (1939), and others were unable to find any D-glutamic acid in tumour tissue. Kogl and Erxleben (1939 A) attributed their failure to find D-glutamic acid to differences in analytical procedure. Johnson reported finding D-glutamic acid in normal rat liver and Chibnall found the compound in a number of plant proteins as well as in normal animal tissue (1940). The utilization of D-isomers of amino acids by microorganisms was reported by Webster and Bernheim (1936) when they stated that Bacillus pyocyaneus (*Pseudomonas aeruginosa*) utilized both isomers of tyrosine, proline, and to a slight extent those of valine and phenylalanine. Dunn, Camien, Rockland, Shankman and Goldberg (1944) suggested that D-glutamic acid might be utilized by Lactobacillus arabinosus 17-5. Lymann and Kuiken (1948) found that the ability of Lactobacillus arabinosus to utilize the D- forms of the amino acids was greatly increased by the substitution of pyridoxamine for pyridoxine. They also found that Streptococcus faecalis did not utilize D-methionine in a medium buffered with acetate but utilized substantial amounts in a medium buffered with citrate. Camien and Dunn (1949) reported that D- and DL-glutamic acids under some conditions are more active than L-glutamic acid in promoting growth of Lactobacillus arabinosus 17-5, but that increased amounts of aspartic acid in the basal medium partly or completely suppressed the activity of D-glutamic acid for this organism. Camien and Dunn (1950) showed that Lactobacillus arabinosus 17-5 utilized D-methionine and DL-methionine equally as well as L-methionine in a synthetic

medium containing at least one per cent of pyridoxamine or 10 per cent of pyridoxal. At lower concentrations of these vitamins, the utilization of D-methionine was reduced or eliminated. Pyridoxine and other nutrients in relatively high concentrations were ineffective in promoting utilization of D-methionine.

MATERIALS, METHODS AND DATA

A culture of Leuconostoc mesenteroides P-60 was obtained from the American Type Culture Collection. In order to have a pure strain, it was necessary to isolate a single cell of the organism. For this purpose a Chambers micromanipulator was used. The general procedure followed for single cell isolation was that outlined by Hildebrand (1938). Two modifications were made on this method. The first was to use a different moist chamber than that described by Hildebrand. Hildebrand's moist chamber did not provide sufficient moisture for the dry air of this atmosphere. The moist chamber outlined by Richter (1948) proved much more satisfactory. The dilution was made greater than that described by Hildebrand. The dilution was such that rarely were two organisms contained in a single drop. A drop was placed on the cover plate, examined and, if it had only one organism, the cover plate was transferred to a hanging drop slide for incubation. If the drop had no organism another drop was deposited and examined for a single organism. This was repeated until a drop was deposited with a single organism. If more than one organism appeared in a drop the cover plate was discarded.

After incubation in the hanging drop slide the organisms were transferred to a tryptose phosphate medium and maintained until identification. The organisms were identified according to the procedure outlined for Leuconostoc mesenteroides in Bergey's Manual (1948).

After identification the organisms were transferred to an agar stab. This stab consisted of basal medium I, enriched according to Henderson and Snell (1948) by 0.2 per cent Bacto-tryptone, 0.5 per cent Bacto-yeast extract and 2 per cent Bacto-agar. The cultures were incubated at 35°C for 36 hours when heavy growth was observed. After incubation the cultures were stored in a refrigerator until used. The inoculum medium was the same as the above, except that agar was omitted.

Three different basal media were used. Basal medium I (Johnson, Leon H., unpublished data) is shown in Table I.

Basal Medium II is the same as that outlined in Table I except that 20 grams of sodium acetate was substituted for the 20 grams of sodium citrate.

Basal Medium III was the medium described by Horn, Jones and Blum (1950). It is shown in Table II.

These media were adjusted to a pH of 6.8 as described by Henderson and Snell (1948), and 4 ml was pipetted into each tube with an automatic pipette. The volume was doubled by addition of 4 ml of a solution of aspartic acid or water. Forty tubes were placed in a rack and covered with a metal cover about two inches deep. The cover was loosely lined with gauze to permit it to fit tightly over each tube. These media were steam sterilized under 12 pounds pressure for 10 minutes.

Before inoculation into the assay medium, the organisms were revitalized by passing through five transfers. The cultures were incubated 24

TABLE I

BASAL MEDIUM I

Amino acids in mg

DL-alpha alanine	500	L-Methionine	100
L-cysteine	100	DL-Phenylalanine	100
L-arginine HCL	200	L-Proline	50
L-glutamic	500	DL-Serine	100
Glycine	100	DL-Threonine	100
L-Histidine	100	L-Tyrosine	100
DL-Isoleucine	200	DL-Tryptophan	100
L-Leucine	100	DL-Valine	200

Glucose 20 grams

Salts in grams

Sodium citrate	20	KH ₂ PO ₄	1
Sodium acetate	1	MgSO ₄	0.2
Ammonium chloride	3	FeSO ₄	0.01
K ₂ HPO ₄	1	NaCl	0.01

Purines in mg

Adenine	10	Uracil	10
Guanine	10	Xanthine	10

Vitamins in mg

Thiamine	0.5	Ca pantothenate	0.5
Riboflavin	0.5	Niacin	1.0
Pyridoxal	0.3	p-aminobenzoic acid	0.1

Micrograms of the following

Biotin	10	Folic acid	10
Reticulogen	0.2		

Quantities are based on 500 ml volume

hours at 35°C between transfers. After the fifth transfer a centrifuge tube containing 3.5 ml of medium was inoculated and incubated for 24 hours at 35°C. Following incubation, the cells were centrifuged and washed twice with a normal physiological saline solution. After the final washing the cells were again centrifuged and suspended in 7 ml of normal saline solution. Approximately 0.1 ml of this suspension was placed in each tube of assay medium.

Assays were run with various concentrations of D- and L-aspartic acid with all three media, and with basal medium I varying the amounts of glutamic acid and alanine. Varying incubation periods of 12, 24, 36, 48, 60, 72, and 84 hours were employed.

