THE AVAILABILITY OF THE ESSENTIAL VITAMINS AND AMINO ACIDS FOR LACTOBACILLUS PLANTARUM IN CUCUMBER FERMENTATIONS

By

Ralph Norman Costilow

A THESIS

Submitted to the School of Graduate Studies of Michigan
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Lactobacillus plantarum, which is responsible for the desirable acid fermentation of cucumbers for salt stock, is known to require a number of vitamins and amino acids for growth. Therefore, these essential nutrients must be available in cucumber brines for a desirable fermentation to occur. If the concentration of any one or more of these nutrients was low enough to limit the growth of _L. plantarum_ very little acid production would be expected. This study included investigations of the vitamin and amino acid requirements of _L. plantarum_ isolates from cucumber fermentations and the availability in cucumber brines of those found essential.

Ten commercial and three laboratory fermentations were characterized for microbiological activity as a basis for the nutrition study. The vitamin and amino acid requirements of _L. plantarum_ were determined by titrating the acid produced by various isolates of this organism in lots of semi-synthetic or synthetic media deficient in the individual nutrients. Microbiological assay techniques were employed to determine the available concentrations in cucumber brines of the vitamins and amino acids found to be required by _L. plantarum_.

Under commercial conditions the acid-forming bacteria attained their maximum activity in the first 3 to 6 days and declined throughout the remainder of the fermentations period studied. Yeast populations declined during the first few days and then multiplied rapidly reaching their maximum in from 10 to 20 days after brining. The coliform organisms played no significant role in these fermentations. The microbiological activity in laboratory fermentations was similar except for the yeast picture; yeast activity was variable in these brines. The principal acid-forming organism was *L. plantarum*, and the most active yeast was *Torulopsis holmii*. However, in the laboratory fermentations and in one commercial tank *Torulaspora rosei* was predominant in the yeast flora.

L. plantarum isolates from cucumber fermentations were shown to require the vitamins--biotin, niacin, and pantothenic acid; and the amino acids--leucine, isoleucine, valine, glutamic acid, cystine, tryptophane, and threonine in the basal medium used. The strains tested were variable in their requirements for *p*-aminobenzoic acid, alanine, phenylalanine, arginine, and tyrosine.

Biotin, niacin, and pantothenic acid became available in cucumber brines very rapidly and the concentrations were relatively constant after the first 5 to 7 days. No great influence of fermentation on the concentrations of these vitamins was evident. Although the amino acids were considerably slower in reaching their maximum concentrations in the brine than the vitamins, in most fermentations they were present in sufficient concentrations within 24 hours to support the growth of *L. plantarum*.

Tryptophane was the only essential amino acid which was consistently affected by the fermentation. The level of this amino acid was markedly reduced in the brines at the same period when the yeast activity was greatest, and was also reduced by pure cultures of these microorganisms and by *L. plantarum* in control brine. Leucine, isoleucine, and valine were also rendered less available by the growth of brine yeasts in control brine and available cystine and glutamic acid concentrations were greatly lowered by the growth of a coliform isolate from cucumber fermentations.

The available concentrations of all of the nutrients studied were influenced by the size of the cucumbers; brines containing smaller cucumbers were richer.

The results indicated that the vitamins and amino acids were not limiting to L. plantarum in cucumber fermentations under these conditions. However, in fermentations where Aerobacter activity is prevalent during the first few days, cystine concentrations might well be reduced to a critical level. Also, it was indicated that excessive yeast activity early in the fermentation might result in very low tryptophane concentrations.

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THE AVAILABILITY OF THE ESSENTIAL VITAMINS AND AMINO ACIDS FOR LACTOBACILLUS PLANTARUM IN CUCUMBER FERMENTATIONS

INTRODUCTION

A large percentage of the cucumbers harvested yearly is preserved in the form of salt stock pickles until such time as they are needed. To prepare salt stock the cucumbers are placed in a large tank and covered with a brine ranging from 22° to 40° salometer (5.5 to 10.5 percent salt) depending on the salter and the climate. The brine strength is increased gradually over a period of several weeks until a salt concentration of 14.5 to 18.5 percent is reached. The osmotic influence of the brine solution withdraws the juice from the cucumbers which in turn supplies the nutrients necessary for fermentation. In a desirable fermentation much of the carbohydrate material in the cucumber juice is converted to lactic acid by lactic acid bacteria. The resulting acid and the salt produces an excellent medium which preserves the salt stock for extended periods of time--as long as three or four years.

The lactic fermentation of cucumbers for salt stock is dependent on the rapid utilization of sugars with acid production by the lactobacilli. Many of the microorganisms introduced into the brine with the cucumbers are inhibited by the relatively high salt concentration. However, there are some haloduric and halophilic organisms which may compete with the acid-formers for the sugar available. The rapid growth of such organisms results in an undesirable type of fermentation.

Lactobacillus plantarum is the organism commonly responsible for the acid fermentation. This species is notably fastidious in its nutritional requirements and is used in many microbiological assays for vitamins and amino acids. While there is no doubt that the nutritive elements in most brines are adequate to support the growth of this organism, it is possible that one or more elements may approach the critical level. Thus, in some cases an abnormally low level of proper nutrients may delay the development of *L. plantarum* to such an extent that an undesirable fermentation is obtained. It is also possible that the acid fermentation might be initiated in all fermentations more rapidly with less danger of spoilage organisms growing if only small amounts of some critical element were supplied initially.

Besides the acid fermentation by lactobacilli, definite gaseous fermentations due to *Aerobacter* and to many species of yeasts have been described. These organisms also may have a bearing on the nutritive elements available in the brine.

In this work several commercial and laboratory fermentations have been studied throughout the acid fermentation period to determine the organisms active in them and the nutritive elements available to these organisms. The vitamins and amino acids found to be essential for *L. plantarum* have been considered in the nutrition study.

REVIEW OF LITERATURE

Microbiology of Cucumber Fermentations

That *Lactobacillus plantarum* is the principal species involved in the acid fermentation of salt stock cucumbers has been well established by Etchells and Jones (16) and Rosen and Fabian (51). The former workers isolated and studied 49 strains of acid producing organisms from small scale commercial fermentations. All strains were found to answer the description of the species *L. plantarum*. Nineteen isolates from laboratory fermentations ranging from 2 to 20 days in fermentation age were identified by Rosen and Fabian as *L. plantarum*.

The rate of growth, total population attained, and the amount of acid produced by these organisms probably depends on a great number of factors. In studying the effect of various concentrations of salt, Fabian *et al.* (18) observed that the population of acid producing organisms reached a peak earlier and was higher in a 30° salometer brine (8 percent salt) than in a 40° salometer (10.6 percent salt) and the percent titratable acidity increased much more rapidly in the former. Etchells and Jones (15) and Jones and Etchells (29) using 20° (5.3 percent salt) and 40° salometer brines obtained similar results. They reported that the titratable acidity reached a maximum of about 0.7 percent in 8 to 9 days in the lower brine concentration and attained a maximum of only 0.4 percent in the higher salt concentration after a period of 12 to 13 days.

Other factors which have been investigated as to their possible influence on fermentation are bacteriophage, antibiotics, and changes in Eh (21). It was demonstrated by these workers that in certain cases bacteriophage from the soil, antibiotics produced by microorganisms, and/or low Eh values due to the growth of certain types of organisms may result in little or no lactic fermentation.

The next most prevalent and pronounced phase of the cucumber fermentation is that of the yeasts. The work of Etchells and his co-workers is notable in this field (11, 13). In fermentations under Southern conditions (11), the first and most prominent yeast activity was found to be predominantly that of a highly fermentative, non-sporulating yeast which was classified as a new species; *Torulopsis caroliniana*[1/]. This yeast became active in 4 to 5 days from the start of the fermentation, attained maximum populations of over one-million per ml in from 10 to 15 days, and died off very rapidly in 20 to 30 days. Following the *Torulopsis*, another non-sporulating species predominated; this yeast was also named as a new species--*Brettanomyces versatilis*[2/]. Although this yeast never attained the high populations of the *Torulopsis*, it was found consistently throughout the remainder of the fermentation period up to 100 days at which time the study was concluded. Two other yeasts which were believed to play some role in the fermentation were *Hansenula subpelliculosa* and an unnamed species of *Zygosaccharomyces*. In a study of the Northern fermentations over a 3 year period, Etchells *et al*. (13) found a somewhat similar pattern. The largest variation noted from the Southern fermentations was that *Torulopsis holmii* dominated the early fermentation period in place of Torulopsis caroliniana. However, the overall pattern of yeast fermentation in the Northern brines was quite similar to that observed in the South. Torulaspora rosei 3/ was believed to be active in a number of the fermentations but not all.

In Rosen and Fabian's work (51) with laboratory fermentations, no attempt was made to follow the predominating yeasts occurring in the brines since isolated colonies were picked at random. However, three species of yeasts were obtained that were found by Etchells et al. (13) to be common to Northern cucumber fermentations; viz., Hansenula subpelliculosa, Torulaspora rosei, and Torulopsis holmii.

A hydrogen fermentation due to Aerobacter which occurs soon after brining has been definitely established in commercial cucumber fermentations in the South (14, 15). Eighteen of 20 isolates from such fermentations were identified by Etchells et al. (14) as Aerobacter cloacae and the other two were considered as varieties. These authors noted that Aerobacter activity occurred most consistently in brines of 60° salometer (15.8 percent salt), while they were active in only some of the brines having an initial concentration of 20° or 40° salometer. However, the hydrogen fermentation was observed earlier in the brines of lower salt concentration than in the higher. Rosen and Fabian (51) noted an early Aerobacter fermentation in two laboratory experiments in

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1/ The Dutch authorities, Lodder and Kreger-Van Rij (40), have reclassified this yeast as Torulopsis lactis-condensi, 2/ as Torulopsis versatilis, and 3/ as Saccharomyces rosei. However, since this new classification scheme has not come into wide acceptance at this time, to avoid confusion, the original names will be used in this manuscript.

which the brines had initial salt concentrations of 30° salometer. In one instance a maximum population of about 5×10^5 per ml was reached in 2 days. These populations declined rapidly in both instances and were practically insignificant in from 4 to 5 days after brining. Twenty-nine isolations were made at random of these organisms, and all proved to belong to the genus *Aerobacter*. Twenty-one isolates were identified as *Aerobacter cloacae*.

In addition to the above types of fermentations, luxuriant films due to oxidative yeasts are always found where the brining tanks are not exposed to the direct rays of the sun and the surface is not disturbed. These yeasts have been studied extensively by Mrak and Bonar (46) and Etchells and Bell (12). In both studies, the predominating yeast growing on the surface of salt stock fermentations was found to belong to the genus *Debaryomyces*. Etchells and Bell (12) classified this yeast *Debaryomyces membranaefaciens* var. *Hollandicus*.

Vitamin and Amino Acid Requirements of *L. plantarum*

Great progress has been made in the last two decades in the study of the nutritional requirements of microorganisms and their application to microbiological assay techniques for various vitamins and amino acids. The lactic acid bacteria have been the subject of many investigations due to their requirements for many of these nutrients.

The species *L. plantarum* is one of the lactic acid organisms used most commonly in microbiological assays. This review will be restricted to the requirements of this organism and its synonyms[1/].

Vitamin requirements. In a comprehensive review of the relation of bacteria to vitamins and other growth factors, Peterson and Peterson (47) noted that biotin, niacin, pantothenic acid, and riboflavin were the most frequently reported as essential for microorganisms. All of these vitamins except riboflavin have been established as essential for *L. plantarum*. Thus, Snell and his co-workers (60, 61, 62) have demonstrated the requirements of *L. arabinosus* 17-5 for nicotinic acid, pantothenic acid, and biotin. Cheldelin *et al* (9) demonstrated that 33 strains of lactic acid bacteria including one labeled as *L. plantarum*, two as *L. arabinosus*, and two as *L. pentosus* required pantothenic acid. Rosen and Fabian (51) observed that 10 isolates of *L. plantarum* from cucumber fermentation all required biotin, niacin, and pantothenic acid as did *L. arabinosus* 17-5. Similar results were reported by Kreuger and Peterson (32) for *L. pentosus* 124-2.

By increasing the concentrations of the vitamins and amino acids several fold over those commonly used and lengthening the incubation time up to about 7 days, Shankman *et al*. (56) obtained greatly different results than those previously noted. Thus, it was observed that biotin, pantothenic acid, and niacin were not essential for *L. arabinosus* 17-5 when a 7-day incubation period was used but were necessary for maximum

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1/ The 6th edition of Bergey's Manual (5) lists 19 probable synonyms of this species including *Streptobacterium plantarum*, *Lactobacillus arabinosus*, *Lactobacillus pentosus*, *Lactobacillus brassicae*, and *Bacillus cucumeris fermentatae*. Therefore, studies of the nutrient requirements of bacteria designated by these names are included in this review.

acid production during a short incubation period. With L. pentosus 124-2 and L. brassicae (8041) nicotinic acid was still noted as essential after 7 days incubation and the acid produced in the absence of either biotin or pantothenic acid was less than one-half that in the control.

There are conflicting reports in the requirements of L. plantarum for p-aminobenzoic acid. Snell and Mitchell (58) noted that this vitamin was non-essential for L. arabinosus 17-5 and for L. pentosus, while Isbell (37) and Lewis (35) observed it to be essential for L. arabinosus. Krueger and Peterson (32) found p-aminobenzoic acid to be stimulatory for L. pentosus when specially prepared casein was used. However, in the concentrated synthetic medium of Shankman et al. (56) the absence of this vitamin had no apparent affect on the acid production of either L. arabinosus 17-5 or L. pentosus 124-2.

Snell and Strong (59) noted that riboflavin was not required by L. arabinosus 17-5, L. pentosus 124-2, nor by L. brassicae (8041). Similar results were obtained by Campbell and Hucker (8) with cultures labeled L. arabinosus F-17-5, Bacillus cucumeris fermentatae L-25, and two strains identified as L. plantarum. However, two other cultures labeled L. plantarum would not grow in the medium regardless of the riboflavin content, and Lactobacillus plantarum var. rudensis was noted to require this vitamin.

Bohonos et al. (3, 4) reported that pyridoxine was not required by either L. arabinosus or L. pentosus but was synthesized by them. However, Shankman et al. (56) noted that both L. arabinosus 17-5 and L. brassicae (8041) were stimulated by pyridoxine when short incubation periods were used. No stimulation of L. pentosus was observed.

Neither thiamine or folic acid were noted bo be essential for L. arabinosus 17-5 by Shankman et al (56) or Baumgarten et al (1). However, the latter workers have noted some stimulation by folic acid. L. pentosus did not require these two vitamins (32, 56).

Snell and Wright (62) observed that pyridoxine, thiamine, and riboflavin stimulated the growth of L. arabinosus 17-5 during the first few hours of incubation. Therefore, the addition of these vitamins to assay media for this organism was considered desirable.

Amino acid requirements. The findings of the various workers on the amino acid requirements of L. plantarum are still more conflicting than on the essential vitamins. Starting with the report of Möller (45) who reported that Streptobacterium plantarum 10S required glutamic acid, leucine, aspartic acid and valine for growth in a chemically defined medium, practically every investigation has resulted in different observations as to the essential amino acids for this organism. Even on the strain of L. arabinosus 17-5 there have been considerable differences in the observations made in various laboratories. The results obtained in five investigations of the requirements of this strain are summarized in Table 1.

The number of amino acids noted to be required by L. arabinosus 17-5 varied from 10 (24) to 5 (10), but five amino acids (viz, glutamic acid, valine, leucine, isoleucine, and tryptophane) were noted to be essential in all five investigations. Cystine was found to be essential in all instances except when a greatly enriched medium was used (10). Even in this medium some of the data indicate that there was some

TABLE 1

The amino acid requirements of Lactobacillus arabinosus 17-5 as noted by various workers

	Shankman (55)	Kuiken et al. (33)	Hegsted (24)	Lyman et al. (43)	Dunn et al. (10)
Glutamic acid	+ 1/	+	+	+	+
Valine	+	+	+	+	+
Leucine	+	+	+	+	+
Isoleucine	+	+	+	+	+
Tryptophane	+	+	+	+	+
Cystine (or cysteine)	+	+	+	+	-
Methionine	+	-	+	-	± 2/
Arginine	-	-	+	-	±
Phenylalanine	±	+	+	-	-
Tyrosine	±	-	+	-	-
Threonine	+	+	-	-	-
Lysine	-	+	-	-	-

1/ A + indicates that the amino acid was noted to be essential, and a - that it was not essential.

2/ ± The results of some experiments indicated these amino acids were required while in other instances they were not.

stimulation of acid production when cysteine was added. Riesen _et al._ (49), also noted that cystine was not absolutely essential for _L. arabinosus_ 17-5 but was stimulatory.