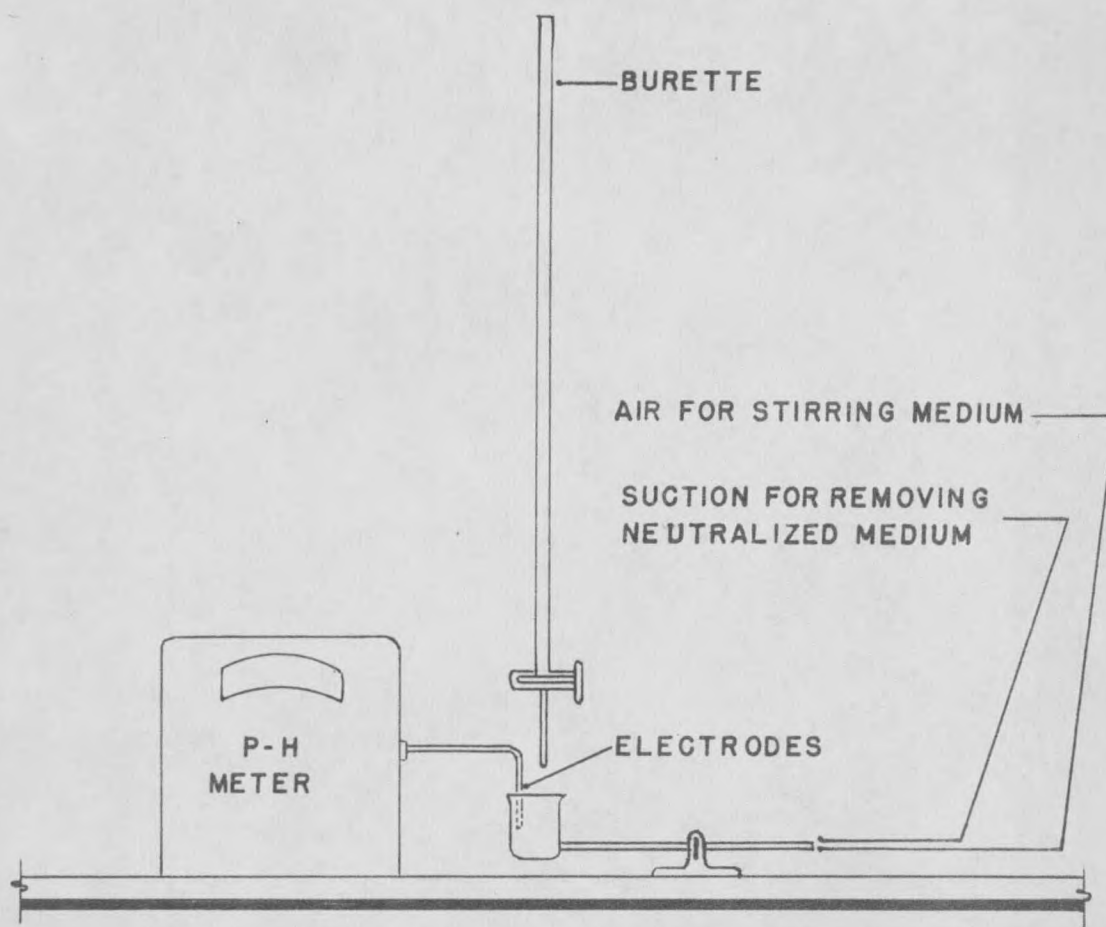
TABLE II

BASAL MEDIUM III

Nutrient	Amount	Nutrient	Amount
Glucose	20gm	Biotin	.01mg
Sodium acetate (anhydrous)	12gm	Folic acid	.002mg
Salts A:		DL-Alanine	.80mg
K_2HPO_4	1gm	L-Arginine hydrochloride	96mg
KH_2PO_4	1gm	DL-Aspartic acid	240mg
Salts B:		L-Cystine	400mg
$MgSO_4 \cdot 7H_2O$	400mg	DL-Glutamic acid H_2O	940mg
$MnSO_4 \cdot 4H_2O$	20mg	Glycine	400mg
NaCl	20mg	L-Histidine hydrochloride H_2O	54mg
$FeSO_4 \cdot 7H_2O$	20mg	L-Hydroxyproline	20mg
Adenine	100mg	DL-Isoleucine	50mg
Guanine	100mg	DL-Leucine	400mg
Uracil	100mg	DL-Lysine hydrochloride	300mg
Thiamine chloride	2.0mg	DL-Methionine	200mg
Pyridoxamine dihydrochloride	.4mg	DL-Norleucine	400mg
Calcium pantothenate	.4mg	DL-Phenylalanine	160mg
Riboflavin	.4mg	L-Proline	140mg
Nicotinic acid	.8mg	DL-Serine	240mg
p-Aminobenzoic acid	.4mg	DL-Threonine	180mg
DL-Tryptophane	400mg	L-Tyrosine	130mg
DL-Valine	240mg		

Solution brought to 1,000 ml volume, pH 6.8.

FIG. 1



TITRATION APPARATUS

EXPERIMENTAL RESULTS

The response of *Leuconostoc mesenteroides* P-60 in each of the three basal media to D and L aspartic acid, varying periods of incubation and concentration of aspartic acid, are reported in Tables III to IX. The lactic acid produced was titrated with 0.092 N NaOH. All values are corrected to zero blank value. Figures 2 to 18 illustrate the results expressed in these Tables graphically.

TABLE III

BASAL MEDIUM I

ug of aspartic acid per tube	36 hour culture				60 hour culture			
	ml NaOH		ml NaOH		ml NaOH		ml NaOH	
	D	L	D	L	D	L	D	L
40	0.7	1.4	0.6	1.7	1.2	2.6	1.3	2.3
80	1.6	2.8	1.5	3.0	2.9	4.6	3.1	4.3
160	2.4	3.7	2.3	3.6	4.6	6.4	4.1	6.1
240	3.3	5.1	3.3	4.8	5.3	7.4	5.3	7.5
320	3.7	5.2	3.6	4.9	6.7	8.1	6.6	8.1

TABLE IV

BASAL MEDIUM I

ug of aspartic acid per tube	12 hour culture		18 hour culture		36 hour culture		60 hour culture		84 hour culture	
	ml NaOH		ml NaOH		ml NaOH		ml NaOH		ml NaOH	
	D	L	D	L	D	L	D	L	D	L
40	0.0	0.7	0.4	1.0	0.5	1.8	1.1	2.4	2.2	3.5
80	0.0	1.1	0.4	1.8	1.4	3.0	2.9	4.4	4.4	5.8
160	0.7	1.3	0.4	2.4	2.3	3.9	4.7	6.3	6.3	7.4
320	0.8	1.2	1.5	2.6	3.4	5.1	6.5	7.7	7.1	8.5
640	0.8	1.1	2.4	2.0	5.0	5.2	6.6	8.1	7.7	8.6

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TABLE V

BASAL MEDIUM I

ug of aspartic acid per tube	24 hour culture		36 hour culture		48 hour culture		60 hour culture	
	ml NaOH		ml NaOH		ml NaOH		ml NaOH	
	D	L	D	L	D	L	D	L
40	0.6	1.4	0.9	2.1	1.3	2.7	2.4	3.5
160	2.5	4.0	3.8	5.5	5.5	6.6	6.6	7.5
640	4.4	5.5	6.2	6.7	7.5	8.0	9.0	9.9
1280	4.9	5.0	6.4	6.4	8.3	7.8	9.7	8.9

TABLE VI

BASAL MEDIUM I

ORGANISMS PASSED THROUGH 10 TRANSFERS IN MEDIUM CONTAINING
ONLY THE D-FORM OF ASPARTIC ACID

60 hour culture

ug of aspartic acid per tube	ml NaOH	
	D	L
40	1.8	2.7
160	6.8	7.3
320	8.2	8.9
640	10.4	10.6
960	11.0	11.1
1280	11.1	10.3

TABLE VII

BASAL MEDIUM II

ug of aspartic acid per tube	12 hour culture		24 hour culture		36 hour culture		60 hour culture	
	ml NaOH		ml NaOH		ml NaOH		ml NaOH	
	D	L	D	L	D	L	D	L
40	0.2	0.8	0.5	1.3	1.1	2.3	2.2	2.6
160	0.4	2.1	2.1	3.5	2.8	4.7	4.8	5.7
640	0.4	2.2	2.2	4.0	3.2	5.8	5.3	7.9
1280	0.6	2.2	2.3	4.0	3.3	5.8	5.3	7.7

TABLE VIII.

BASAL MEDIUM III

ug of aspartic acid per tube	36 hour culture ml NaOH		60 hour culture ml NaOH	
	D	L	D	L
40	0.4	1.6	0.4	2.4
80	0.9	1.9	1.0	2.9
160	1.5	2.1	1.6	2.9
240	1.8	2.1	1.7	2.9
320	1.9	2.1	2.2	2.9

TABLE IX

BASAL MEDIUM I

Glutamic acid varied ----- 60 hour culture

Amount of glutamic acid per tube

ug of aspartic acid per tube	50 ug ml NaOH		100 ug ml NaOH		500 ug ml NaOH		1 mg ml NaOH		4 mg ml NaOH	
	D	L	D	L	D	L	D	L	D	L
40	0.8	1.7	1.5	2.0	1.2	2.0	0.9	2.0	1.5	2.0
80	1.2	1.6	2.2	2.8	1.8	3.0	2.2	3.4	2.7	3.5
120	0.8	1.2	2.6	3.1	2.5	4.3	2.8	4.3	3.8	4.7
160	0.9	0.9	2.8	3.3	2.8	4.9	3.7	5.2	3.9	5.3

TABLE X

BASAL MEDIUM I

Per cent Response of D-Aspartic Acid in Terms of L-Aspartic Acid
at Concentrations of 40 to 320 ug per Tube

ug of aspartic acid per tube	36 hour culture per cent	60 hour culture per cent
40	50	60
80	56	70
160	43	45
240	42	46
320	47	54

TABLE XI

BASAL MEDIUM I

Per cent Response of D-Aspartic Acid in Terms of L-Aspartic Acid
At Concentrations of 40 to 640 ug per Tube

ug of aspartic acid per tube	12 hour culture per cent	18 hour culture per cent	36 hour culture per cent	60 hour culture per cent
40	0	40	40	50
80	0	20	46	65
160	25	10	30	50
320	15	15	15	43
640	7.5	-	-	-

TABLE XII

BASAL MEDIUM II

Per cent Response of D-Aspartic Acid in Terms of L-Aspartic Acid
At Concentrations of 40 to 160 ug per Tube

ug of aspartic acid per tube	36 hour culture per cent	60 hour culture per cent
40	40	90
80	80	75
160	25	45

TABLE XIII

BASAL MEDIUM III

Per cent Response of D-Aspartic Acid in Terms of L-Aspartic Acid
At Concentrations of 40 to 320 ug per Tube

ug of aspartic acid per tube	36 hour culture per cent	60 hour culture per cent
40	18	15
80	20	18
160	23	14
240	25	10
320	25	11

TABLE XIV

BASAL MEDIUM I

Per cent Response of D-Aspartic Acid in Terms of L-Aspartic Acid
 at Concentrations of 40 to 320 ug per Tube
 With Glutamic acid varied

Amount of glutamic acid per tube

ug of aspartic acid per tube	50 ug per cent	100 ug per cent	500 ug per cent	1 mg per cent	4 mg per cent
40	30	60	62	41	70
80		60	40	58	71
120		57	53	53	75
160		50	50	60	63

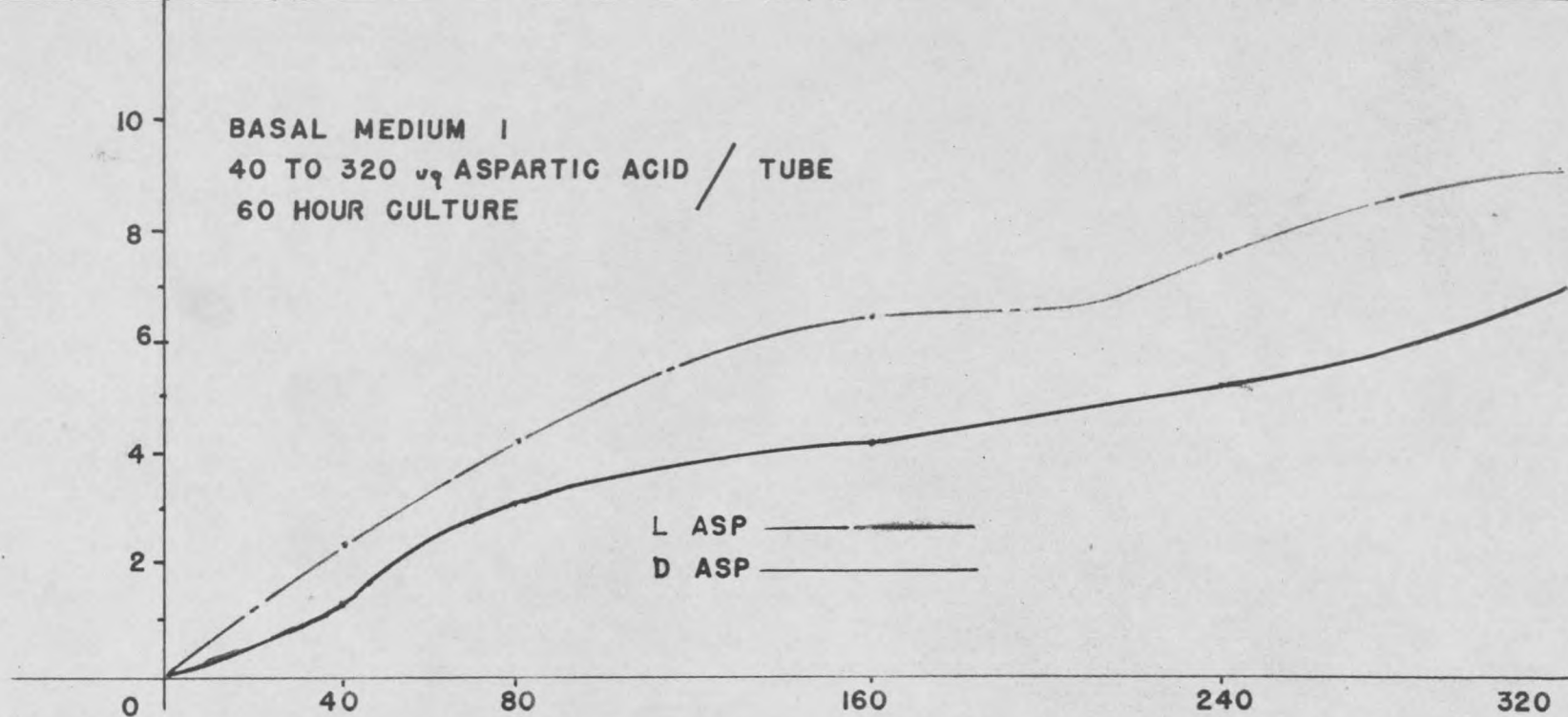


FIG. III

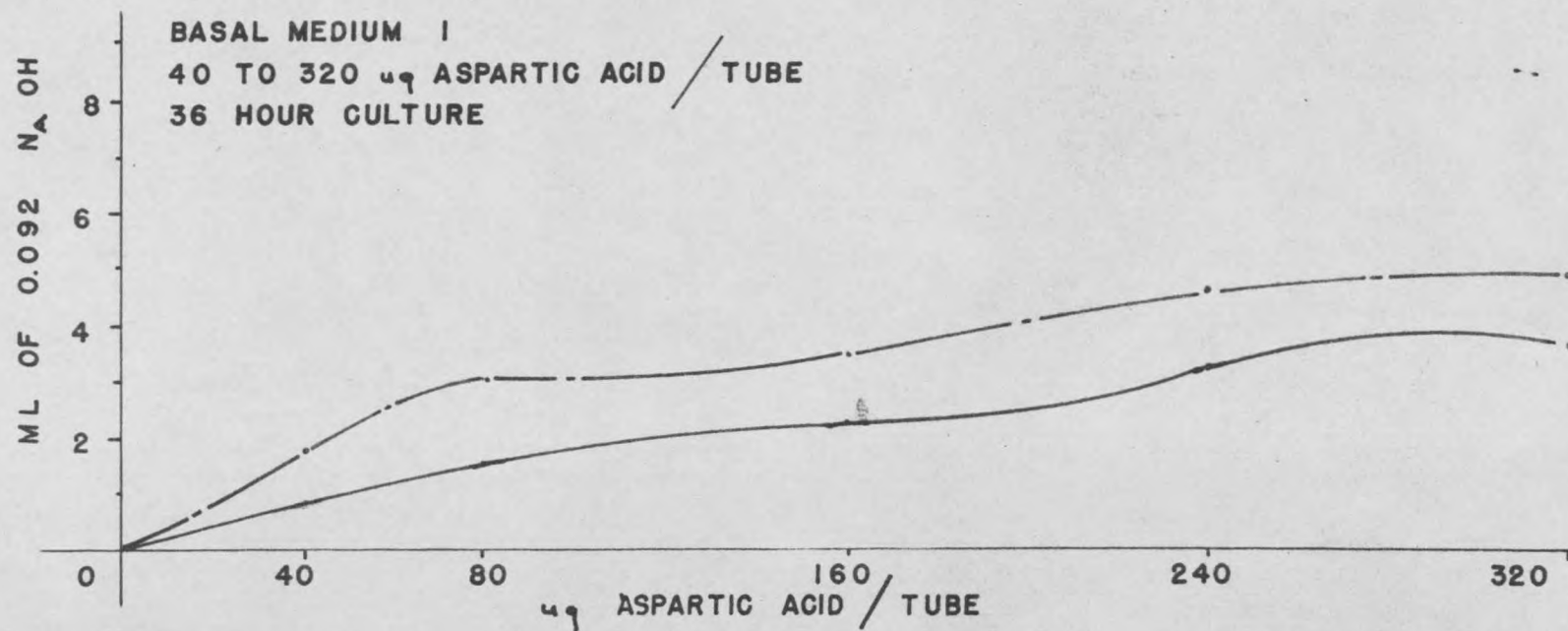
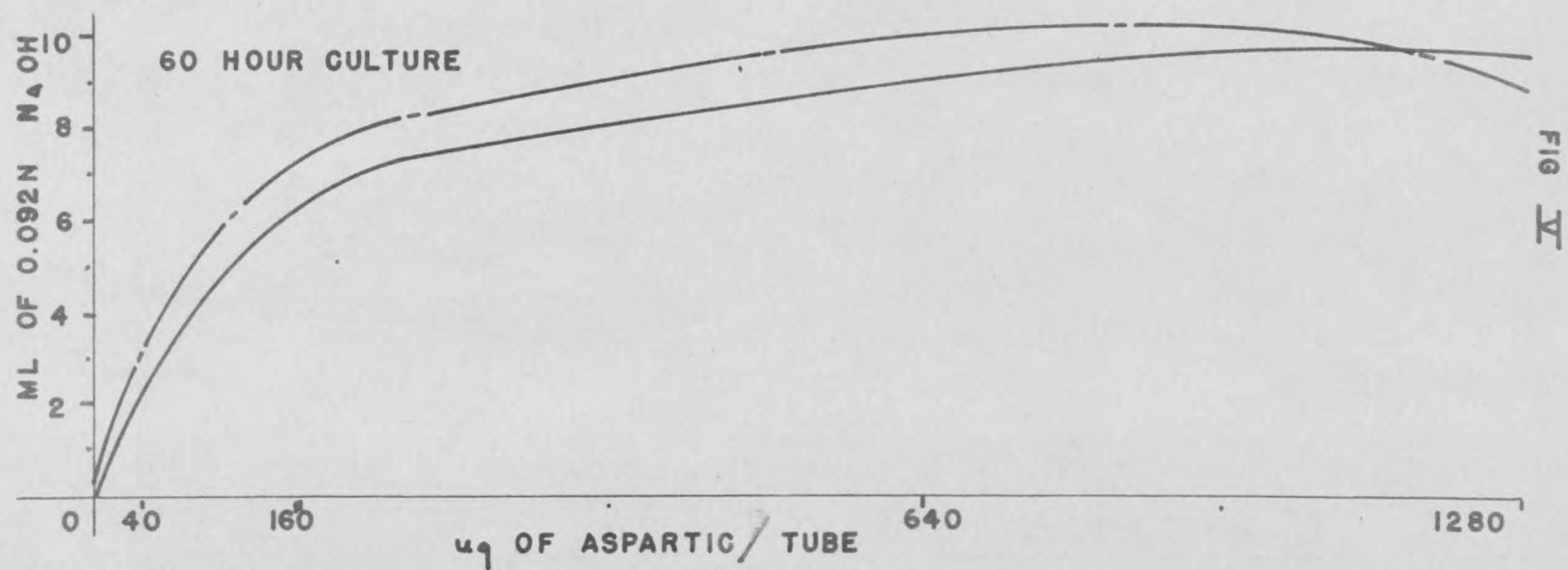
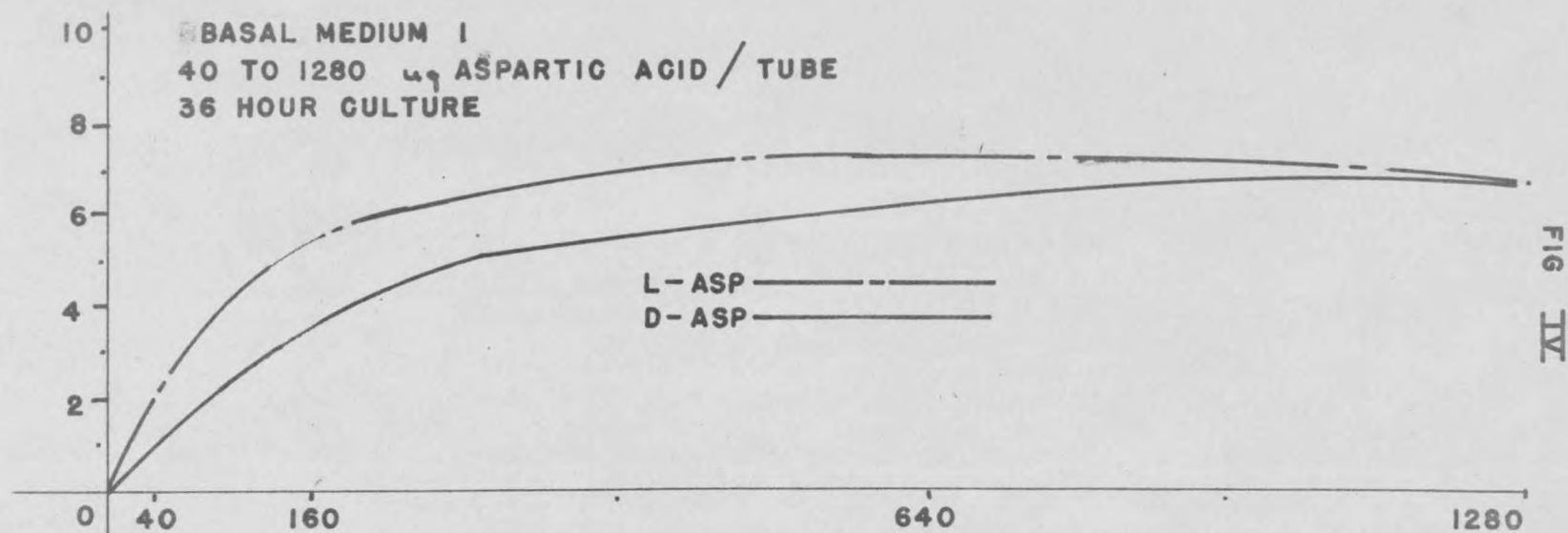