Such variations in results indicate that either variations occurred in the cultures or that differences in the methods of determining the amino acid requirements influenced the response of the organism. Strains of _L. arabinosus_ which did not require tyrosine and others which did not require tryptophane were developed by James (28) and by Wright and Skeggs (71) respectively by growing on media deficient in these amino acids. Dunn _et al._ (10) have demonstrated that six different cultures of _L. arabinosus_ 17-5 carried for more than two years on three different media all had the same amino acid requirements.

Lyman _et al._ (43) have conclusively demonstrated a relationship between the composition of the medium used for testing and the amino acids found to be essential. Thus, these workers observed that threonine, lysine, and alanine were essential for _L. arabinosus_ 17-5 when pyridoxine was omitted, but not essential in its presence. Arginine, phenylalanine, and tyrosine were noted to be essential in the presence of pyridoxine if CO_2 was not available, but not required in the presence of both vitamin B_6 and CO_2. However, pyridoxine was not required by this organism when all these amino acids were present. The requirements for valine, leucine, isoleucine, tryptophane, cystine, and glutamic acid were unchanged by the presence of pyridoxine or of both CO_2 and pyridoxine.

Stokes and Gunness (64) noted that pyridoxamine in a basal medium would eliminate the requirements of _L. arabinosus_ 17-5, _L. casei_ and _L. delbruckii_ for lysine, threonine, and alanine but pyridoxine would not.

However, if a basal medium containing pyridoxine was sterilized at 15 pounds for 30 minutes similar results were obtained as when pyridoxamine was used. It was suggested that pyridoxamine may be formed during the sterilizing process when pyridoxine is present. These observations might well account for many of the conflicting results obtained in the various investigations.

Impurities in individual amino acid preparations are another source of variation in results. Hegsted and Wardwell (26) have shown that some commercial samples of synthetic DL-leucine contained appreciable amounts of isoleucine. A similar observation was made on one commercial batch of L-leucine in this investigation (unpublished data).

That there may be considerable variation between strains of the same species was demonstrated by Dunn *et al.* (10). Thus, one strain of *Leuconostoc mesenteroides* was found to require 15 amino acids while another strain required only two. In this same report, it was noted that *L. pentosus* and *L. brassicae* had identical amino acid requirements; glutamic acid, valine, isoleucine, leucine and cysteine were essential for both. *L. arabinosus* 17-5, however, did not require cysteine, but required tryptophane and in some instances methionine and arginine. *L. plantarum* (strain No. 8004 from the American Type Culture Collection) would not grow in the enriched medium used by Dunn *et al.* (10).

Krueger and Peterson (32) reported valine, leucine, isoleucine, glutamic acid, and phenylalanine as essential for *L. pentosus* 124-2. Cystine, threonine, and alanine stimulated growth, but the omission of tryptophane from the basal medium had no effect on this strain.

In most instances, only the naturally occurring isomers (the L-forms) have been found to be effective in promoting growth of L. plantarum. Thus, Kuiken et al. (33) have shown that only the isomers having the L-configuration of leucine, isoleucine, and valine were capable of promoting growth of L. arabinosus 17-5. Hegsted (25) obtained similar results except that some response to D-leucine was noted after long incubation periods. However, this response was not believed to be great enough to influence the results of assays for this vitamin when DL-leucine was used to prepare the standard curves.

Snell (57) and Greene and Black (22) noted that while only the L-form of tryptophane was active with L. arabinosus, both indole and anthranilic acid would replace tryptophane, but that these two naturally occurring substances could be readily removed from samples by ether extraction. Snell (57) observed that L. pentosus would not utilize either indole or anthranilic acid in place of tryptophane.

Microbiological assays for glutamic acid using L. arabinosus 17-5 are not specific as glutamine has been shown to produce a greater growth response of this organism than glutamic acid (23, 36, 41). The D-form of glutamic acid was noted to have some activity in the absence of the naturally occurring isomer (41). Pollack and Lindner (48) observed that glutamine would also replace glutamic acid for L. pentosus.

Riesen et al. (49) noted that L-cysteine had about the same activity as L-cystine for L. pentosus 124-2. Other sulfur containing compounds were also found to have some activity.

Nutrients Essential for L. plantarum in Cucumbers, Cucumber Brines and Pickles

Until recently, the only essential nutrient for the acid fermentation of cucumbers which had been investigated as such was reducing sugar. Thus, Fabian and Wickerham (20) noted that the addition of sucrose late in the fermentation resulted in an increase in the number of acid producing bacteria. However, Jones *et al.* (30) and Veldhuis *et al.* (67) demonstrated that while the addition of sucrose brought about an increase in the population of acid-forming bacteria, the titratable acidity formed in such brines was no higher than that in control lots. This was observed in both dill and salt stock fermentations. Yeast populations were also noted to be higher in fermentations to which sucrose was added. From these results it would appear that some other factor was limiting the amount of acidity formed.

Vitamins. Practically no information was available as to the concentrations of biotin, niacin and pantothenic acid in cucumbers and in the brines of fermenting cucumbers until the recent report of Rosen and Fabian (51). Due to their importance to this investigation the results obtained by these workers are briefly summarized as follows:

1. In a study of the biotin, pantothenic acid and niacin available to *L. arabinosus* 17-5 in the extracts of six different varieties of cucumbers the following observations were made:

    a. Considerable variation occurred in the vitamin concentration in the juice from different cucumbers but no significant difference between varieties of cucumbers was evident.

    b. Biotin concentrations ranged from 5.2 to 33.0 mμg/ml, niacin from 1.83 to 5.05 μg/ml and pantothenic acid from 1.05 to 2.42 μg/ml of cucumber juice.

2. The available concentrations of these three vitamins in the brines of two laboratory fermentations were studied through the first 20 days of fermentation. It was noted that the concentration of all three reached their maximum in the first 5 to 6 days, and did not greatly change thereafter. Sufficient vitamins were available throughout the fermentation to support the growth of *L. plantarum*.

3. The vitamin content of the cucumbers was greatly diminished after 20 days in brine.

While no effort was made to extract the bound forms of these vitamins, the concentrations observed by Rosen and Fabian are those of importance to cucumber fermentations. According to the work of Lampen *et al.* (34), most vegetables have a water extractable form of biotin so the concentrations of this vitamin observed by Rosen and Fabian are probably close to the total concentrations. In a compilation of nutrient data for various foodsby the Heinz Co. (70) the concentration of nicotinic acid in cucumbers is given as 0.2 mg. per 100g (2 μg per g); this compares favorably with the data noted above.

Amino acids. No information was found in the literature on the amino acid composition of cucumbers or pickles. However, Camillio *et al.* (7) noted the crude protein content of various cucumbers to vary from 0.7 to 1.4 percent, and this was reduced significantly after fermentation for salt stock or dill pickles. In the nutritive charts prepared by the Heinz Co. (70) the average total protein content of fresh cucumbers is given as 1.1 percent, while that for fresh cucumber pickles is 0.8 percent and for dill pickles is 0.7 percent.

Effect of Brine Microorganisms on Vitamins and Amino Acids

While there has been considerable work on the synthesis of vitamins and some reports on the destruction of vitamins by microorganisms, most of these reports are not pertinent to this study as the activity of various species and groups of microorganisms are greatly different. This review is restricted to those investigations of microorganisms which are closely related to those found in cucumber fermentations.

Since *L. plantarum* requires niacin, biotin, and pantothenic acid for growth, it would be expected to lower the concentrations of these vitamins in the growth medium. Rosen and Fabian (52) noted, however, that while the biotin content of an assay medium was lowered greatly, the concentration of biotin in cucumber juice was not greatly affected by *L. plantarum*. Since it was found that Tween 80 had a biotin sparing action in the assay medium, it was suggested that cucumber juice may have a lipoidal substance present which is capable of substituting for biotin.

The relationship of oleic acid and of Tween 80 (an esterified form of oleic acid) to biotin requirements of lactic acid organisms has been observed by Kodicek and Worden (31), Williams and Fieger (69), and Williams *et al.* (68).

A number of investigators have noted that the coliform group of organisms are capable of synthesizing vitamins (6, 44, 66). However, Rosen and Fabian (51, 52) found that *Aerobacter cloacae* from cucumber fermentations would also destroy biotin in cucumber juice and other media if a substantial quantity of biotin is present initially. This organism synthesized this vitamin in the same medium when no biotin was added. No appreciable effect of *Aerobacter cloacae* was noted on the niacin and pantothenic acid content of cucumber juice (51).

Various species of yeasts vary greatly in their vitamin requirements and in their ability to synthesize vitamins. In a study of 18 species of osmophilic yeasts, all belonging to the sub-genus *Zygosaccharmyces*, Lochhead and Landerkin (38) noted that all strains required biotin and some required pantothenate for growth. Rogosa (50) noted that 114 strains of lactose fermenting yeasts required nicotinic acid or nicotinamide. *Torulopsis utilis* was noted by Lewis *et al*. (37) to synthesize significant quantities of biotin, niacin, and pantothenic acid in media such as fruit and prune juice, and molasses.

Rosen and Fabian (51) studied the effect of six species of yeasts isolated from cucumber fermentations on the biotin, niacin and pantothenic acid content of cucumber juice. The growth of four species (*viz*, *Hansenula subpelliculosa*, *Torulopsis caroliniana*, *Torulopsis holmii*, and *Torulospora rosei*) resulted in a marked decrease in the biotin level. The niacin and pantothenic acid concentrations were not greatly affected although some increase in the concentration of the latter was noted with three yeast species.

No information was found concerning the effect of brine microorganisms on the various amino acids. However, since *L. plantarum* has been shown to require several amino acids for growth this organism must deplete the concentration of these amino acids in the growth medium.

EXPERIMENTAL PROCEDURES AND METHODS

Fermentations Studied and Methods of Sampling

A total of 13 fermentations were studied--10 commercial and 3 in the laboratory. The 10 tanks of fermenting cucumbers under commercial conditions were located at the salting station of the H. W. Madison Co. at Mason, Michigan. All fermentations were brined within a 20 day period during the month of August 1952. The commercial tanks were of the regular wooden variety and ranged in size from 700 to 1100 bushels capacity. Five tanks were filled with size No. 1 cucumbers and the other five were filled with a mixture of sizes No's. 2 and 3 cucumbers. The 3 laboratory experiments were carried out in 5-gallon crocks over which an ultraviolet light was used to prevent growth of film yeasts. The same variety of cucumbers, Davis, grown on the same plot of ground was used in filling all three crocks. Crock FC 1 contained size No. 1 cucumbers, crock FC 2 contained size No. 2 and crock FC 3 was filled with No. 3 size cucumbers.

Samples of the brine were taken daily from all fermentations for a period of 15 days and then at increasingly greater intervals. The last samples of the laboratory brines were taken 36 days after the cucumbers were salted and from the commercial brines at from 51 to 71 days, depending on the tank, after the cucumbers were salted.

The sampling technique used for the large tanks was essentially that recommended by Etchells and Jones (17) except that only three perforations (about 2mm in diamter) were made in the 3/16-inch (inside diameter) stainless steel tube. These perforations were spaced 18 inches apart, starting at the sealed end of the tube. The tube was 6 feet long and the tanks about 7 to 8 feet deep. Thus, a composite sample was obtained including brine from various depths of the tank. The laboratory brines were sampled in much the same manner except that a glass tube was used and the open end moved slowly up from the bottom to the top as the sample was being withdrawn.

On removal from the tanks the brine samples were immediately returned to the laboratory and chemical and bacteriological analysis made. The samples were then held at -18.5°C (0°F) until time was available for the nutritional studies which were made later.

Chemical and Bacteriological Methods

Brine subsamples were titrated with standard NaOH to determine titratable acidity and with standard $AgNO_3$ using dichlorofluorscein as an indicator to determine percent salt.

The V-8 agar with brom cresol green indicator devised by Fabian *et al.* (19) was used in the enumeration and isolation of acid-producing bacteria, and dextrose agar acidified with 5 ml of 5 percent tartaric acid per 100 ml of medium for yeasts. Lauryl-tryptose broth tubes were inoculated in triplicate with various dilutions of the samples to determine members of the coliform group. All incubation was done at 30° C. Three days incubation was allowed for the acid-formers, two days for the coliforms, and five days for the yeasts.

Microbiological Assays for Vitamins and Amino Acids

This study was concerned with the vitamins and amino acids available in cucumber brines for the growth of *L. plantarum*. Therefore, the brine samples were not treated to release the bound vitamins or hydrolyzed to free amino acids from protein material prior to running the microbiological assays.

The brine samples were removed from the freezer and allowed to thaw in a refrigerator at about 4.5° C just prior to running the assays. The acid in measured portions of the brine samples from fermenting cucumbers was neutralized with N NaOH and the samples made up to volume with distilled water to give either a 1:5 or 1:10 dilution as desired. This solution was stored in the refrigerator under toluene. Further dilutions were made at the time of the individual assays from this original as needed.

The dehydrated media prepared by Difco Laboratories, Inc. were used to assay the samples for biotin, niacin and pantothenic acid. *L. plantarum* 17-5 was used as the test organism. The techniques employed in the assays were essentially the same as those described in "Methods of Vitamin Assay" (65).

Assays for leucine, isoleucine, valine, and glutamic acid were run as outlined by Sauberlich and Baumann (54) using medium I and *L. plantarum* 17-5 as the test organism. Difco's dehydrated medium was used for tryptophane assays with *L. plantarum* 17-5. The method of Lyman *et al.* (42) as modified by Sarkar *et al.* (53) was used for the determination of cystine. *Leuc. mesenteroides* P-60 was the test organism used in this determination.

The individual amino acids used were obtained from either Nutritional Biochemicals Corp., Merck and Co., or Pfanstiehl Chemical Co.

RESULTS

Microbiology of Fermentations

Sequence of organisms in the fermentations. With a few exceptions, the ten commercial fermentations studied were found to have similar patterns with respect to acid-forming organisms, yeasts, and coliforms. The general pattern of acid-forming bacteria and yeast activity in the fermentations studied is illustrated in Fig. 1. This represents the average populations of the 10 fermentations at each time interval. The complete results of this study are given in Tables 2-11.

In general the acid-forming bacteria count was found to rise sharply within 1 to 3 days after brining; reaching a peak within 5 to 6 days. The total numbers of these organisms declined rapidly for the following 5 to 10 days, and then continued to decline at a slower rate for the remainder of the fermentation period studied. However, the maximum populations obtained in the various brines varied from 5.5×10^7 (Table 6) to 1.29×10^9 per ml (Table 4). Six of the ten fermentations had maximum populations ranging from 3×10^8 to 6×10^8 per ml.

In contrast to the acid-producing bacteria, the yeasts were generally found to decline in number during the first few days after brining, started rapid growth after about 5 days, and reached their maximum population in from 10 to 20 days of fermentation. Thereafter, there was a steady decline in yeast population. The maximum populations attained were quite variable, ranging from 1.57×10^5 per ml in tank 23 (Table 6) to 2.6×10^6 per ml in tank 14 (Table 3).