FIG. II



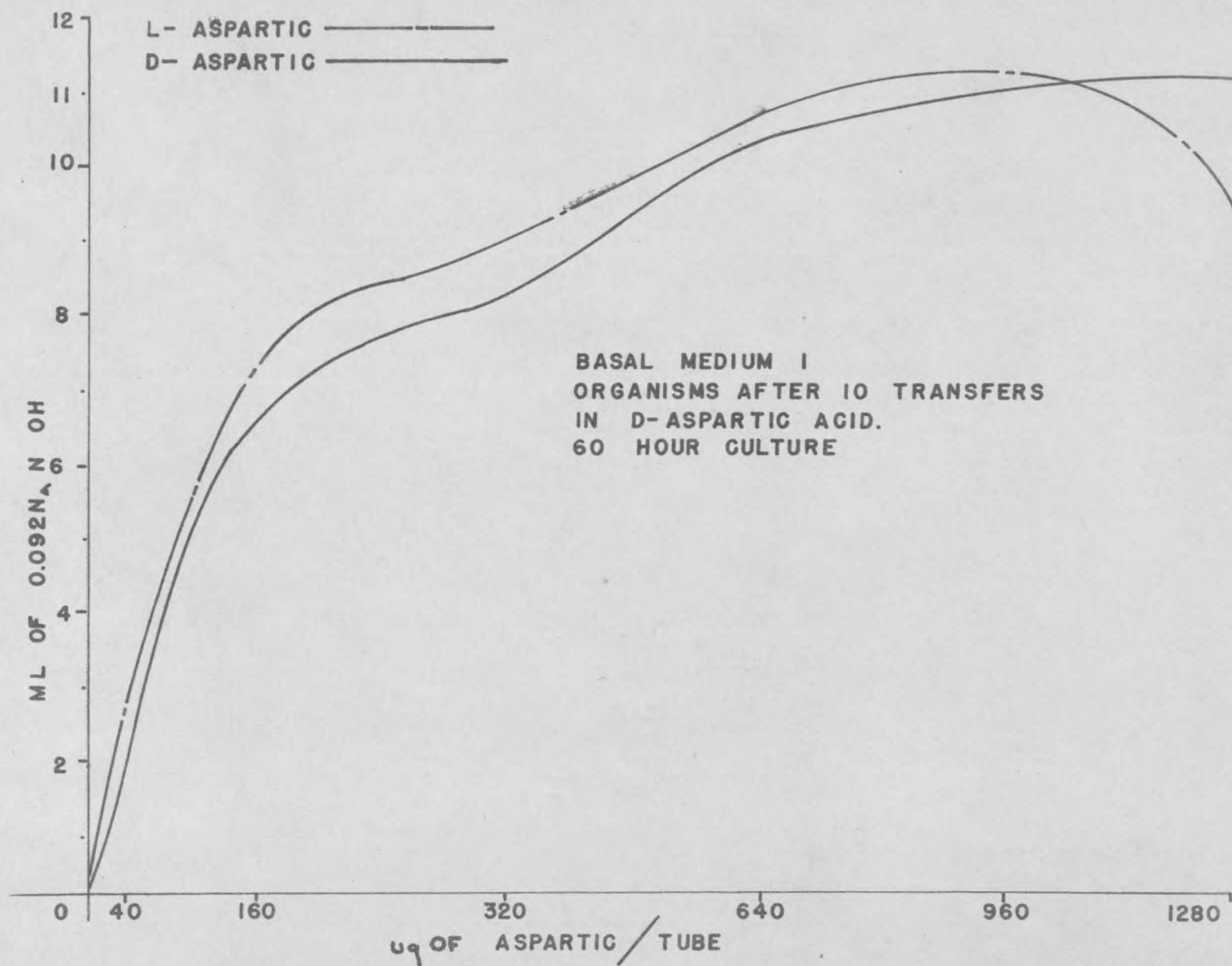


FIG. VI

BASAL MEDIUM III
40 TO 320 μ g ASPARTIC ACID/TUBE
36 HOUR CULTURE