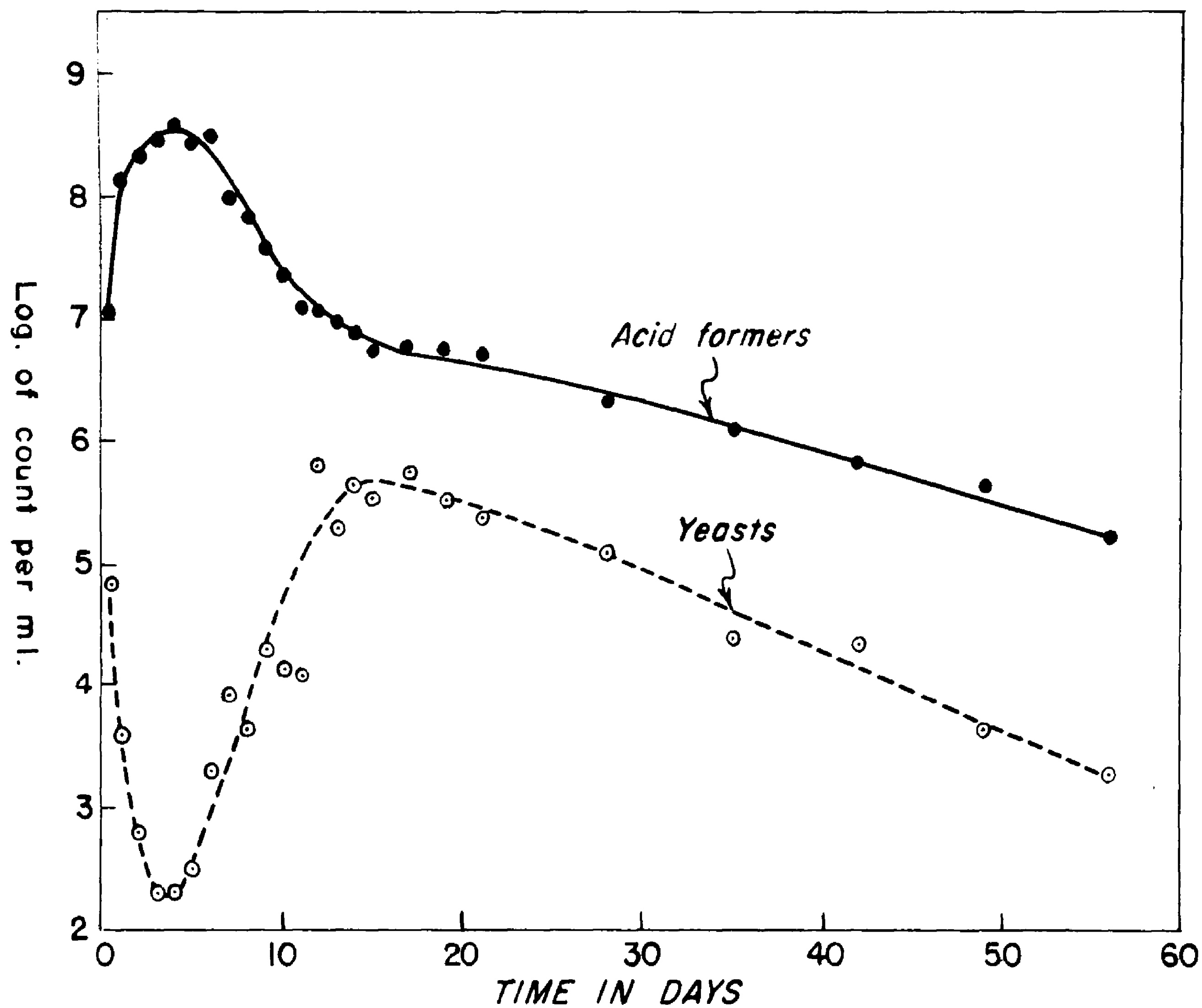

Fig. 1. Average fermentations by acid-forming bacteria and yeasts in 10 commercial tanks of cucumbers.

TABLE 2

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 13 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	6.25	0.059	95.0	1.7	4,300,000
1	7.30	0.263	620.0	0.2	1,500
2	6.85	0.398	540.0	0.1	4,300
3	6.65	0.435	610.0	-	430
4	8.30	0.368	134.0	0.5	24
5	7.75	0.383	50.0	-	250
6	8.50	0.390	11.9	1.0	*
7	8.55	0.345	16.3	1.0	43
8	8.25	0.443	53.0	5.6	43
9	8.40	0.480	44.0	7.5	*
10	8.50	0.510	34.0	3.6	*
11	8.70	0.510	10.6	4.6	*
12	8.55	0.495	15.2	3.3	*
13	8.80	0.525	21.0	2.3	*
14	8.70	0.525	5.7	0.9	*
15	9.10	0.503	5.4	14.0	*
17	8.90	0.615	8.8	140.0	*
19	9.30	0.555	13.2	-	*
21	9.55	0.533	16.4	310.0	*
24	9.70	0.465	15.1	51.0	*
29	10.00	0.503	4.6	4.2	*
36	10.20	0.435	4.1	2.0	-
43	11.15	0.443	0.9	3.0	-
50	10.90	0.420	0.3	1.7	-
57	11.20	0.450	0.14	2.7	-
64	11.00	0.437	0.004	-	-

1/ Tank No. 13 contained 718 bushels of No. 1 size cucumbers.

2/ The zero time sample was taken eight to ten hours after covering the cucumbers with brine.

* = Less than 10/ml.

- = No determination made.

TABLE 3

Percent acid and salt and the populations of various groups of microorganisms in tank No. 14 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	11.10	0.008	*	**	43,000
1	6.60	0.008	0.1	12.0	920,000
2	7.50	0.015	0.3	***	9,200
3	8.95	0.045	4.3	1.0	25,000
4	8.40	0.135	125.0	***	9,200
5	9.10	0.218	360.0	-	250
6	8.40	0.308	360.0	****	*****
7	9.95	0.210	95.0	****	*****
8	10.90	0.255	80.0	****	*****
9	8.90	0.375	68.0	0.1	*****
10	8.50	0.405	28.0	0.5	*****
11	9.20	0.360	14.5	-	*****
12	9.65	0.330	5.4	29.0	*****
13	9.65	0.405	2.8	540.0	*****
14	10.15	0.345	2.5	119.0	*****
15	9.50	0.450	2.8	590.0	*****
17	9.65	0.428	3.4	2,600.0	*****
19	9.60	0.495	1.3	680.0	*****
21	9.85	0.488	0.72	310.0	*****
24	10.95	0.390	1.48	250.0	*****
28	11.05	0.465	0.64	93.0	-
35	10.70	0.548	1.04	23.0	-
42	10.75	0.525	0.70	1.3	-
49	11.10	0.563	0.44	0.60	-
56	10.80	0.555	0.25	0.1	-

1/ Tank No. 14 contained 706 bushels of No. 1 size cucumbers.
2/ The first sample was taken one to three hours after the cucumbers were covered with brine.
  * = Less than 10 million/ml.
  ** = Less than 10 thousand/ml.
  *** = Less than 1 thousand/ml.
  **** = Less than 100/ml.
  ***** = Less than 10/ml.
  - = No determination made.

TABLE 4

Percent acid and salt and the populations of various groups of microorganisms in tank No. 21 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	8.60	0.008	5.6	-	920
1	7.95	0.128	215.0	0.1	4,300
2	7.95	0.263	850.0	*	43,000
3	6.60	0.323	1,290.0	*	430
4	8.10	0.375	1,060.0	*	920
5	7.95	0.368	520.0	*	43
6	8.20	0.420	540.0	**	43
7	8.80	0.405	118.0	*	25
8	8.25	0.398	87.0	0.49	***
9	8.35	0.383	19.3	5.0	250
10	8.30	0.428	14.7	5.9	***
11	8.30	0.443	15.0	19.0	***
12	8.45	0.450	17.1	480.0	25
13	8.90	0.465	22.0	-	***
14	8.45	0.495	20.0	1,290.0	***
15	8.80	0.488	7.2	680.0	***
17	9.00	0.510	12.0	830.0	***
19	9.10	0.510	11.4	350.0	***
21	9.10	0.548	11.2	79.0	***
24	9.55	0.525	8.1	24.0	***
30	9.90	0.518	2.6	6.8	-
37	9.90	0.525	0.28	0.30	-
44	9.90	0.525	0.19	0.9	-
51	10.20	0.570	0.028	0.1	-
58	10.20	0.533	0.10	0.8	-

1/ Tank No. 21 contained 717 bushels of No. 1 size cucumbers.

2/ The first sample was taken six to eight hours after the cucumbers were covered with brine.

* = Less than 100/ml.

** = Less than 1,000/ml.

*** = Less than 10/ml.

\- = No determination made.

TABLE 5

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 22 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	9.35	0.015	1.7	3.0	9,200,000
1	4.25	0.173	440.0	4.0	2,500,000
2	8.00	0.195	490.0	0.5	43,000
3	8.60	0.248	400.0	*	2,500
4	8.30	0.315	249.0	-	**
5	8.70	0.360	118.0	*	250
6	8.60	0.360	137.0	*	43
7	8.30	0.353	38.0	*	25
8	8.30	0.413	40.0	280.0	**
9	8.40	0.428	48.0	-	**
10	8.20	0.420	11.2	64.0	**
11	8.50	0.428	13.8	320.0	**
12	9.10	0.435	12.2	2,400.0	**
13	9.25	0.390	1.9	123.0	**
14	8.60	0.435	2.2	1,360.0	**
15	8.45	0.533	2.6	1,170.0	**
17	9.00	0.518	4.1	500.0	**
19	9.20	0.510	5.4	23.0	**
21	9.50	0.510	6.6	44.0	**
24	10.10	0.480	6.3	4.9	**
28	9.85	0.443	4.8	0.9	**
35	10.50	0.375	0.93	0.5	-
42	10.80	0.383	0.45	0.2	-
49	10.80	0.390	0.34	3.2	-
56	11.40	0.390	0.13	1.0	-
63	10.85	0.375	0.22	0.21	-

1/ Tank No. 22 contained 702 bushels of No. 1 cucumbers.
2/ The first sample was taken five to eight hours after the cucumbers were covered with brine.
* = Less than 100/ml.
** = Less than 10/ml.
\- = No determination made.

TABLE 6

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 23 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	10.68	0.034	1.4	6.3	920
1	11.95	0.045	1.6	1.2	4,300
2	10.95	0.062	24.3	2.0	43,000
3	11.30	0.117	55.0	*	430
4	10.50	0.189	54.0	0.7	920
5	10.70	0.230	37.0	*	43
6	11.05	0.199	41.0	*	43
7	10.35	0.276	39.0	*	25
8	10.90	0.203	7.6	*	**
9	10.45	0.300	17.0	3.9	250
10	10.70	0.293	6.4	9.7	**
11	10.60	0.300	8.8	157.0	**
12	10.50	0.323	7.0	-	25
13	10.60	0.330	2.6	104.0	**
14	11.15	0.315	3.12	67.0	**
15	10.90	0.315	1.24	89.0	**
17	11.10	0.308	0.64	6.0	**
19	10.45	0.423 3/	0.07	125.0	**
21	10.85	0.525	2.29	95.0	**
24	10.40	0.623	0.83	74.0	**
29	11.00	0.578	1.67	6.7	**
36	10.80	0.638	0.84	3.6	**
43	11.00	0.585	0.17	15.7	-
50	11.30	0.578	0.39	2.3	-
57	11.30	0.593	0.041	2.3	-
64	11.50	0.615	0.003	2.4	-
71	11.45	0.600	0.0003	1.0	-

1/ Tank 23 contained 701 bushels of No. 1 size cucumbers.

2/ The first sample was taken eight to ten hours after the cucumbers were covered with brine.

3/ Two-thirds of a barrel of vinegar and a barrel of brine from an actively fermenting tank were added to tank 23 between the 17th and 19th days of sampling.

* = Less than 100/ml.

** = Less than 10/ml

\- = No determination made.

TABLE 7

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 24 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	13.60	0.027	0.68	4.2	2,500
1	12.80	0.039	0.99	3.0	9,200
2	9.90	0.032	2.2	0.4	25,000
3	9.80	0.075	29.0	0.24	9,200
4	9.70	0.143	35.0	*	**
5	8.80	0.209	73.0	0.53	15
6	11.30	0.201	81.0	3.3	92
7	9.70	0.228	57.0	0.43	43
8	9.70	0.248	46.0	102.0	25
9	9.65	0.240	37.0	10.7	25
10	9.65	0.255	19.0	4.0	15
11	9.45	0.270	27.0	79.0	**
12	9.70	0.300	26.4	-	25
13	9.50	0.345	8.5	-	**
14	9.70	0.354	8.7	240.0	**
15	9.50	0.398	3.1	210.0	**
17	9.50	0.443	5.1	300.0	**
19	9.40	0.495 3/	2.1	770.0	**
21	9.90	0.480	0.26	160.0	**
24	9.55	0.585	0.26	114.0	**
29	10.75	0.555	0.93	240.0	**
36	10.60	0.570	0.091	17.6	**
43	11.10	0.548	0.020	114.0	-
50	11.15	0.563	0.015	16.0	-
57	10.95	0.570	0.023	6.0	-
64	12.80	0.548	0.015	5.7	-
71	11.40	0.525	0.009	3.1	-

1/ Tank 24 contained 712 bushels of sizes No's. 2 and 3 cucumbers.

2/ The first sample was taken eight to ten hours after the cucumbers were covered with brine.

3/ One-third of a barrel of vinegar and one barrel of brine from an actively fermenting tank were added to tank 24 between the 17th and 19th days of sampling.

* = Less than 100/ml.

** = Less than 10/ml.

\- = No determination made

TABLE 8

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 32 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	14.40	0.008	0.002	0.1	4,300
1	10.10	0.015	0.002	0.2	2,900
2	8.60	0.045	0.013	0.2	4,300
3	8.55	0.045	0.120	*	2,500
4	7.90	0.248	236.0	*	43
5	7.75	0.285	340.0	*	**
6	8.40	0.293	162.0	-	**
7	8.45	0.330	81.0	*	**
8	8.45	0.375	58.0	*	**
9	8.75	0.420	35.0	*	**
10	8.40	0.480	19.5	*	**
11	8.70	0.480	16.3	*	**
12	8.90	0.480	19.2	*	**
13	8.65	0.555	18.3	0.2	**
14	8.90	0.570	18.1	4.0	**
15	8.95	0.585	19.7	5.5	**
17	9.20	0.615	8.9	10.0	**
19	9.30	0.623	11.2	165.0	**
23	9.50	0.653	1.4	350.0	-
30	9.75	0.668	0.98	235.0	-
37	9.75	0.630	1.1	17.6	-
44	10.00	0.690	0.43	11.2	-
51	10.00	0.690	0.46	14.1	-

1/ Tank No. 32 contained 1100 bushels of No's. 2 and 3 sizes cucumbers.
2/ The first sample was taken eight to ten hours after covering the cucumbers with brine.
* = Less than 100/ml.
** = Less than 10/ml.
- = No determination made.

TABLE 9

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 33 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0	10.60	0.003	0.006	0.3	430
1	9.25	0.015	0.016	1.0	2,500
2	9.55	0.023	0.015	*	2,500
3	7.85	0.038	0.44	0.1	1,500
4	6.95	0.128	94.0	**	74
5	8.60	0.075	25.1	**	92
6	7.50	0.263	204.0	**	***
7	7.40	0.300	230.0	**	***
8	7.60	0.323	128.0	0.2	***
9	8.30	0.323	54.0	-	***
10	8.50	0.360	45.0	2.1	***
11	8.60	0.360	9.6	1.4	***
12	8.55	0.413	13.0	0.72	***
13	8.80	0.450	6.6	13.1	***
14	8.60	0.458	9.8	16.5	***
15	8.60	0.480	6.9	52.0	***
17	9.00	0.510	8.1	79.0	***
19	8.70	0.518	7.2	180.0	***
22	9.05	0.555	3.4	520.0	***
26	9.40	0.548	1.9	148.0	-
33	9.75	0.548	2.2	64.0	-
40	9.85	0.578	2.5	41.0	-
47	10.20	0.555	1.9	1.3	-
54	10.15	0.563	0.67	0.3	-

1/ Tank 33 contained 1153 bushels of sizes No's. 2 and 3 cucumbers.
* = Less than 1,000/ml.
** = Less than 100/ml.
*** = Less than 10/ml.
\- = No determination made.

TABLE 10

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 34 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	9.80	0.008	0.1	0.4	14,000
1	7.00	0.030	0.2	1.4	92,000
2	6.10	0.128	175.0	1.0	2,500
3	6.30	0.218	280.0	0.18	25
4	6.10	0.263	440.0	0.10	*
5	6.25	0.278	460.0	0.20	*
6	6.60	0.270	231.0	0.40	*
7	6.40	0.293	157.0	0.50	*
8	6.95	0.338	88.0	0.55	*
9	7.80	0.405	31.0	24.0	*
10	7.85	0.450	28.9	28.0	*
11	8.00	0.503	7.4	131.0	*
12	8.10	0.495	7.5	150.0	*
13	8.60	0.510	5.3	-	*
14	8.80	0.495	1.3	187.0	*
15	8.95	0.518	4.8	430.0	*
17	8.80	0.525	5.8	550.0	*
19	8.90	0.533	3.8	183.0	*
21	9.40	0.503	4.3	256.0	*
24	9.80	0.510	2.8	340.0	*
30	10.15	0.548	1.7	200.0	-
37	10.00	0.563	0.97	13.4	-
44	10.00	0.563	0.54	10.3	-
51	10.00	0.585	0.19	1.2	-
58	10.25	0.585	0.064	1.1	-

1/ Tank No. 34 contained 1149 bushels of sizes No's. 2 and 3 cucumbers.

2/ The first sample was taken six to eight hours after the cucumbers were covered with brine.

* = Less than 10/ml.

- = No determination made.

TABLE 11

Percent acid and salt and the populations of various groups of micro-organisms in tank No. 35 1/

Time-days	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
0 2/	5.20	0.008	0.19	*	92,000
1	6.50	0.023	0.21	1.0	14,700
2	5.85	0.045	9.6	0.2	25,000
3	5.95	0.188	348.0	*	4,300
4	5.45	0.263	1,230.0	*	4,300
5	6.20	0.248	670.0	0.8	430
6	7.30	0.248	470.0	9.0	920
7	6.80	0.345	173.0	56.0	250
8	6.80	0.405	93.0	35.0	25
9	7.30	0.413	15.0	79.0	25
10	7.75	0.420	4.4	-	**
11	7.90	0.435	2.6	220.0	**
12	8.15	0.503	2.8	230.0	**
13	8.05	0.473	2.9	690.0	**
14	7.90	0.473	2.5	220.0	**
15	8.15	0.503	2.8	230.0	**
17	8.20	0.480	1.1	207.0	**
19	8.45	0.495	1.4	340.0	**
21	9.15	0.488	0.64	260.0	**
23	9.00	0.495	1.1	280.0	**
27	9.60	0.503	1.2	230.0	-
34	9.65	0.488	1.3	97.0	-
41	9.70	0.458	1.0	10.7	-
48	10.30	0.480	0.14	0.7	-
55	10.50	0.428	0.14	1.5	-

1/ Tank No. 35 contained 1125 bushels of sizes No's. 2 and 3 cucumbers.

2/ The first sample was taken eight to ten hours after the cucumbers were covered with brine.

* = Less than 1,000/ml.
** = Less than 10/ml.
- = No determination made.

Although some large variations may be noted in the populations of coliforms present initially in the various brines, these organisms disappeared from all 10 fermentations rapidly. None were found after 13 days. Only in 5 of the 10 tanks studied was there any increase in the population of coliforms and these increases were not large. Thus, it is believed that the coliforms played no significant part in these fermentations. Rather, it is believed that the coliforms entered the brines on the cucumbers, found conditions unfavorable for growth and died.

The percentages of salt and of titratable acidity, expressed as percent lactic acid, at various stages in the commercial fermentations are given in Tables 2-11. No evident correlation exists between the total titratable acidity formed and the maximum populations of acid-formers found in the various brines. However, in the cases of tanks 23 and 24 where quite low populations of the acid producing bacteria were noted, vinegar and brine from an actively fermenting tank were added between the 17th and 19th day of fermentation. Thus, the measurements of the total acidity is not an accurate picture in these two instances. The initial salt concentration in these two tanks was excessively high and this explains possibly the low maximum populations of acid-forming bacteria present, as well as the slow formation of acid. The trend lines for the 10 fermentations indicating the changes in acid and salt concentrations are shown in Fig. 2.

The population of acid-formers, yeasts, and coliforms in three laboratory fermentations are presented in Fig. 3 and in Tables 12, 13, and 14. The curves representing the acid-forming bacteria counts are

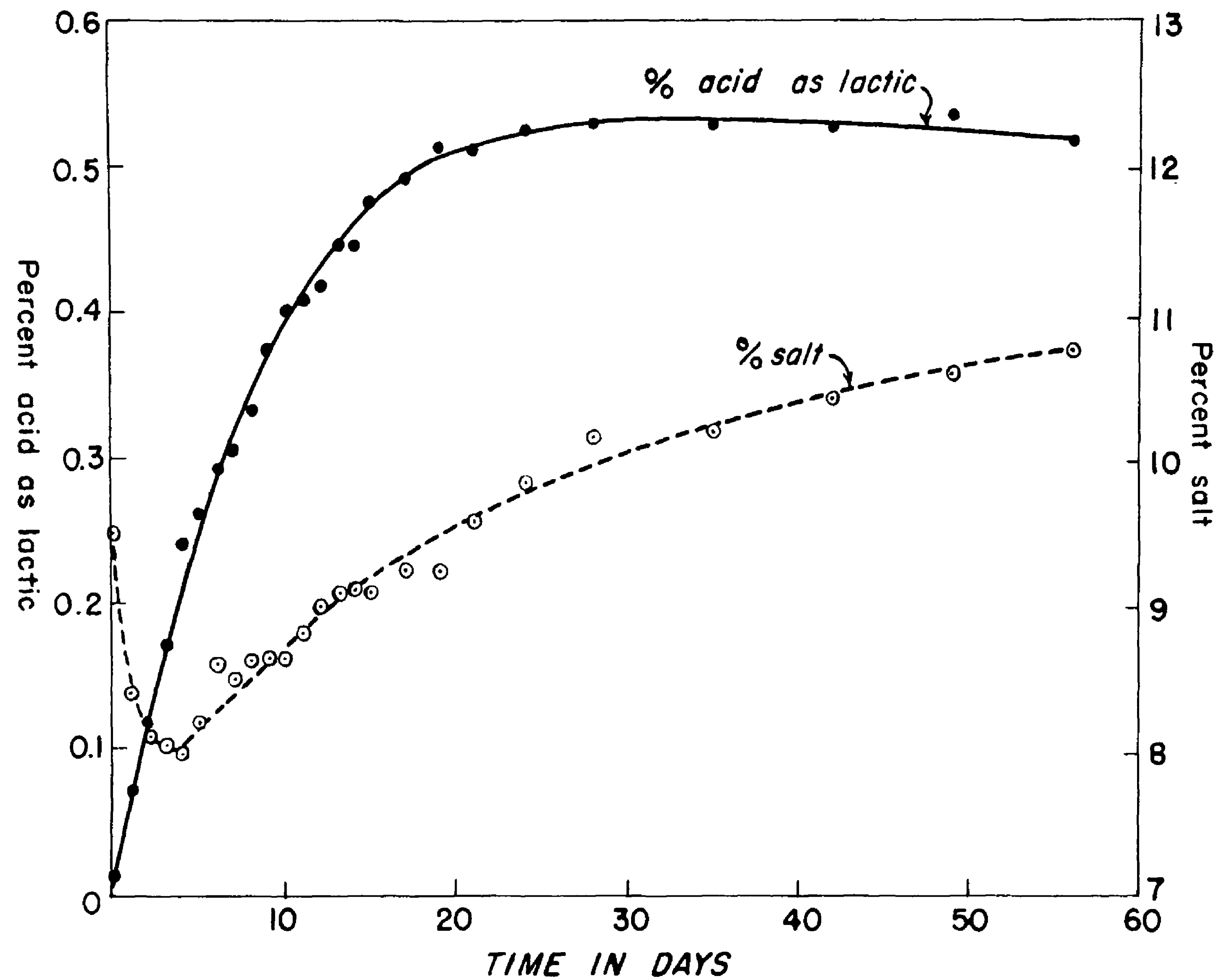

Fig. 2. Average changes in acid and salt concentrations during the fermentation of 10 commercial tanks of cucumbers.

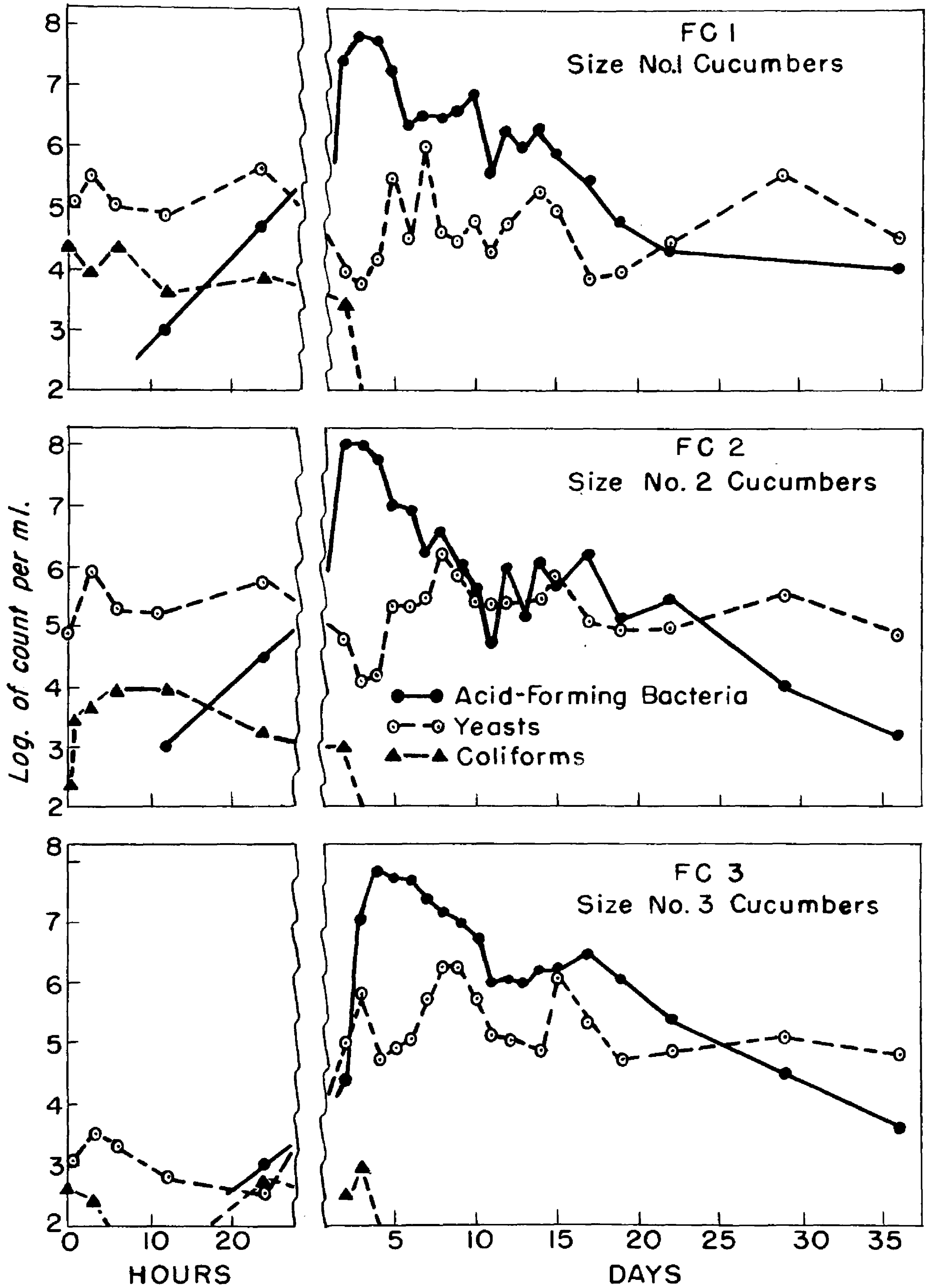

Fig. 3. Populations of various microorganisms in three fermentations under laboratory conditions.

TABLE 12

Acid and salt concentrations and the populations of various groups of microorganisms in laboratory fermentation FC 1.

Time	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
Hours					
0	9.7	0.000	*	-	25,000
1	8.7	0.000	*	129.0	25,000
3	7.5	0.003	**	340.0	9,200
6	6.6	0.005	**	125.0	25,000
12	5.9	0.008	0.001	81.0	4,300
Days					
1	5.3	0.015	0.05	480.0	9,200
2	6.95	0.105	24.8	9.4	4,300
3	7.10	0.218	68.0	6.7	***
4	7.50	0.300	58.0	15.0	***
5	7.20	0.300	16.9	320.0	***
6	7.60	0.488	2.4	32.0	***
7	7.40	0.540	3.4	1,120.0	***
8	7.80	0.605	3.4	42.0	***
9	8.05	0.645	4.1	28.0	***
10	8.05	0.643	7.7	64.0	***
11	8.25	0.668	0.40	19.0	***
12	8.45	0.668	2.0	54.0	***
13	8.25	0.630	1.1	-	***
14	8.10	0.632	1.9	190.0	***
15	8.20	0.632	0.79	89.0	***
17	9.15	0.632	0.30	7.0	***
19	9.10	0.632	0.063	9.0	***
22	9.20	0.630	0.023	28.0	***
29	9.70	0.578	**	350.0	-
36	10.40	0.548	0.011	33.0	-

* = Less than 1,000/ml.
** = Less than 10,000/ml.
*** = Less than 10/ml.
\- = No determination made.

TABLE 13

Acid and salt concentrations and the populations of various groups of microorganisms in laboratory fermentation FC 2.

Time	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
Hours					
0	9.85	0.000	*	88.0	240
1	9.35	0.000	*	113.0	2,400
3	8.30	0.000	**	840.0	4,300
6	7.20	0.002	*	193.0	9,200
12	6.25	0.003	0.001	159.0	9,200
Days					
1	5.45	0.018	0.030	580.0	1,500
2	6.30	0.143	103.0	60.0	920
3	6.70	0.345	111.0	12.2	***
4	7.20	0.398	54.0	14.0	***
5	7.00	0.450	9.5	210.0	***
6	7.50	0.488	7.7	200.0	***
7	7.75	0.555	1.6	250.0	***
8	7.40	0.615	3.1	1,600.0	***
9	7.70	0.638	0.95	750.0	***
10	7.80	0.675	0.38	210.0	***
11	8.25	0.675	0.050	230.0	***
12	8.30	0.675	0.85	230.0	***
13	8.30	0.690	0.16	-	***
14	8.75	0.630	1.0	280.0	***
15	8.90	0.653	0.46	740.0	***
17	9.30	0.555	1.3	128.0	***
19	9.30	0.600	0.12	79.0	***
22	9.30	0.533	0.25	97.0	***
29	9.70	0.638	**	340.0	-
36	10.35	0.563	**	53.0	-

* = Less than 1,000/ml.
** = Less than 10,000/ml.
*** = Less than 10/ml.
\- = No determination made.

TABLE 14

Acid and salt concentrations and the populations of various groups of microorganisms in laboratory fermentation FC 3

Time	Percent salt	Percent acid as lactic	Acid-formers x 10^6/ml	Yeasts x 10^3/ml	Coliforms No./ml
Hours					
0	9.65	0.000	*	1.3	430
1	9.35	0.000	*	4.2	920
3	8.90	0.000	*	3.3	240
6	8.20	0.000	*	2.0	91
12	7.35	0.002	*	0.70	36
Days					
1	6.35	0.003	0.001	0.34	430
2	6.80	0.053	0.029	110.0	250
3	7.00	0.083	10.6	650.0	920
4	7.10	0.150	67.0	55.0	43
5	7.15	0.218	50.0	84.0	**
6	7.65	0.195	44.0	110.0	**
7	7.50	0.248	21.0	470.0	**
8	8.25	0.270	13.5	1,860.0	**
9	8.05	0.305	8.0	1,830.0	**
10	8.20	0.375	5.3	530.0	**
11	7.85	0.458	0.81	122.0	**
12	7.90	0.495	1.15	112.0	**
13	7.75	0.540	0.94	-	**
14	8.50	0.493	1.40	71.0	**
15	8.30	0.525	1.72	1,220.0	**
17	8.85	0.510	2.45	190.0	**
19	9.45	0.488	1.06	47.0	**
22	9.20	0.570	0.20	75.0	**
29	9.55	0.585	0.03	124.0	-
36	10.35	0.563	0.004	56.0	-

* = Less than 1,000/ml.
** = Less than 10/ml.
- = No determination made.

very similar to those obtained in the commercial fermentations studied, and are in close agreement with the findings of Rosen and Fabian (51) in the study of two laboratory fermentations. However, the maximum populations of acid-forming bacteria attained in these lots as well as in those reported on by Rosen and Fabian were considerably lower than the maximum counts noted in the commercial fermentations. No significant difference was noted in the acid fermentations of sizes No. 1 (crock FC 1) and 2 (crock FC 2) cucumbers but in both of these crocks the acid-formers started rapid growth about one day earlier than with the No. 3's (crock FC 3).

The total titratable acidity correlated with the acid-forming bacteria in that the rapid development of acid started more slowly with the large size (No. 3) than with the two smaller sizes. Also, the maximum acidity was not as great as in the crock containing large cucumbers (Fig. 4).

The greatest difference in the fermentations in the laboratory as compared to those under commercial conditions was in the yeast fermentation. While a rather definite yeast phase of the fermentation was evident in the commercial tanks, as outlined previously, the yeast populations in the laboratory experiments were quite variable and, on the whole, were at a fairly high level throughout the fermentation period studied. Pronounced differences were evident in the yeast species responsible for the fermentations and will be discussed in detail later.