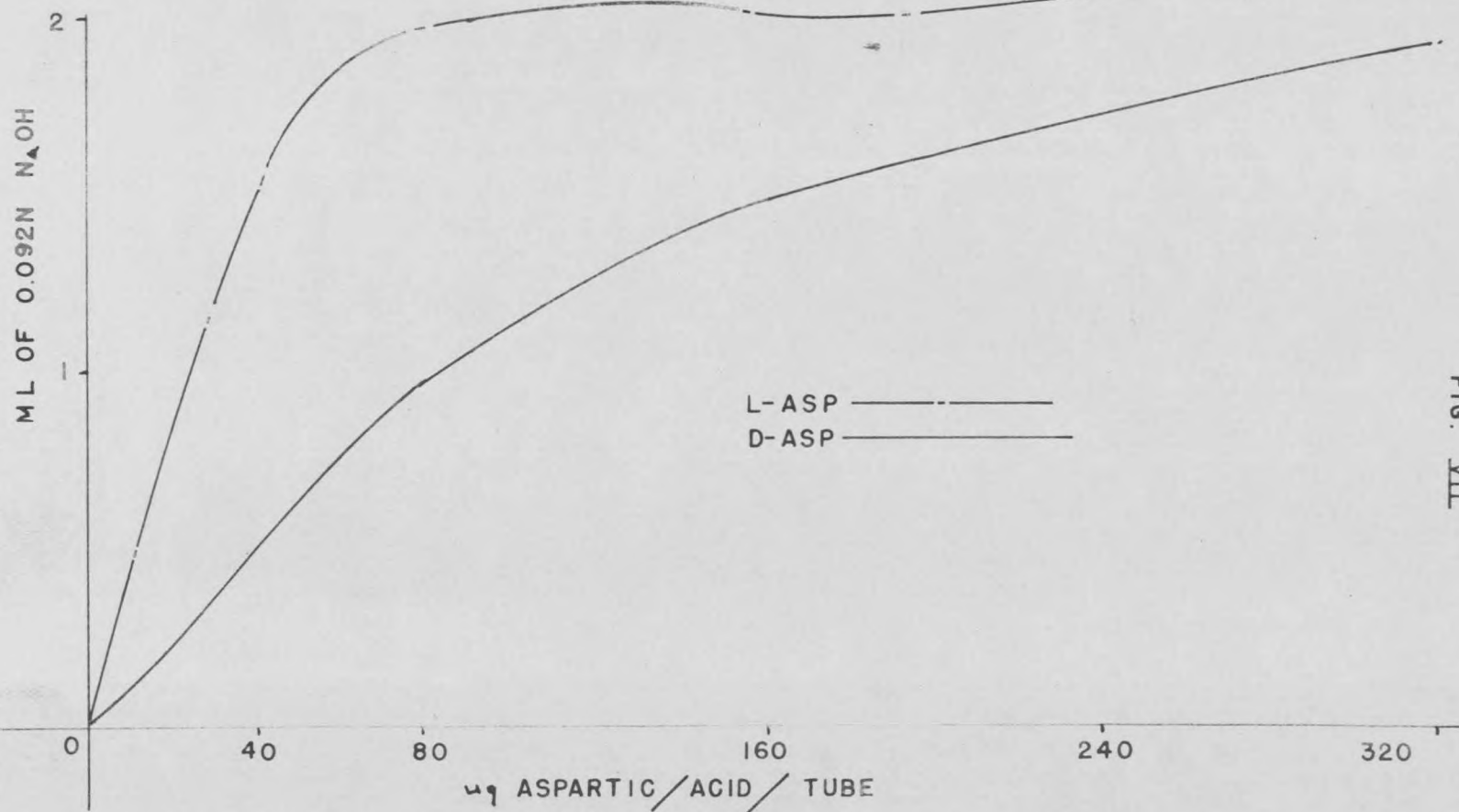


FIG. VII

BASAL MEDIUM III
 40 TO 320 ASPARTIC ACID / TUBE
 60 HOUR CULTURE

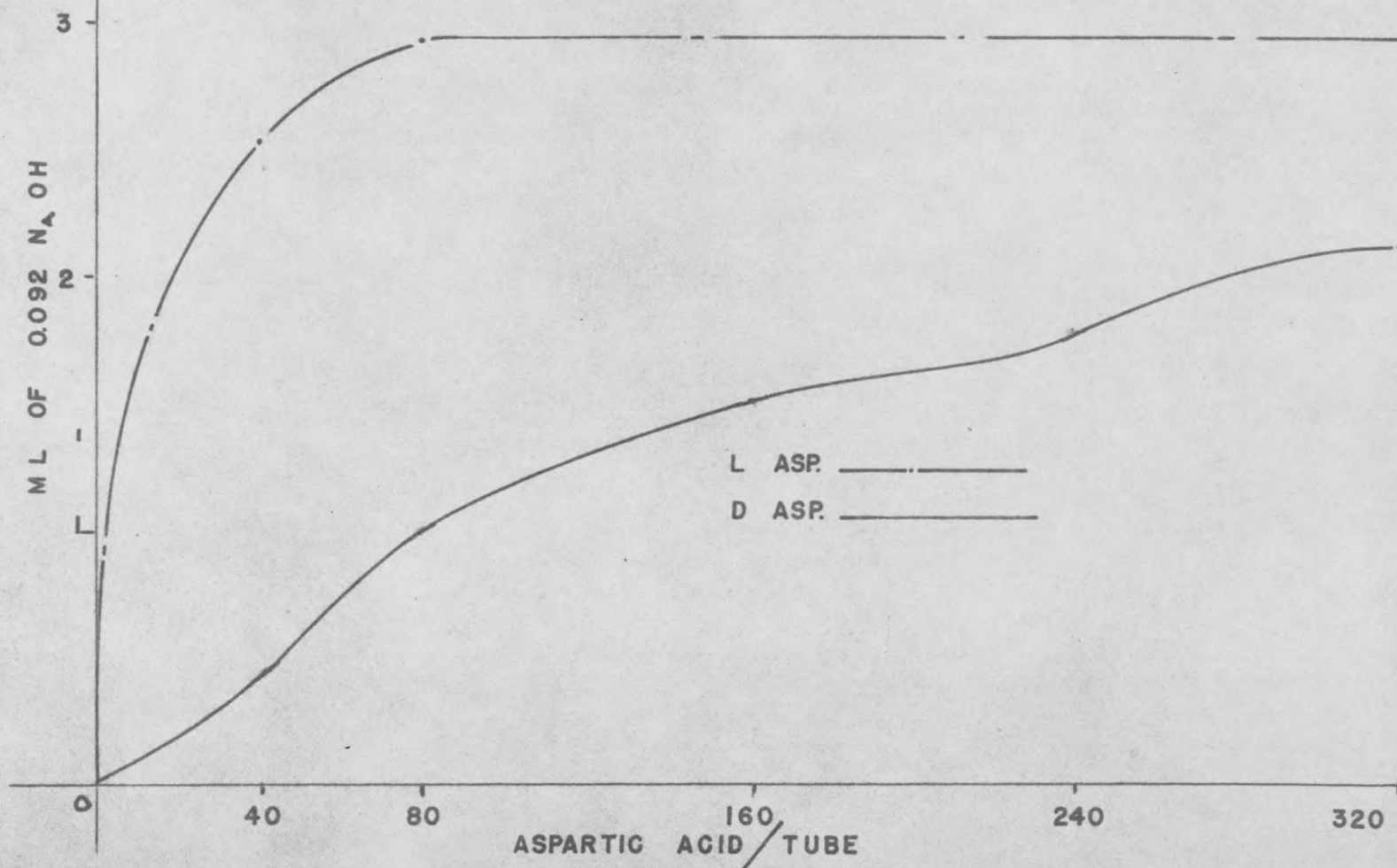