The coliform populations were even more insignificant in the laboratory fermentations than they were in the commercial. The highest count observed was 2.5×10^4 per ml (Table 12) and that was in a sample taken

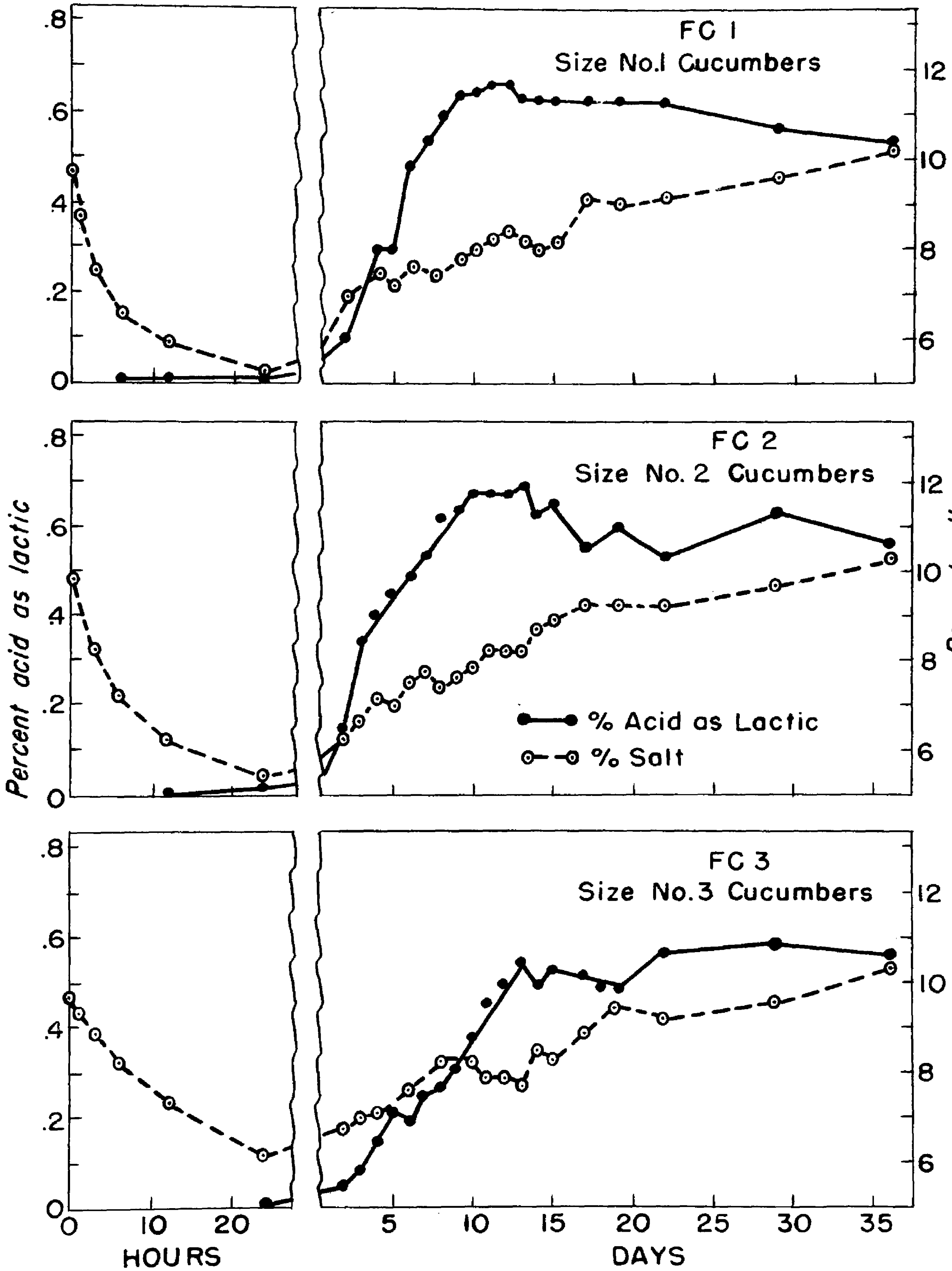

Fig. 4. Changes in acid and salt concentrations during the fermentation of three lots of cucumbers in the laboratory.

immediately after covering the cucumbers with brine. No coliforms were found to be present after the fifth day of fermentation in any of the three crocks.

Identification of acid-forming organisms. Since previous work by Etchells and Jones (16) and Rosen and Fabian (51) has quite definitely established that *L. plantarum* was the principal acid-forming organism in cucumber fermentations, only a few random isolations were made of these bacteria. However, of 20 isolates obtained by picking isolated colonies from the V-8 agar plates only 16 were definitely identified as *L. plantarum* according to the description of this species in Bergey's manual (5). The other four isolates were gram-positive cocci similar to *Leuconostoc mesenteroides* but failed to produce slimy growth on sucrose-gelatin agar or CO_2 in dextrose broth as determined by use of the Eldridge tube technique.

Yeasts from commercial fermentations. Since marked variations in the various yeast species active in different cucumber fermentations have been noted by Etchells *et al.* (11, 13), it was necessary to isolate and screen several of these organisms from each tank throughout the fermentations in order to characterize their activity. A total of 279 isolations were made from the commercial brines by picking isolated colonies from the highest dilutions showing growth on the acidified dextrose agar onto vegetable juice sporulation medium[1/]. The distribution

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1/ This medium was prepared as described by Etchells and Bell (11) except that the V-8 juice was filtered through a single layer of cheese cloth in a Buchner funnel in order to prevent excessive foaming in the tube and wetting of the cotton plugs.

of these isolates in relation to the tanks and fermentation age is given in Table 15.

All 279 isolates were screened by microscopic examination of the vegetable juice agar slant cultures after 2 to 4 weeks incubation at room temperature. On the basis of distinctive morphological characteristics and the general appearance of slant growth, the isolates were broken down into five groups; viz., Group 1, 123 isolates of asporogenic yeasts similar to Torulopsis holmii; Group 2, 84 isolates with morphological characteristics of Torulaspora rosei; Group 3, 18 sporulating cultures with hat-shaped spores and pleomorphic cells similar to Hansenula subpelliculosa; Group 4, 8 cultures similar to Brettanomyces versatilis; and, Group 5, comprised the remaining 46 isolates which were a group of miscellaneous yeasts.

Complete identification studies using the methods described by Etchells and Bell (11) and the classification systems of Stelling-Dekker (63), Lodder (39), and Bedford (2) were made on 25 of the isolates taken at random in Group 1, 42 of those in Group 2, and on all of those in Groups 3, 4, and 5. All 25 isolates of Group 1 which were studied to completion were positively identified as Torulopsis holmii. Thus, it was not believed necessary to run the physiological tests on the remaining 98 isolates of this group. The probability is that all 123 isolates were of the same species and will be referred to as such for the purposes of this paper. All except one of the 42 cultures of Group 2 were identified as Torulaspora rosei, and the one isolate not so classified was a non-sporulating yeast with similar cell morphology but different sugar fermentaions than noted for this species. On the basis of

TABLE 15

Distribution of yeast isolates as to tanks and fermentation age

Time days	No. of isolates obtained from various tanks									
	13	14	21	22	23	24	32	33	34	35
0-4	5	2	0	0	4	3	7	1	0	1
5-8	7	3	3	1	3	8	0	0	5	6
9-12	7	4	5	7	6	2	1	3	6	6
13-16	3	10	7	7	4	1	5	4	3	5
17-20	1	3	3	3	2	5	5	2	3	4
21-24	2	3	3	2	4	3	0	2	2	3
26-30	1	2	0	2	1	1	2	2	1	1
33-37	1	1	1	1	2	1	2	2	1	1
40-44	2	1	1	0	1	1	1	1	1	1
47-51	1	1	1	1	1	1	1	1	1	1
54-58	1	2	3	2	2	2	0	1	1	1
63-71	0	0	0	3	3	4	0	0	0	0
TOTAL No.	31	32	27	27	33	32	24	19	24	30
							GRAND TOTAL			279

these results, 83 of the 84 isolates in Group 2 will be referred to as Torulaspora rosei. The 18 sporulating cultures in Group 3 were identified as Hansenula subpelliculosa, and the 8 isolates in Group 4 plus 8 more from Group 5 were identified as Brettanomyces versatilis. The 39 remaining isolates were broken down into several groups, but were not completely identified and will be referred to as miscellaneous yeasts.

Based on the total 279 isolates from the 10 commercial fermentations, Torulopsis holmii represented 44.1 percent; Torulaspora rosei, 29.8 percent; Hansenula subpelliculosa, 6.4 percent; Brettanomyces versatilis, 5.7 percent; and the miscellaneous group accounted for the remaining 14.0 percent.

The occurrence of the two predominating species in the various fermentations was of particular interest, Table 16. While both yeasts were found in all 10 fermentations, Torulopsis holmii was apparently in great predominance in 7 of the tanks, and in the other 3 tanks (No's. 13, 23, 24) Torulaspora rosei appeared to predominate. Based on only those isolates from brines having a population of over 1 x 10^4 per ml the predominance of Torulopsis holmii in the seven tanks was even more pronounced, and in tanks No's. 13 and 24 the number of isolates of the two major species were not greatly different. However, in tank 23, Torulaspora rosei was apparently responsible for the major yeast fermentation.

From the percentage of isolates obtained at various stages in the fermentation there appeared to be a significant sequence of the various yeasts. This is illustrated in Fig. 5; based on average data given in Table 17. The first few days after brining a miscellaneous group of

TABLE 16

Distribution of the various species of yeasts according to the fermentation from which they were isolated

Tank No.	1/	Total No. of Isolates	Percent of total isolates: Torulopsis holmii	Torulaspora rosei	Hansenula subpelliculosa	Brettanomyces versatilis	Misc.
13	(a)	31	9.7	35.5	29.0	16.1	9.7
	(b)	4	50.0	50.0	0.0	0.0	0.0
14	(a)	32	62.5	25.0	0.0	3.1	9.4
	(b)	20	80.0	20.0	0.0	0.0	0.0
21	(a)	27	48.2	18.5	0.0	11.1	22.2
	(b)	15	80.0	20.0	0.0	0.0	0.0
22	(a)	27	44.4	22.2	0.0	14.8	18.5
	(b)	16	68.7	25.0	0.0	0.0	6.3
23	(a)	33	3.0	75.8	6.1	3.0	12.1
	(b)	12	0.0	91.7	8.3	0.0	0.0
24	(a)	32	28.1	40.7	18.8	6.3	6.3
	(b)	18	38.8	55.6	0.0	0.0	5.6
32	(a)	24	58.3	16.7	0.0	0.0	25.0
	(b)	11	100.0	0.0	0.0	0.0	0.0
33	(a)	19	78.9	15.8	0.0	0.0	5.3
	(b)	13	92.3	7.7	0.0	0.0	0.0
34	(a)	24	62.5	25.0	0.0	3.1	9.4
	(b)	17	82.4	5.9	0.0	0.0	11.7
35	(a)	30	63.3	20.0	0.0	0.0	16.7
	(b)	24	75.0	12.5	0.0	0.0	12.5
Grand	(a)	279	44.1	29.8	6.4	5.7	14.0
Totals	(b)	150	68.7	26.0	0.6	0.0	4.7

1/ The values of (a) are based on the total number of isolates studied during the entire fermentation, while the (b) values represent only those isolates obtained from brine samples having a population of 10,000 per ml. or above.

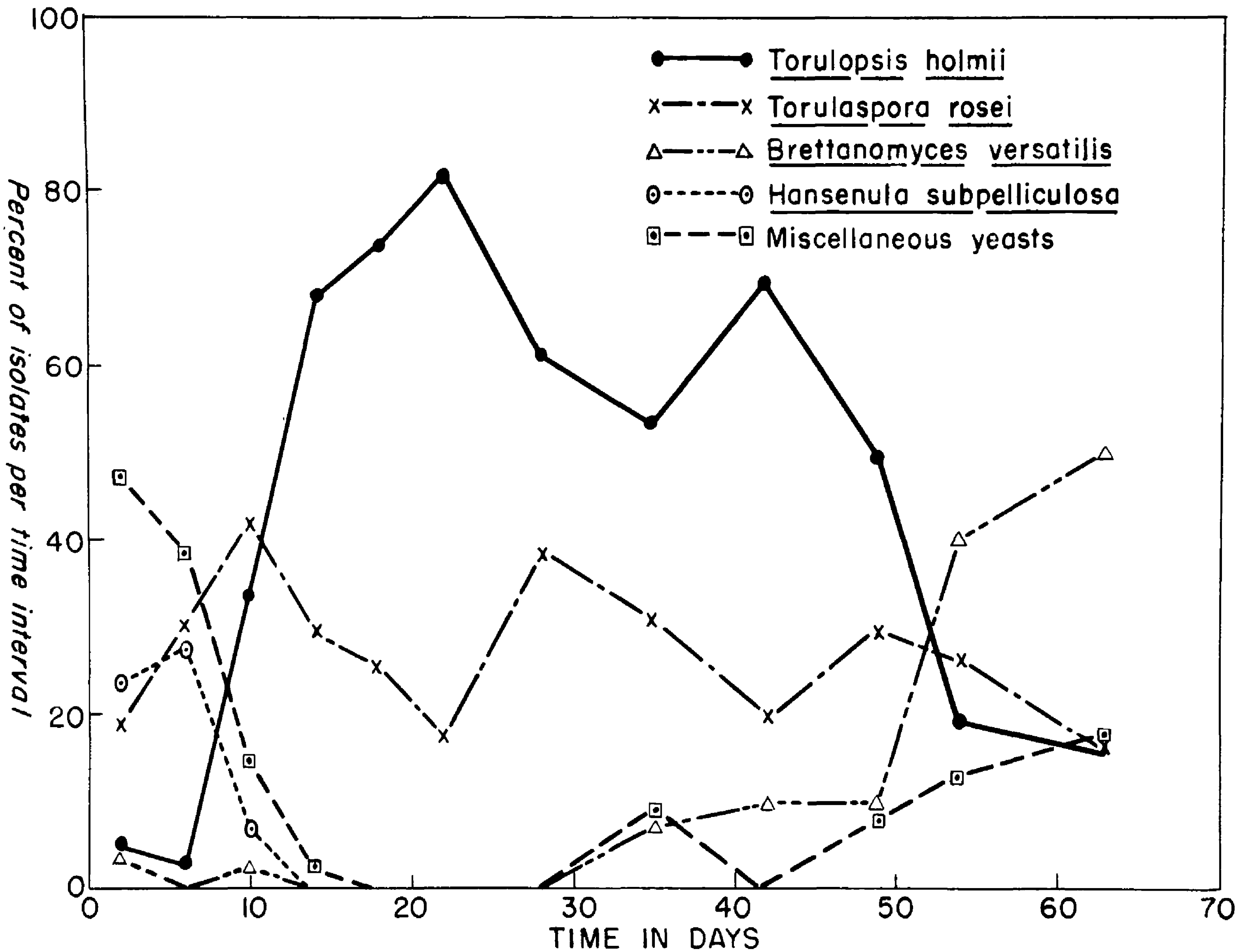

Fig. 5. Estimated sequence of various yeast species in the fermentation of cucumbers under commercial conditions.

TABLE 17

Distribution of yeast species according to the time of fermentation when isolated

Time days		Torulopsis holmii	Torulaspora rosei	Hansenula subpelliculosa	Brettanomyces versatilis	Misc.	Total No.
1-4	No. %1/	1 4.8	4 19.00	5 23.8	1 4.8	10 47.6	21
5-8	No. %	1 2.7	11 30.6	10 27.8	0 0	14 38.9	36
9-12	No. %	16 34.0	20 42.6	3 6.4	1 2.1	7 1.49	47
13-16	No. %	32 68.1	14 29.8	0 0	0 0	1 2.1	47
17-20	No. %	23 74.2	8 25.8	0 0	0 0	0 0	31
21-24	No. %	19 82.6	4 17.4	0 0	0 0	0 0	23
26-30	No. %	8 61.5	5 38.5	0 0	0 0	0 0	13
33-37	No. %	7 53.8	4 30.8	0 0	1 7.7	1 7.7	13
40-44	No. %	7 70	2 20	0 0	1 10	0 0	10
47-51	No. %	5 50	3 30	0 0	1 10	1 10	10
54-58	No. %	3 20	4 26.7	0 0	6 40	2 13.3	15
63-64	No. %	1 16.7	1 16.7	0 0	3 50	1 16.6	6

1/ Percentage of total number of isolates in each age group.

yeasts apparently predominated. During the most active phase of yeast fermentation the predominating yeast was *Torulopsis holmii*, which was replaced by *Brettanomyces versatilis*. *Torulaspora rosei* was found frequently throughout the fermentations. As noted above, this sequence does not necessarily occur in all cucumber fermentations but is merely an overall estimate of the general yeast activity in these 10 tanks.

Yeasts from laboratory fermentations. A total of 109 isolates of yeasts from the three laboratory fermentations were studied. However, 22 of these were found to be film-forming types and probably are not active in the sub-surface fermentation. Such yeasts are found growing luxuriantly on tanks which are protected from the direct rays of the sun (12) but are rarely found in brines from unprotected tanks. Heavy films did not form on the laboratory brines as they were kept under ultraviolet irradiation. Nevertheless, a little scum did form occasionally around the edges of the crocks where the brine surface was protected from the ultraviolet light, and this was thought to be the source of these 22 isolates. For this reason, only the 87 non-film-forming isolates will be considered here.

Of these 87 cultures, 58 or 66.7 percent were found to be *Torulaspora rosei*; 14 (16.1 percent) were *Hansenula subpelliculosa*, and 15 (17.2 percent) were unclassified. The unclassified group represented a number of different species and will be referred to here as miscellaneous yeasts. The distribution of these isolates as to the source is given in Table 18.

TABLE 18

Yeast isolates from laboratory fermentations

Crock No.	Torulaspora rosei		Hansenula subpelliculosa		Miscellaneous		Total No.
	No.	%	No.	%	No.	%	
FC 1	18	81.8	0	0.0	4	18.2	22
FC 2	13	44.8	8	27.6	8	27.6	29
FC 3	27	75.0	6	16.7	3	8.3	36
Grand Totals	58	66.7	14	16.1	15	17.2	87

In all three lots the principal yeast appeared to be *Torulaspora rosei*. This was in marked contrast to the commercial fermentations where *Torulopsis holmii* predominated in all instances but three and was isolated from every fermentation. Not a single isolate of this yeast was obtained from the laboratory fermentations.

Identification of coliform organisms. Twenty isolates of members of this group were obtained by streaking eosin-methylene blue agar plates from positive lauryl-tryptose broth tubes and then picking isolated colonies from the agar plates onto nutrient agar slants. All cultures proved to be gram-negative, non-spore forming, aerobic or facultative anaerobic, short rods, and produced acid and gas in lactose broth. Therefore, they were members of the coliform group. Further identification was not made as the significance of this group in the fermentations studied was very doubtful.

Vitamin and Amino Acid Requirements of *L. plantarum*

The vitamin and amino acid requirements of *L. plantarum* 17-5 have been the subject of many investigations, and the requirements of this strain have been well established. The results of investigations on other strains of *L. plantarum* from various sources, however, have been quite variable.

With the exception of the work by Rosen and Fabian (51), who demonstrated that 10 isolates of *L. plantarum* from cucumber fermentations required biotin, niacin, and pantothenic acid, no study of the vitamin and amino acid nutrition of isolates from this source was found in the literature. Thus, four isolates obtained from four different commercial cucumber fermentations were screened for their vitamin and amino acid requirements in comparison to *L. plantarum* 17-5.

Vitamin requirements of L. plantarum. The medium used to test for vitamin requirements was made up from individual ingredients according to the formula given for the pantothenate assay medium of Difco Laboratories, Inc. except that 0.002g per liter of calcium pantothenate was added. Individual vitamins were omitted from various lots of the medium to test for the effect of their omission on the five strains of *L. plantarum*.

The same procedures for the preparation of the inoculum and for inoculation and incubation of the cultures were followed as in the vitamin assays except that an incubation temperature of 30°C was used instead of 37°C.

As in the work of Rosen and Fabian (51), all strains of L. plantarum tested were noted to require biotin, niacin, and pantothenic acid (Table 19). In addition, both riboflavin and p-aminobenzoic acid were essential for two strains, L-20 and L-15. Strains 17-5 , L-10, and L-5 were not appreciably affected by the omission of riboflavin from the medium, but isolate L-10 required p-aminobenzoic acid and 17-5 and L-5 were greatly stimulated by this vitamin. Pyridoxine and thiamine were not essential for any of the 5 strains tested but the former was stimulatory for strain L-5.

TABLE 19

The effect of the omission of various vitamins from a synthetic medium on the acid produced by various strains of L. plantarum

Vitamin omitted	Ml NaOH (ca. 0.1N) to titrate acid produced by various strains				
	17-5	L-20	L-15	L-10	L-5
None	15.50	13.05	13.60	12.90	13.70
Biotin	1.60[1]	0.10	0.20	0.10	0.40
Niacin	0.30	0.30	0.20	0.30	0.45
Pantothenate	0.20	0.00	0.05	0.00	0.00
Riboflavin	14.20	0.50	0.40	9.50	14.00
p-amino-benzoic acid	4.95	0.95	0.50	0.50	7.20
Pyridoxine	11.60	11.60	12.10	11.30	6.40
Thiamine	13.05	12.25	13.15	12.20	12.65

[1] All results are underlined where the amount of NaOH necessary to titrate the acid produced was less than one-half that of the control.

The response of the L. plantarum isolates from cucumber brines and of strain 17-5 to various concentrations of biotin, niacin, and pantothenic acid was tested in the same manner as in the preparation of standard curves for vitamin assays. The results are given in Table 20. The response of the isolates from cucumber fermentations to biotin and niacin was quite similar to that of the 17-5 strain. However, in the case of pantothenate the 17-5 culture was noted to produce much more acid in the presence of comparatively smaller concentrations than the other four strains. These results are comparable to those obtained by Rosen and Fabian (51).

TABLE 20

Response of L. plantarum to biotin, niacin, and pantothenate

Vitamin	Concentration /10 ml	Ml NaOH (ca. 0.1N) to titrate acid produced by various strains				
		17-5	L-20	L-15	L-10	L-5
Biotin	0.0 mμg	1.60	0.10	0.30	0.10	0.40
	0.2 mμg	4.95	1.80	2.35	2.20	4.20
	0.8 mμg	9.70	6.10	6.85	5.95	10.20
	1.4 mμg	11.85	7.50	9.30	8.40	11.00
	2.0 mμg	12.20	7.90	10.10	9.50	12.05
Niacin	0.0 μg	0.30	0.30	0.20	0.30	0.45
	0.2 μg	5.45	3.80	3.55	3.90	4.55
	0.8 μg	11.10	9.20	10.20	9.65	10.85
	1.4 μg	12.80	11.55	12.55	11.50	11.95
	2.0 μg	13.30	13.10	13.65	13.05	13.60
Pantothenate	0.0 μg	0.20	0.00	0.05	0.00	0.00
	0.05 μg	6.35	0.55	0.60	0.50	0.50
	0.10 μg	11.70	1.65	1.70	1.50	1.90
	0.25 μg	15.30	5.90	7.00	5.50	6.80
	0.40 μg	16.80	13.00	13.40	12.80	11.20

Amino acid requirements of L. plantarum. The basal medium I proposed by Sauberlich and Baumann (54) for microbiological assays using *L. plantarum* 17-5 as the assay organism was used as the basal medium to determine the amino acid requirements of these isolates. The five strains of *L. plantarum* were screened for amino acid requirements in the same manner as for vitamins.

The effect of the omission of the various amino acids from the basal medium on the acid produced by those various strains is given in Table 21. The five *L. plantarum* cultures were constant in their requirements for tryptophane, leucine, isoleucine, valine, glutamic acid, cystine, and threonine. Phenylalanine and tyrosine were required by three strains (L-20, L-15, and L-10), arginine by one (L-5), and alanine was noted to be very stimulatory to the four isolates from cucumber fermentations. Alanine also appeared slightly stimulatory for 17-5. Some stimulation of all five strains was noted with histidine. The other amino acids did not greatly influence the acid produced by these cultures.

By variations in the basal medium used for testing, other workers have shown that the requirements of *L. plantarum* 17-5 may be reduced to include only five amino acids; *viz*, tryptophane, leucine, isoleucine, valine, and glutamic acid. However, cystine has been noted to be essential except in a very enriched medium (10). Since the amino acid requirements of the isolates from cucumber fermentations appeared quite similar to the 17-5 culture, except for variations among individual strains, the above six essential amino acids were selected for further study.

TABLE 21

The effect of the omission of various amino acids from a synthetic medium on the acid produced by various strains of *L. plantarum*

Amino acid omitted	Ml NaOH (ca. 0.1N) to titrate acid produced by various strains				
	17-5	L-20	L-15	L-10	L-5
None	11.95	9.65	9.75	9.90	10.05
L-tryptophane	0.00[1/]	0.00	0.00	0.00	0.00
L-leucine	1.35	0.00	1.30	1.20	1.35
DL-isoleucine	3.30	1.30	2.90	2.75	2.60
DL-valine	0.80	0.70	0.65	0.70	0.40
L-glutamic acid	0.80	0.80	0.70	0.85	0.60
L-cystine	0.00	0.00	0.00	0.00	0.00
L-asparagine	10.70	8.50	8.40	8.70	7.55
L-lysine	11.40	8.55	9.50	8.75	10.60
DL-threonine	2.55	3.90	3.05	2.90	2.00
DL-alanine	7.75	3.20	3.75	3.55	4.85
DL-methionine	12.15	8.65	9.35	9.40	8.20
DL-phenylalanine	11.20	0.40	0.40	0.35	8.40
L-arginine	11.85	8.20	8.05	7.90	0.75
L-tyrosine	10.40	0.40	0.45	0.40	8.70
L-histidine	7.90	7.75	7.50	7.50	8.00
DL-serine	11.75	8.90	9.55	9.35	8.20
L-proline	11.30	9.00	9.45	9.05	7.95
Glycine	11.90	9.50	9.95	10.20	9.80

1/ The results are underlined in all instances where the titration value was less than one-half that of the control.

The response of the five strains of L. plantarum to graded amounts of these six amino acids is given in Table 22[1]. These data are shown in Figs. 6 and 7 for the 17-5 strain and for the strains showing the greatest and the lowest response of the four isolates from cucumber fermentations. The type of curves obtained were similar for all five cultures tested.

With leucine, isoleucine, tryptophane, and cystine the concentrations employed were sufficient to approach the maximum response in the complete basal medium (Table 23). The decline in the total acid produced by the L. plantarum isolates when the concentration of L-cystine was increased from 70 µg per ml to 100 µg per ml is of doubtful significance since the concentration of L-cystine was 200 µg per ml in the complete basal medium. It is possible that the pH of the medium was changed significantly when sufficient L-cystine solution to attain a concentration of 100 µg per ml was added directly to the tubed medium, as HCl is used for solution of this amino acid. Thus, the rate of growth and acid production by L. plantarum would be affected.

With L-valine and L-glutamic acid it was evident that higher concentrations than those tested would be required to attain maximum acid production by L. plantarum.

1/ All response tests were made in the various media used for the assay of each particular amino acid except in the case of cystine. Medium I of Sauberlich and Baumann (54) was used in this instance.

TABLE 22

Response of *L. plantarum* to six amino acids noted to be essential for this species

Amino acid	µg/ 10 ml	Ml NaOH (ca. 0.1N) to titrate acid produced by various strains				
		17-5	L-20	L-15	L-10	L-5
L-leucine	0.0	1.40	0.0	1.30	1.20	1.35
	10.0	2.80	1.90	2.10	2.00	2.40
	30.0	5.35	3.30	3.95	3.30	4.45
	60.0	8.10	5.25	5.50	5.80	7.10
	100.0	10.45	8.40	8.85	8.90	9.80
DL-isoleucine	0.0	3.30	1.30	2.90	2.75	2.60
	10.0	4.40	2.05	3.70	3.45	3.45
	30.0	6.30	3.75	5.05	4.80	4.85
	60.0	8.90	5.70	6.80	6.65	6.50
	100.0	11.60	7.90	9.00	8.60	8.50
DL-valine	0.0	0.80	0.70	0.65	0.70	0.40
	10.0	1.90	1.40	1.50	1.50	1.30
	30.0	3.95	2.60	2.90	2.80	2.60
	60.0	6.15	3.90	4.65	4.50	4.45
	100.0	8.80	6.10	6.75	6.50	6.60
L-tryptophane	0.0	0.00	0.00	0.00	0.00	0.00
	2.0	2.40	2.30	2.45	2.45	1.90
	5.0	4.45	4.50	4.80	4.50	4.60
	8.0	6.25	6.15	6.60	5.85	6.45
	12.0	8.10	7.90	8.25	7.95	7.70
L-glutamic acid	0.0	0.80	0.80	0.70	0.85	0.60
	10.0	1.85	1.70	1.55	1.80	1.25
	30.0	3.50	3.20	3.00	3.20	2.40
	60.0	4.80	4.70	4.55	5.15	3.85
	100.0	5.60	6.40	5.70	6.55	5.85
L-cystine	0.0	0.80	0.30	0.35	0.40	0.40
	5.0	5.30	1.90	1.55	1.70	1.65
	10.0	6.20	3.60	3.10	3.85	3.10
	20.0	8.80	4.60	4.60	4.85	4.30
	30.0	9.70	5.80	5.20	5.90	5.80
	50.0	11.30	7.85	7.85	8.05	8.30
	70.0	11.70	9.10	9.80	9.15	9.35
	100.0	10.80	8.45	9.00	8.90	9.70

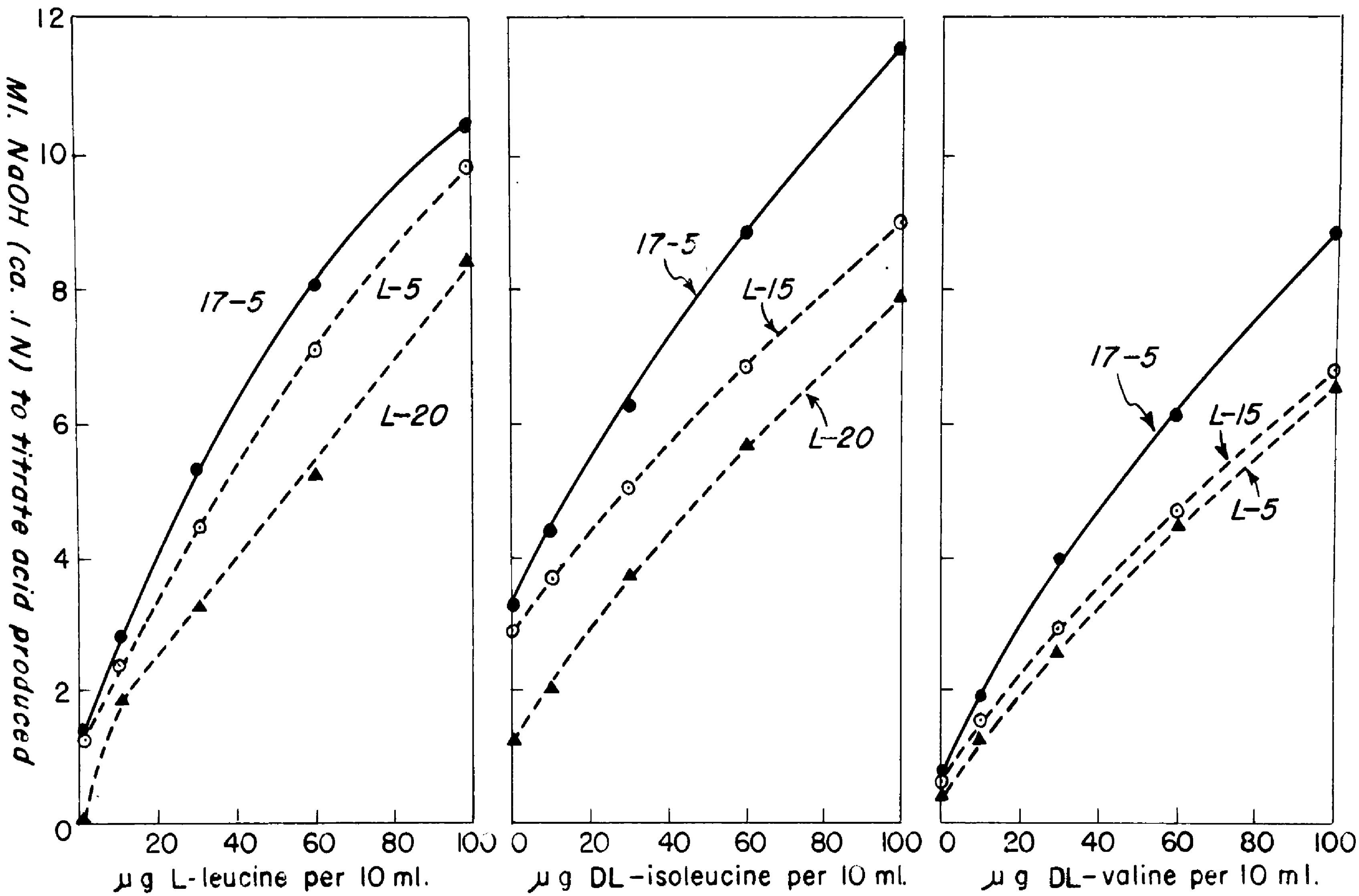

Fig. 6. The response of L. plantarum 17-5 and of two strains of L. plantarum isolated from cucumber fermentations to L-leucine, DL-isoleucine, and DL-valine.