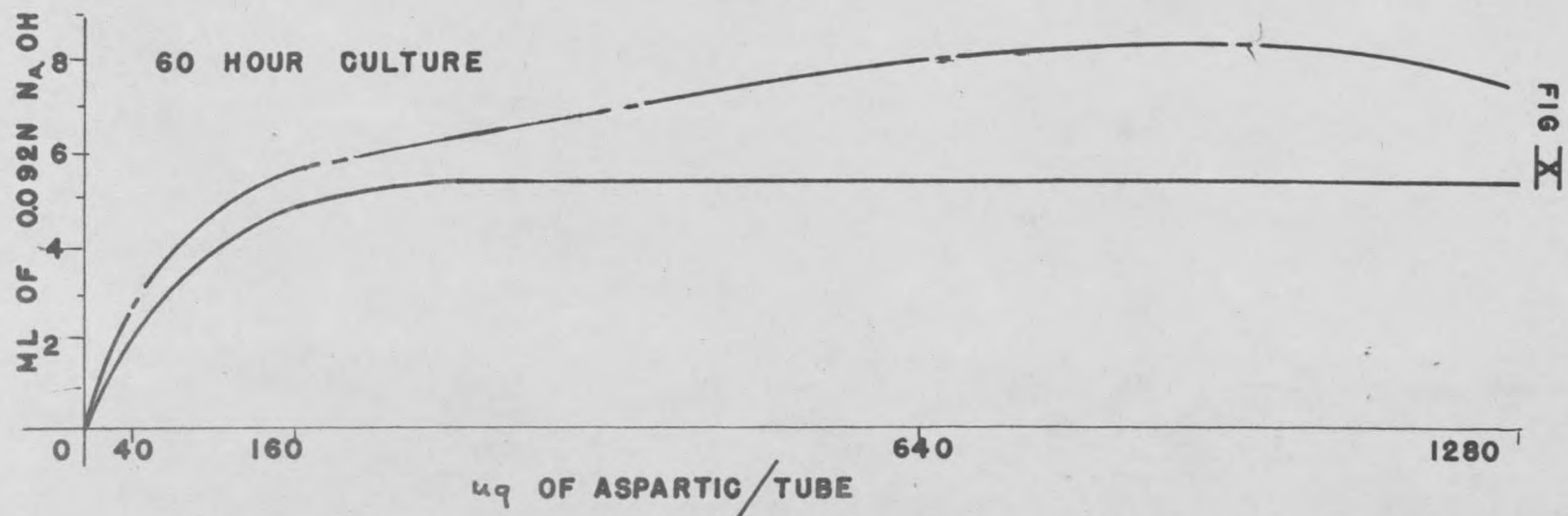
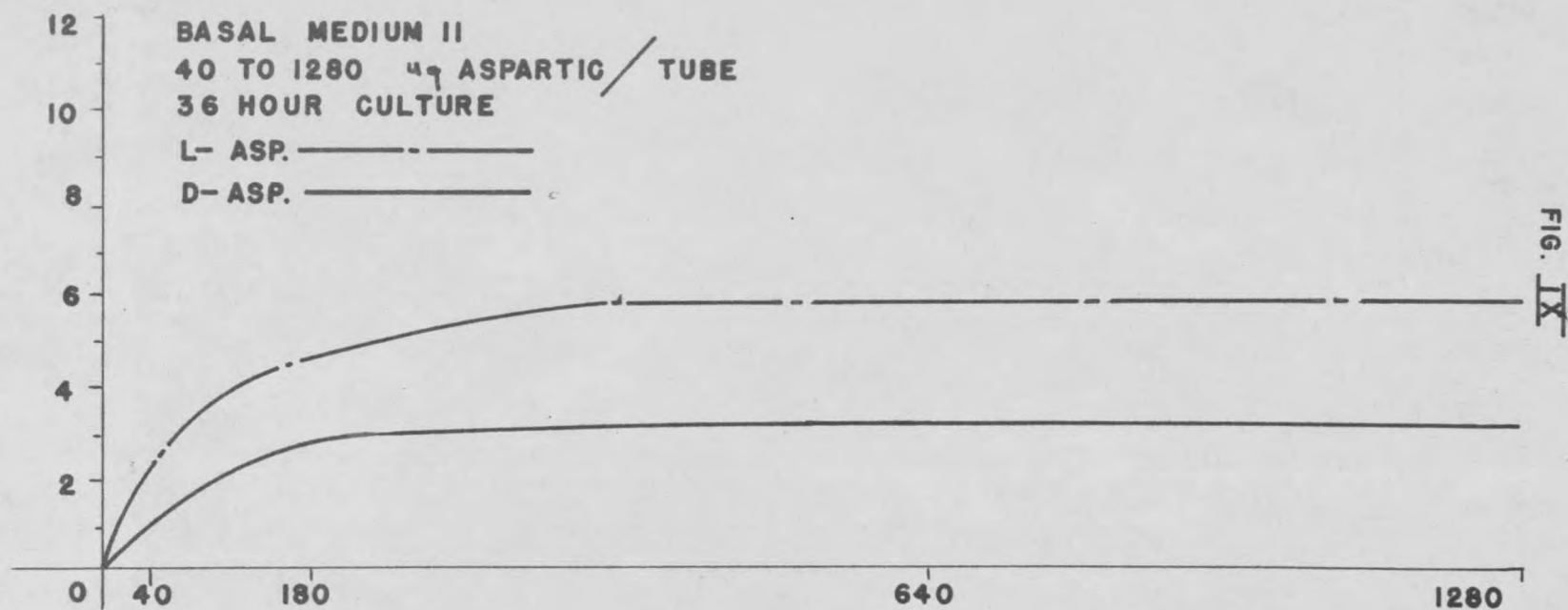
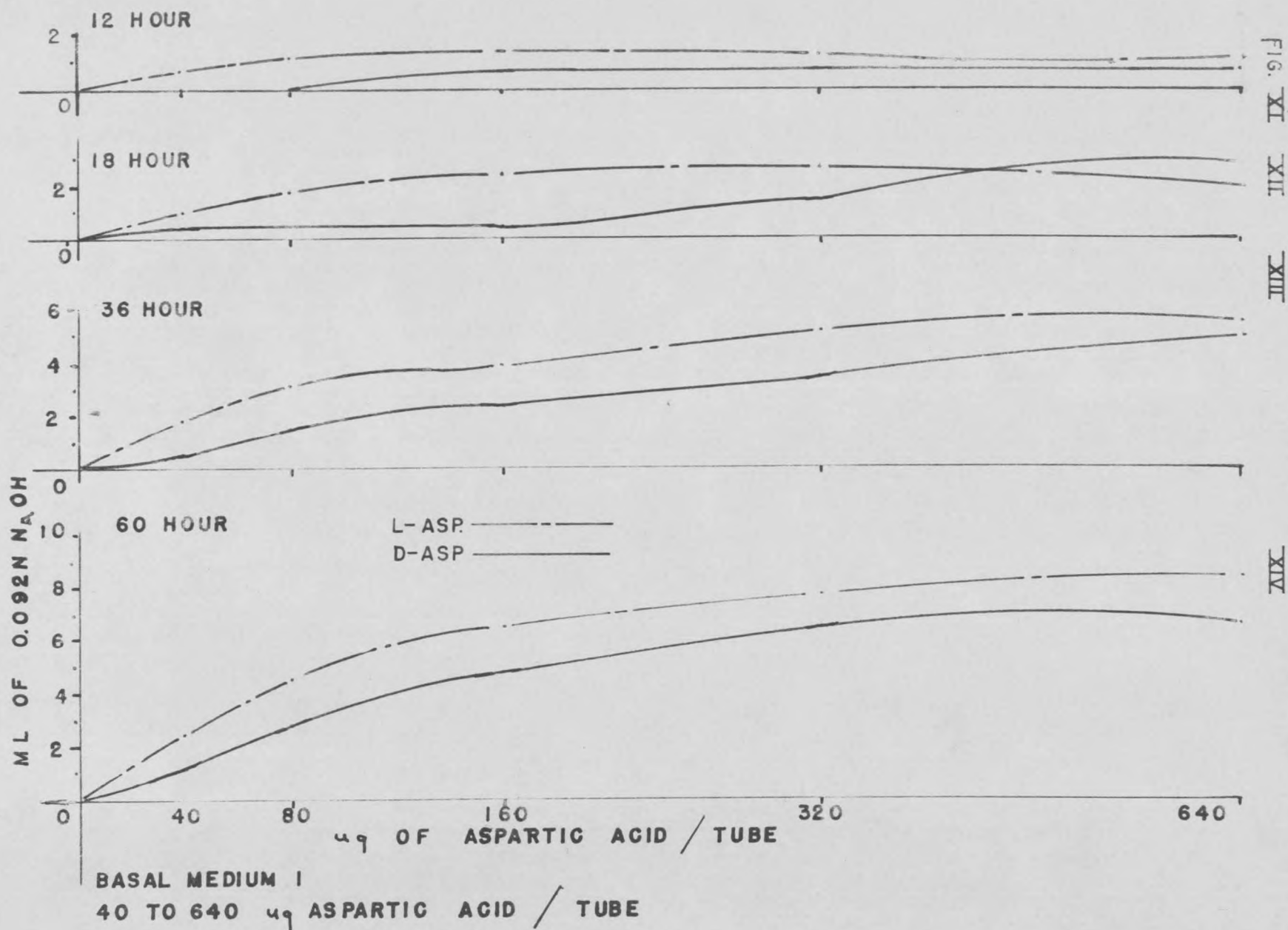