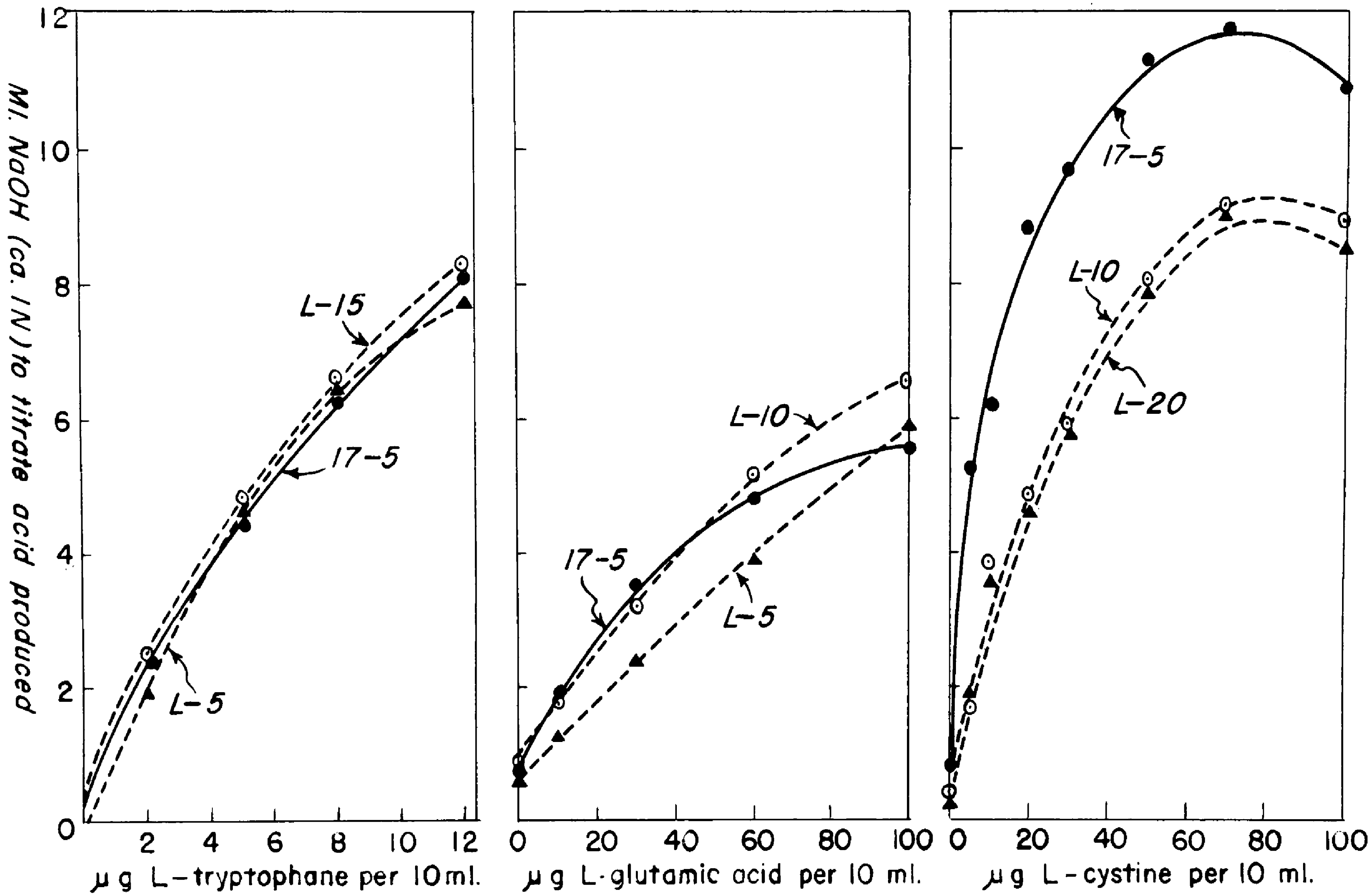

Fig. 7. The response of L. plantarum 17-5 and of two strains of L. plantarum isolated from cucumber fermentations to L-tryptophane, L-glutamic acid, and L-cystine.

TABLE 23

Comparison of the concentration of the amino acids and the acid produced by *L. plantarum* (L-15) in a complete basal medium to their concentrations and the acid produced in assay media[1/]

Amino acid	Basal medium µg/ml	Basal medium ml NaOH[2/]	Level where maximum response was observed µg/ml	Level where maximum response was observed ml NaOH[2/]
L-leucine	200	9.75	10	8.85
L-isoleucine	200[3/]	9.75	5[3/]	9.00
L-valine	200[3/]	9.75	5[3/]	6.75
L-tryptophane	80	9.75	1.2	8.25
L-glutamic acid	800	9.75	10	5.70
L-cystine	200	9.75	7.0	9.80

1/ Compiled from data given in Tables 21 and 22.

2/ Ml of NaOH (ca. 0.1N) to titrate acid produced in 10 ml of medium.

3/ Double this concentration of the DL form was used, but the L-form is the only active fraction.

Essential Vitamins and Amino Acids for *L. plantarum* in Brines of Commercial Cucumber Fermentations

It has been established that *L. plantarum* isolates from cucumber fermentations require at least three vitamins and six amino acids to produce a significant quantity of acid. Therefore, the presence of these nutrients in cucumber brines are essential for the acid fermentation. The concentrations, are also important as the amount of acid produced by *L. plantarum* is limited when the level of any one of these vitamins or amino acids is too low.

As a basis for the study of the nutrients essential for L. plantarum in the brines, 10 commercial fermentations were studied in detail as to their microbiological activity--reported previously. Five of these 10 tanks contained size No. 1 cucumbers and the other 5 contained mixed sizes of No's. 2 and 3. Three of each of these two groups were selected for this study. The six fermentations chosen included both the high and the low extremes of acid forming bacteria and yeast populations noted in the various fermentations, Tables 2-11.

Microbiological assays for the available vitamins and amino acids were run on brine samples taken at short intervals during the active acid fermentation--about the first 20 days of fermentation. A few brine samples taken later in the fermentation were assayed to test for any major changes in the concentration of the available nutrients due to continued yeast activity or other factors.

Vitamins in commercial fermentations. The complete results of the study of the niacin, pantothenic acid, and biotin content of the brines are given in Table 24, and the results of two representative fermentations are shown in Fig. 8. The general trend of the concentrations of these vitamins with time were quite similar in all six tanks. Thus, the maximum concentration of the vitamins in the brine was reached in from 5 to 7 days and a slight decrease in the levels was generally noted thereafter.

There was a significant difference in the concentrations of the three vitamins in the brines from tanks with the small size cucumbers (size No. 1) as compared to those with the larger sizes (No's. 2 and 3).

TABLE 24

Niacin, pantothenic acid, and biotin content of brines during the commercial fermentation of cucumbers.

Time from brining		Tanks filled with size No. 1 cucumbers			Tanks filled with sizes No. 2 and No. 3 cucumbers		
		21	23	14	24	35	33
Niacin		µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
0-10 hrs.		0.653	0.635	0.282	0.178	0.128	0.061
1 day		1.415	0.733	0.613	0.354	0.558	0.310
2		-	-	1.233	-	-	0.493
3		2.880	1.490	1.310	0.723	1.182	0.725
5		2.480	1.202	2.120	0.958	1.548	0.970
7		2.410	1.519	-	0.992	1.676	-
10		2.102	1.420	2.340	1.206	1.366	1.533
14		1.790	1.230	-	0.975	1.090	-
19		1.615	1.248	1.858	0.781	0.979	1.273
23-24	1/	1.743	1.335	-	0.808	1.008	-
30-36		1.785	1.364	-	0.894	0.896	-
37-41		1.663	-	-	-	0.842	-
55-58		1.655	1.335	-	0.921	0.763	-
64		-	1.360	-	0.811	-	-
Pantothenic acid							
0-10 hrs.		0.738	0.774	-	0.167	*	0.158
1 day		1.478	0.861	0.684	0.446	0.632	0.444
2		-	-	1.293	-	-	0.488
3		2.370	1.809	1.305	0.969	1.268	0.843
5		2.140	1.600	2.085	1.320	1.435	1.505
7		2.810	2.321	-	1.395	1.435	-
10		2.620	1.719	2.274	1.615	1.438	1.838
14		2.538	1.575	-	1.875	1.603	-
19		2.394	1.983	2.152	1.958	1.618	1.803
23-24	1/	2.044	1.680	-	1.570	1.400	-
30-36		2.136	1.675	-	1.411	1.337	-
37-41		2.180	-	-	-	1.294	-
55-58		1.996	1.596	-	1.433	1.128	-
64		-	1.508	-	1.407	-	-
Biotin		mµg/ml	mµg/ml	mµg/ml	mµg/ml	mµg/ml	mµg/ml
0-10 hrs.		15.20	11.75	5.84	0.856	1.860	0.452
1 day		17.70	10.99	12.72	1.512	4.604	1.580
2		-	-	12.00	-	-	2.280
3		-	16.33	16.70	3.509	5.835	3.200
5		19.74	13.00	16.99	4.758	6.895	5.500
7		19.26	14.75	-	4.835	7.670	-
10		-	14.16	19.78	6.540	6.325	6.160
14		17.90	13.94	-	5.165	7.395	-
19		18.20	13.72	18.30	5.200	7.550	6.240
23-24	1/	17.50	14.18	-	5.053	7.313	-
30-36		17.40	13.32	-	4.750	5.962	-
37-41		19.26	-	-	-	5.865	-
55-58		18.40	12.13	-	4.492	5.145	-
64		-	11.57	-	4.487	-	-

1/ Day varies with the individual tanks.
* = Less than 0.02 µg/ml.
- = No determination made.

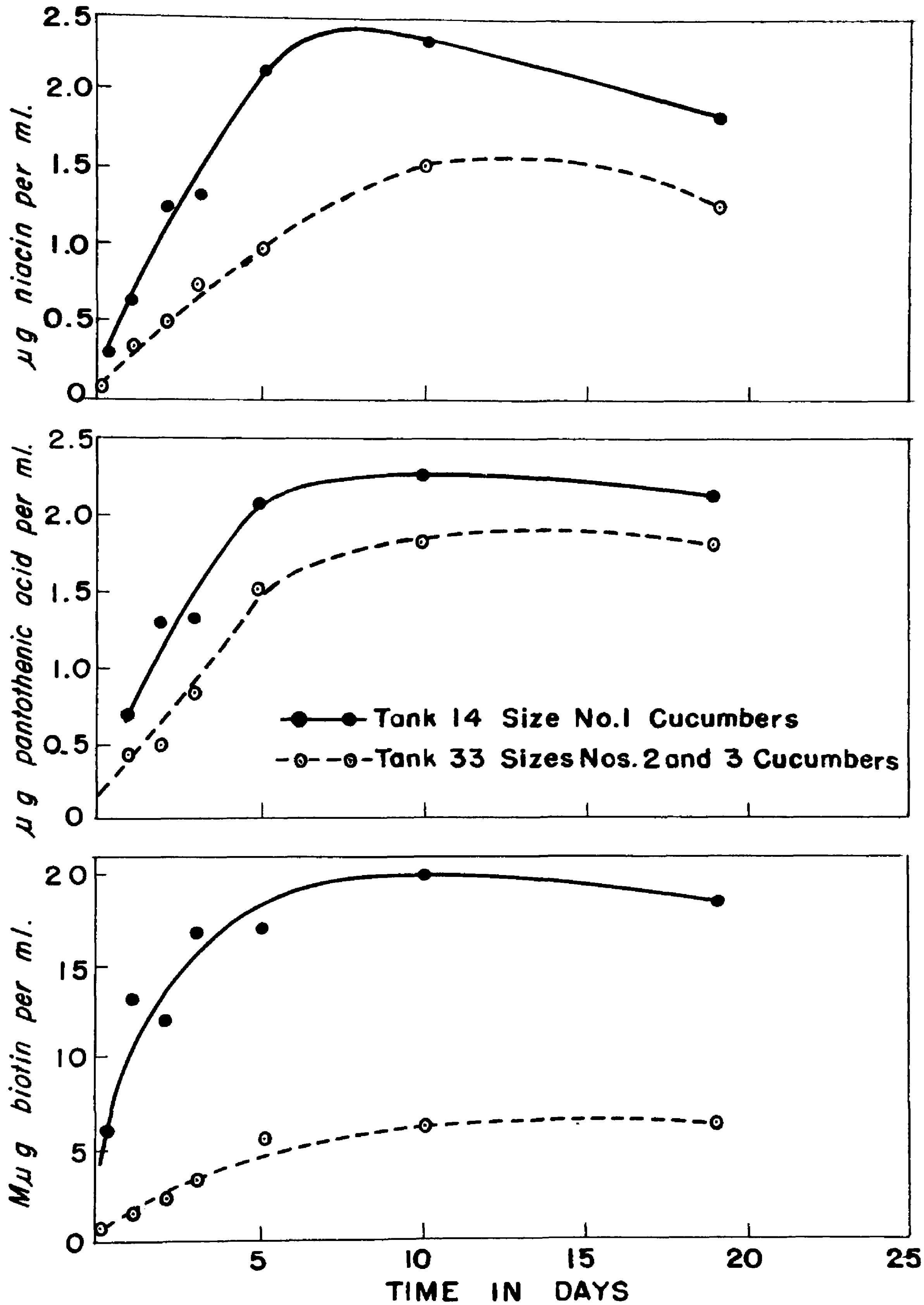

Fig. 8. Niacin, pantothenic acid, and biotin concentrations in the brines of two commercial cucumber fermentations.

The most striking difference may be noted in the biotin levels; the biotin concentrations in the brines from tanks filled with No. 1 cucumbers are from 2 to 4 times as high as those in brines covering the larger cucumbers. However, it should be noted that in all instances the vitamin levels at one day after brining were well above those required by *L. plantarum* for rapid acid production in the assay medium.

No obvious correlation existed between the vitamin levels in the brines and the microbiological activity. The decline in vitamin content which was observed in brines after the first 10 to 19 days of fermentation occurred at the same time that the yeast activity was greatest in all these brines. However, this may well have been due to a number of factors.

Amino acids in commercial fermentations. The concentrations in the commercial brines of the six amino acids studied are given in Tables 25 and 26 and those in two representative fermentations are shown in Figs. 9 and 10. In general, the maximum concentrations of all the amino acids were reached more slowly than were those of the vitamins; 10 to 19 days being required for the amino acids as compared to 5 to 7 days for the vitamins. This was probably due to the relatively low solubility in brine of protein material and of the amino acids themselves as compared to the three vitamins.

The results of the study of the levels of leucine, isoleucine, and valine were quite comparable over all six fermentations, and is well illustrated in Fig. 9. As with the vitamins the brines from the tanks containing the smaller cucumbers were in practically all instances

TABLE 25

Leucine, isoleucine, and valine content of brines during the commercial fermentation of cucumbers

Time from brining	Tanks filled with size No. 1 cucumbers			Tanks filled with sizes No. 2 and No. 3 cucumbers		
	21	23	14	24	35	33
	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
Leucine						
0-10 hrs.	46.30	25.90	26.20	8.30	6.25	6.40
1 day	114.70	43.80	47.10	24.50	36.70	30.75
2	-	-	109.40	-	-	50.00
3	191.00	121.50	126.20	85.00	94.00	89.50
5	194.70	153.00	208.00	125.00	133.50	152.60
10	250.00	215.00	331.00	185.00	172.50	231.00
19	238.50	228.50	318.50	187.50	181.30	256.00
30-36 1/	268.10	284.00	-	218.40	208.60	-
55-64	271.60	263.60	-	205.80	202.80	-
Isoleucine						
0-10 hrs.	5.45	*	12.90	*	*	*
1 day	42.10	*	24.08	*	16.20	15.38
2	-	-	54.20	-	-	27.50
3	103.90	22.90	62.90	19.00	19.10	50.00
5	108.00	57.50	104.40	44.10	80.20	88.50
10	117.80	102.90	181.00	101.10	80.60	120.00
19	111.20	103.90	184.50	105.90	98.60	133.60
30-36 1/	145.20	144.00	-	117.20	120.80	-
55-64	147.20	142.40	-	112.50	105.40	-
Valine						
0-10 hrs.	26.68	6.90	12.78	*	*	*
1 day	77.50	13.80	27.20	*	22.25	15.05
2	-	-	59.80	-	-	28.40
3	133.00	77.40	70.70	53.50	63.30	54.30
5	150.00	105.00	123.15	86.10	96.50	93.70
10	188.50	154.50	211.00	126.50	117.00	139.80
19	174.50	166.00	214.00	133.50	140.50	153.80
30-36 1/	170.10	182.05	-	129.50	136.00	-
55-64	178.10	164.30	-	128.30	116.00	-

1/ Day varies with the individual tanks.

* = Less than 5.0 µg/ml.