FIG. VIII





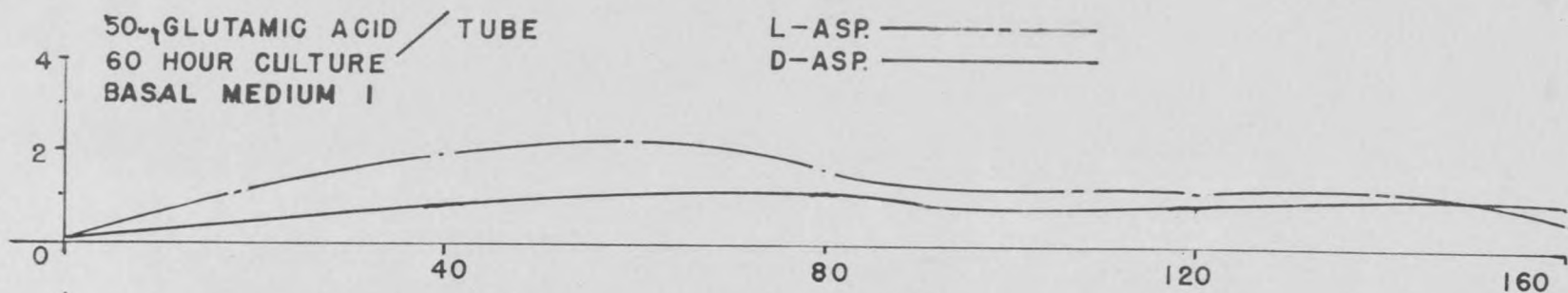


FIG. XV

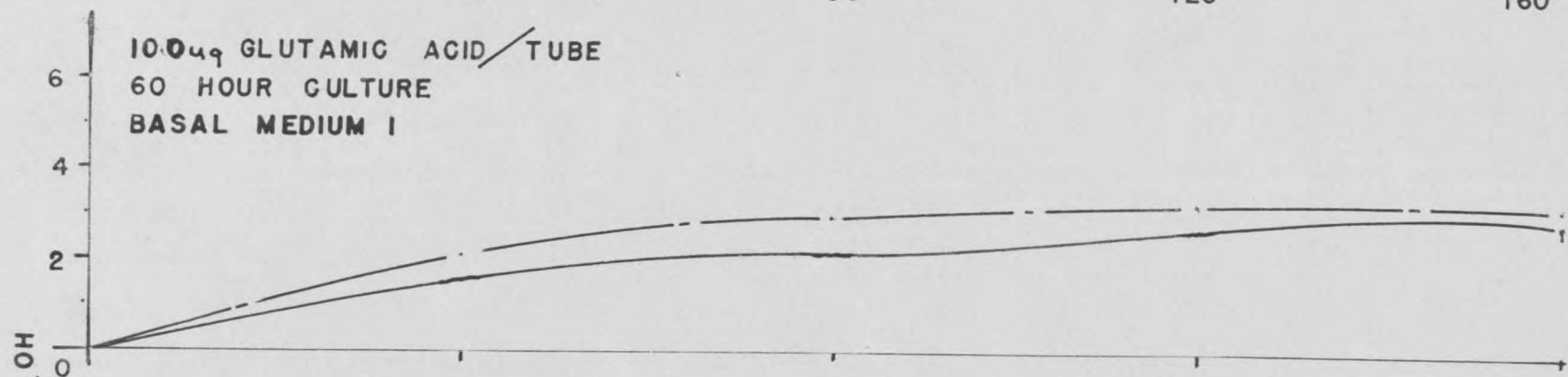


FIG. XVI

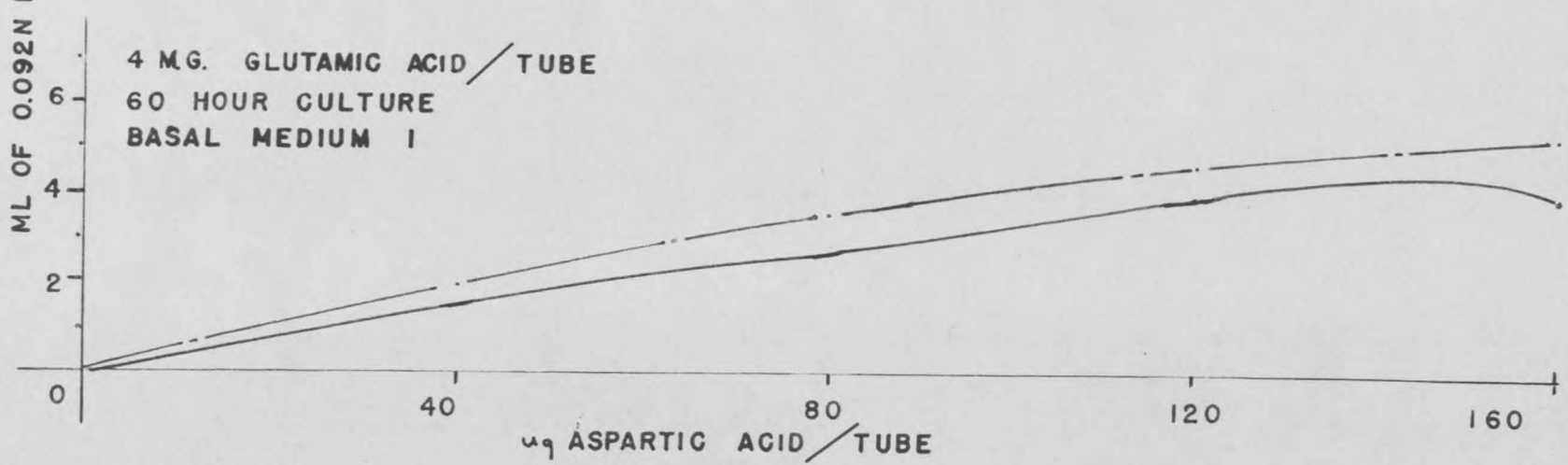


FIG. XVII

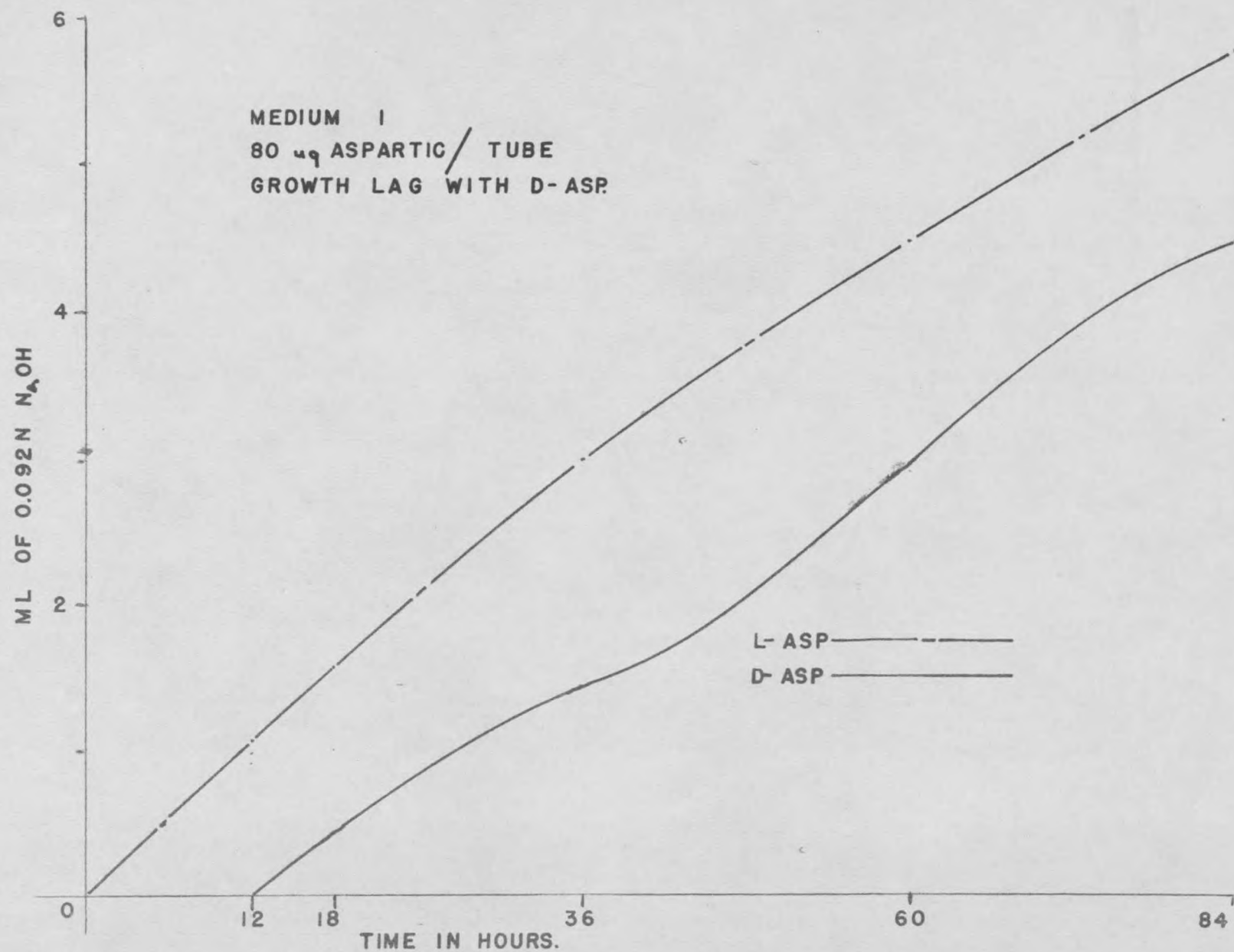


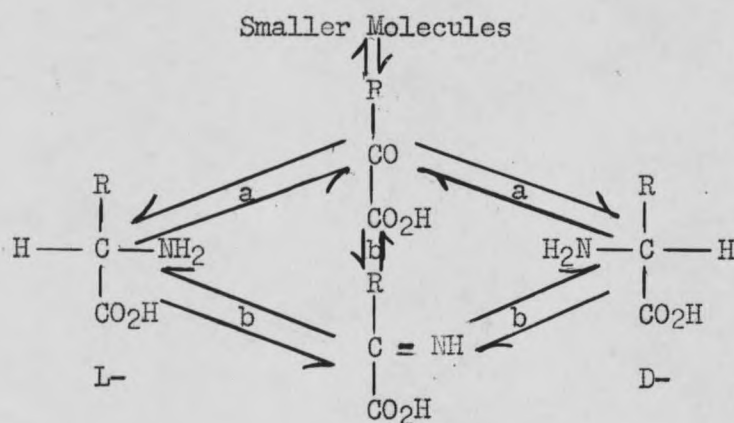
FIG. XVIII

DISCUSSION

In order to explain the results obtained in this work a postulated mechanism for the utilization of the D-form of amino acids will be presented.

Horowitz (1944) studying strains of Neurospora expressed the opinion that cells normally synthesize DL-amino acids and the D-amino oxidase is present to oxidatively deaminate the D-forms. The α -keto analogue is then resynthesized to the L-form. He also stated that this D-amino acid oxidase enabled the organism to utilize D-amino acids from unnatural sources.

Rydon (1948) states that it seems the synthesis and utilization of D-amino acids involve α -keto or α -imino acids as intermediates. He generalizes the scheme thus: Where (a) and (b) are alternate routes.



This is presented as one possibility in the utilization of the D-aspartic acid. If this is correct it must be assumed that the aspartic

acid is used in the synthesis of the protein material of the cell. This does not seem to be an unreasonable assumption since other energy sources should be available and it is unlikely that Leuconostoc mesenteroides P-60 should require aspartic acid as a specific source of energy.

Until this work no evidence had been offered to show that D-aspartic acid was utilized by Leuconostoc mesenteroides P-60, Rydon, (1948). That it is utilized is shown by Tables III to IX. At high concentrations, (i.e. 1160 ug per tube) the response to D-aspartic acid exceeds that of L-aspartic acid. (See Figures V and VI). It will be noted that this is caused by a decrease in the response of L-aspartic acid and a continued increase in the response of D-aspartic acid at these higher concentrations.

It is interesting that in practically all cases, the percent response to D- in terms of L-aspartic acid is greater at lower concentrations of the aspartic acid. This may be a function of pH, because as the concentration of aspartic acid increases the acid production increases. It would also be anticipated that at the greater periods of time, where acid production is greater there would be less D- utilized in terms of L-aspartic acid. This is not true, however, but when the lag in utilization of the D-form is considered the results may not be out of keeping with what might be expected.

The response obtained with the D-form at 60 hours and a concentration of 40 ug of aspartic acid per tube are shown in Table XV.

Medium II exhibits a higher utilization of D-aspartic acid than either Medium I or III. In the case of Medium III low acid production with both

TABLE XV

Comparative Response at Concentration of
40 ug of Aspartic Acid Per Tube

60 hour culture

Basal Medium	ml NaOH		Percent of D- in Terms of L-aspartic acid
	D	L	
I	1.3	2.3	60
II	2.2	2.6	90
III	0.4	2.4	15

D- and L-aspartic acid was noted. This may be attributed to insufficient buffering. The pH fell very rapidly in this medium and perhaps inactivated the enzyme system before much acid could be produced. An explanation for the difference between Medium I and II is not as apparent. A difference in response to the D-form of methionine by Streptococcus faecalis between media buffered with citrate and acetate was reported by Lyman and Kuiken (1948). They found no utilization of the D-form in a medium buffered with acetate, but a considerable utilization in a medium buffered with citrate. The variation in utilization appears to be due to the difference in buffers which is usually not considered as having any influence except on pH. Additional investigations would be helpful here. The difference in response between Medium I and Medium II may also be due to unlike rates of change of pH. Cohen and Lichstein (1945) report that in certain trans-amination reactions there is an optimal pH and temperature.

If it is assumed that pH has an important effect on the utilization of D-aspartic acid it would appear that at the earliest times the greatest

utilization of D-aspartic acid in terms of L-aspartic acid should occur. This is not so. An examination of Tables IV and IX and Figures XI, XII, and XVIII shows that there is practically no utilization of the D-isomer during the first few hours of incubation. This slowness in utilization of the D-form has been noted previously. Rydon (1948) mentions that D-amino acids are utilized quite slowly with reference to L-amino acids, and that the organisms must be growing actively. He reported that Konikova and Nicolle found that D-alanine will substitute for L-alanine with both Salmonella typhimurium and Proteus X-19, but that growth is slower and preceded by a considerable lag. He stated that they were able to train the organisms to utilize D-alanine as readily as the L-isomer. An attempt was made to train Leuconostoc mesenteroides P-60 to D-aspartic acid by passing the organism through ten transfers in a medium containing only the D-isomer of aspartic acid. Although this may not have been sufficient to train the organisms it should have been sufficient to eliminate all but the variant causing utilization of the D-form, if there were such a variant. Training did not occur and that no culture of a variant developed is evidenced from a comparison of Figures V and VI.

The slowness of response to D-aspartic acid indicates that perhaps an enzymatic adaptation is taking place. Spiegelman (Cold Spring Harbor Symposia on Quantitative Biology, XI) defines an enzymatic adaptation thus: ("A population of cells placed in contact with some substrate acquires, after the lapse of some time, the enzymes necessary to metabolize the added substrate".) The production of an enzyme for this assimilation

could be a function of both time and the concentration of the D-form in the substrate. If this is true then the organism meets both of these requirements and the definition of Spiegelman. It appears to the author that this may be an example of enzymatic adaptation.

The effect of varying amounts of glutamic acid upon the utilization of aspartic acid was studied. The results are shown in Figures XV, XVI, and XVIII, and tabulated in Tables IX and XIV. Green, Leloir and Nicito (1945) reported an enzyme which they called aspartic-glutamic acid transaminase. They offered the following reaction: Glutamic acid + Oxalacetic acid $\rightarrow$ α -ketoglutaric acid + Aspartic acid. This reaction was also reported by Cohen and Lichstein (1945).

No evidence of transamination was found in this report. An unexpected decrease in the response to D-aspartic acid was noted at 40 ug of aspartic acid per tube with increasing concentrations of glutamic acid. A smaller decrease was noted at 80 and 120 ug of D-aspartic acid per tube, and the increase anticipated at 160 ug was not observed. The concentration of 4 mg of glutamic acid, which is used in this laboratory for assay, provided the most constant results. It is interesting to note (see Table IX) that at the lowest levels of glutamic acid the utilization of L-aspartic acid decreased as the amounts of this isomer increased. This phenomenon was not apparent with the D-isomer which would seem to indicate that perhaps different mechanisms were being affected.

Alanine was also varied, but it showed no significant changes in response of the organisms at various concentrations.

D- and L-asparagine were substituted for D- and L-aspartic acid in Basal Medium I. No growth was evident with either isomer of asparagine.

Although this work accomplished its major purpose by establishing that Leuconostoc mesenteroides P-60 utilizes the D-form of aspartic acid it left many questions unanswered. No clear explanation as to the mechanism of the utilization of D-aspartic acid was provided. The author's belief is that it is utilized by being oxidatively deaminated and then resynthesized. It is further believed that this oxidation is caused by an enzyme which is elaborated only under special conditions. Part of those conditions must be the presence of D-aspartic acid. A further study adding small amounts of L- to D-aspartic acid using DL-aspartic and the α -keto analogue would be interesting.

If titrations were made at close intervals and the pH controlled by varying the amounts of buffers used until the optimum was obtained, the extent of the lag, or period of adaptation could be much more sharply defined.

SUMMARY

A study of utilization of D-aspartic acid by Leuconostoc mesenteroides P-60 was made. The following conclusions were reached:

1. Leuconostoc mesenteroides P-60 utilizes the D-isomer of aspartic acid.
2. The utilization of the D-isomer is affected by the kind and quantity of the buffers present in the medium. The affect of buffers is in part, at least, due to differences in the rate of change of pH.
3. It is believed that the utilization is made possible by the organism adaptively elaborating enzymes perhaps capable of oxidative deamination and resynthesis.
4. Glutamic acid was varied with different concentrations of both D- and L-aspartic acid, but no final conclusions were drawn.
5. Varying the amounts of alanine has no appreciable affect on the utilization of D- or L-aspartic acid by this organism.
6. D- or L-asparagine will not substitute for D- or L-aspartic acid.

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