- = No determination made.

TABLE 26

Tryptophane, glutamic acid, and cystine content of brines during the commercial fermentation of cucumbers

Time from brining	Tanks filled with size No. 1 cucumbers			Tanks filled with sizes No. 2 and No. 3 cucumbers		
	21	23	14	24	35	33
	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
Tryptophane						
0-10 hrs.	5.70	2.15	1.77	*	*	*
1 day	11.75	4.36	5.22	1.92	4.38	3.01
2	-	-	11.61	-	-	3.76
3	17.60	11.69	14.12	10.28	17.05	10.56
5	24.30	15.50	16.25	9.00	27.50	19.90
10	32.10	17.20	33.46	21.75	35.00	32.44
19	29.60	29.55	6.62	2.94	39.00	34.90
30-36 1/	19.80	24.10	-	7.18	31.10	-
55-64	19.20	18.25	-	9.06	24.24	-
Glutamic acid						
0-10 hrs.	10.35	10.10	26.65	5.20	7.70	5.05
1 day	47.60	18.90	50.00	13.40	15.40	26.20
2	-	-	100.00	-	-	45.30
3	248.00	43.40	119.00	56.60	56.50	81.00
5	207.20	81.20	218.40	76.40	110.40	159.40
10	179.70	110.40	336.00	203.20	70.60	253.15
19	93.60	100.00	326.00	106.60	62.60	259.20
30-36 1/	152.80	151.20	-	141.60	146.80	-
55-64	141.80	144.40	-	122.40	128.20	-
Cystine						
0-10 hrs.	*	*	*	*	*	*
1 day	7.05	*	1.88	*	3.45	2.65
2	-	-	6.80	-	-	3.18
3	16.22	4.78	8.56	2.54	3.60	7.88
5	11.52	6.45	15.24	5.96	7.50	16.88
10	14.02	6.76	18.80	8.92	7.84	14.77
19	11.74	5.68	16.74	7.50	10.04	15.33
30-36 1/	15.91	12.62	-	12.80	17.22	-
55-64	14.20	10.92	-	11.06	14.04	-

1/ Day varies with the individual tanks.

* = Less than 1.0 µg/ml.

- = No determination made.

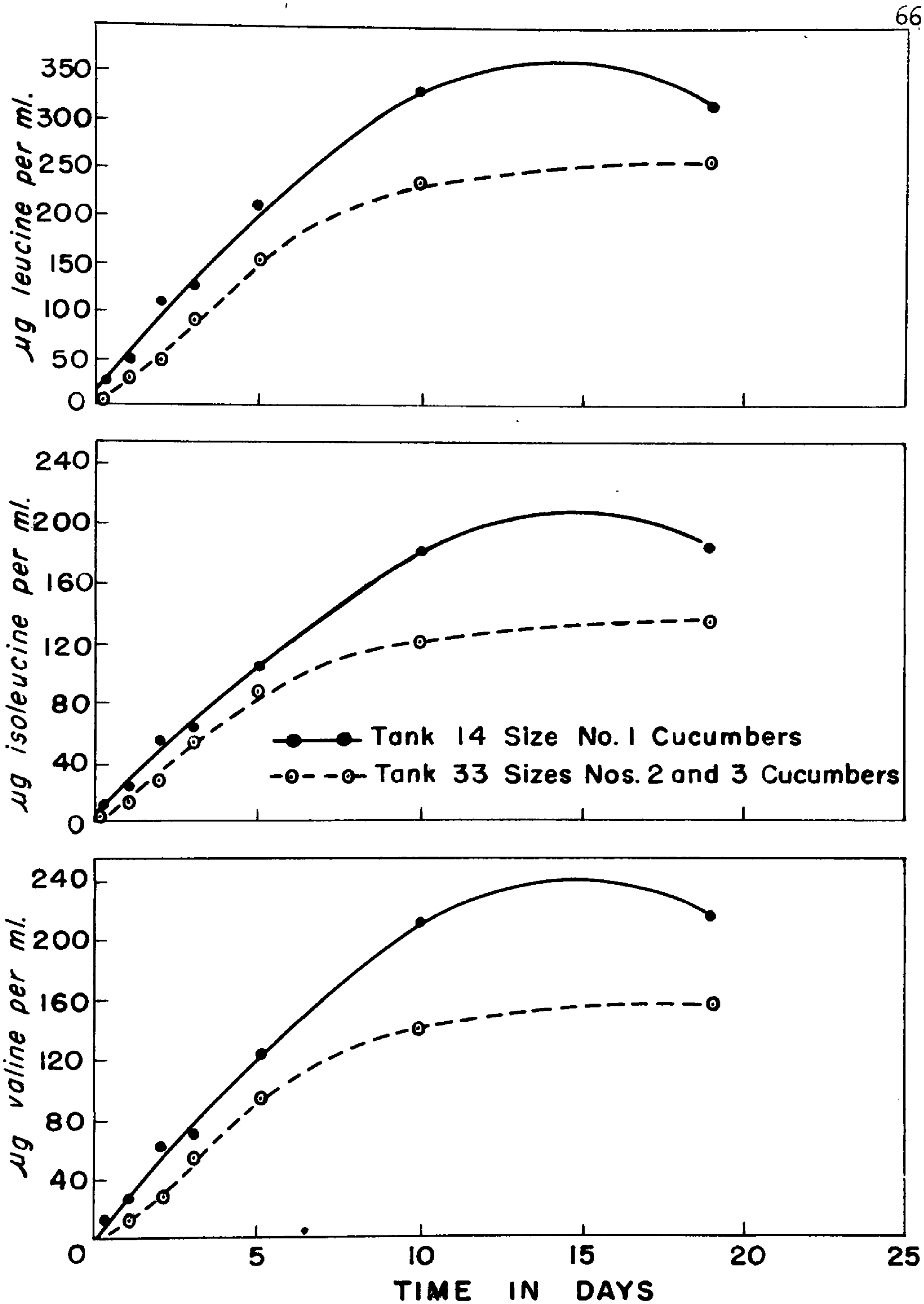

Fig. 9. Leucine, isoleucine, and valine concentrations in the brines of two commercial cucumber fermentations.

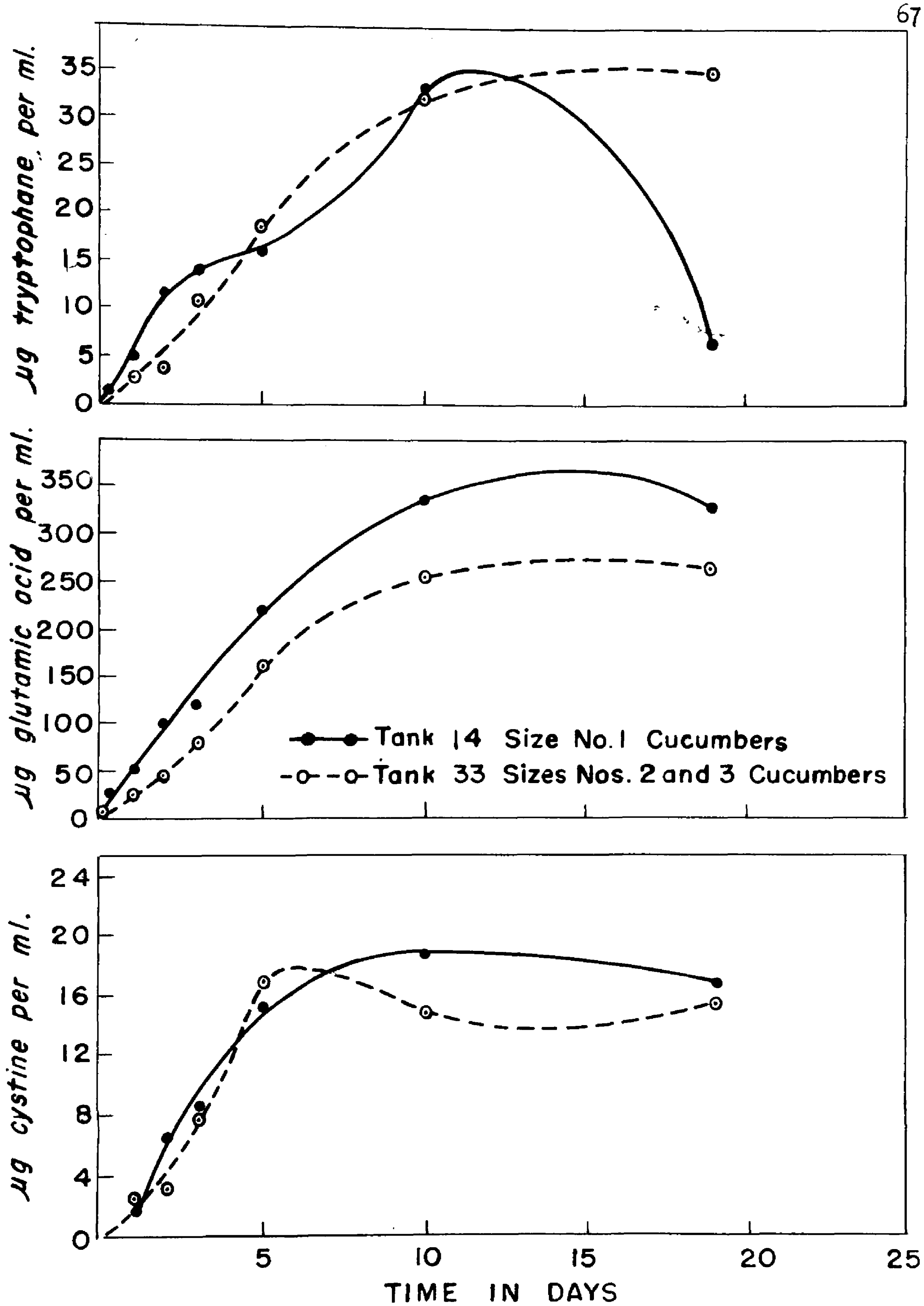

Fig. 10. Tryptophane, glutamic acid, and cystine concentrations in the brines of two commercial cucumber fermentations.

richer in leucine, isoleucine, and valine throughout the fermentation period studied.

The concentrations of isoleucine in tanks 23 and 24 and of valine in tank 24 were still relatively low one day after brining--less than 5.0 µg per ml. However, since 5.0 µg per ml of the L-form of these two amino acids and 10 µg per ml of L-leucine were sufficient for rapid acid production by L. plantarum in the assay media, these three amino acids did not appear to be limiting.

No influence of the size of the cucumbers on the concentration of tryptophane in the brines was evident since the same levels were approached in all six fermentations (Table 26 and Fig. 10). However, in two fermentations, tanks 14 and 24, great reductions in the tryptophane content occurred during the fermentation. Some reduction in the levels of this amino acid also occurred at about the same time in tanks 21, 23, and 35. As the reduction in tryptophane occurred at the same period when yeast activity was greatest in these brines, it is indicated that these microorganisms may either utilize or destroy large quantities of this amino acid.

Considerable variation was noted in the concentrations of glutamic acid present in the brines of the six different fermentations (Table 26). For example, considerable reduction in the glutamic acid content of brines from tanks No's. 21, 24, and 35 were noted 10 to 19 days after the beginning of the fermentations but similar reductions were not observed in the brines of the other three tanks. Also, there were large differences in the maximum concentrations of this amino acid attained in the various tanks.

From the standpoint of the levels of the various amino acids required for rapid acid formation by L. plantarum, the study of the cystine content of these brines was of the most interest. Thus, while 4 to 7 µg per ml of L-cystine was found to be required for optimum acid production, in 3 of the fermentations studied (Tanks 23, 24, and 35) the concentration of this amino acid was below 5.0 µg per ml three days after brining. The cystine content of these brines was still relatively low at 19 days. However, the levels of cystine in these three fermentations approached those of the other three as the fermentation proceeded.

No relationship was evident between cucumber size and the cystine content of the brine.

Essential Vitamins and Amino Acids for L. plantarum in Laboratory Fermentations and Non-fermented Control Brines

It is important for the acid fermentation of cucumbers to be initiated very soon after brining. This decreases the possibility of undesirable groups of organisms utilizing a large quantity of the available sugar resulting in low final acidity. Therefore, it is quite important that the nutrients essential for L. plantarum become available in the brine rapidly. This study was concerned with the rate of exodus of these essential nutrients from cucumbers of various sizes in carefully controlled laboratory experiments.

Rosen and Fabian (51) have shown that yeasts and coliform organisms may influence the concentrations of some vitamins in brine during the fermentation of cucumbers. It is also reasonable that the amino acids may be similarly influenced. Therefore, two lots of each size of cucumbers were brined; one lot was allowed to ferment while the fermentation of the other lot was inhibited by the use of preservatives. By comparison of these fermented and non-fermented lots, it was possible to note any marked effect of the brine organisms on the content of these essential nutrients during the fermentations.

Three bushels of cucumbers were obtained through the courtesy of the H. W. Madison Co. at Mason, Michigan; 1 bushel of each of 3 sizes --No. 1, No. 2, and No. 3. These cucumbers were all of the Davis variety and were grown on the same plot of ground. Each bushel was broken down into two lots and one lot used for the non-fermented control and the other for a laboratory fermentation.

The non-fermented controls were brined in quart fruit jars; 11 jars of each size. Ten size No. 1 cucumbers per jar were brined in lot C 1, 5 size No. 2's per jar in lot C 2, and 2 size No. 3's per jar in lot C 3. After determining the weight of the cucumbers in each jar, they were covered with a 38° salometer (10 percent salt) brine at the ratio of 1.8 ml brine to 1g or cucumbers. This ratio was necessary to completely immerse the cucumbers in the brine. Five ml of toluene and 5 ml of chloroform were added to each jar to prevent fermentation and the jars sealed with regular screw type lids.

Composite samples were taken of each lot by removing 5 ml of brine from each jar and pooling the samples from all 11 jars. The samples were tested for microbiological activity, and for acid and salt concentrations. No organisms were found in any of the samples. All brine samples were frozen until the nutritive study was made.

To prevent depletion of the brine in the jars, 5 ml of a 38° salometer brine was added to each jar when samples were removed for the first 5 days. None was added thereafter.

The fermented lots (FC 1, FC 2, and FC 3) were brined in 5-gallon crocks. Nine kilograms of each size cucumbers were weighed, placed in separate crocks, and covered with 9 liters of 38° brine. Sufficient salt was added within the first 2 days after brining to make the salt concentration about equal to that of the non-fermented controls after equalization--about 27° salometer. The brine strength was increased about 2° salometer per week in the fermented lots.

Sampling of these laboratory fermentations was carried out as described earlier. As fairly large samples were taken, at each interval, about 100 ml, and much evaporation occurred, 38° salometer brine was added as needed to maintain the original brine levels in the crocks.

<u>Vitamins in non-fermented controls and in laboratory fermentations.</u> In general the results of the vitamin study on the laboratory fermentations and the non-fermented control brines were similar, Table 27. The concentrations of the 3 vitamins in the 3 non-fermented control lots (C1, C2, and C 3) are shown in Fig. 11.

The differences in the vitamin levels which were noted in the commercial brines from tanks with different sizes of cucumbers were even more pronounced in these experiments. Thus, the brines covering the No. 1 cucumbers had the greatest and those on the No. 3 size the least concentration of vitamins. As in the study of the commercial brines, the largest differences were noted in the biotin levels, Lot C 2 brine contained only about one-half the amount of biotin that was found in

TABLE 27

A comparison of the niacin, biotin, and pantothenic acid levels in non-fermented and fermented brines covering cucumbers of three different sizes

Time from brining	Size No. 1		Size No. 2		Size No. 3	
	C 1	FC 1	C 2	FC 2	C 3	FC 3 1/
Niacin	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
0 hr.	*	*	*	*	*	*
1	*	0.063	*	*	*	*
3	0.124	0.131	*	0.074	*	*
6	0.255	0.283	0.139	0.154	-	-
12	0.599	0.604	0.353	0.456	0.161	0.097
1 day	0.980	1.062	0.605	0.972	0.320	0.380
2	1.243	1.414	0.836	1.202	0.571	0.624
3	1.288	1.698	0.992	1.690	0.691	0.540
5	1.277	1.764	1.055	1.732	0.836	0.760
7	1.299	1.436	1.028	1.614	0.877	0.726
15	1.305	1.692	1.040	1.324	0.910	0.752
29	1.241	1.782	0.969	1.542	0.913	0.789
Pantothenic acid						
0 hr.	**	**	**	**	**	**
1	0.033	0.093	**	0.052	**	**
3	0.145	0.117	0.084	0.120	0.018	0.012
6	0.365	0.422	0.209	0.204	0.074	0.035
12	0.579	0.950	0.342	0.808	0.149	0.092
1 day	0.826	1.875	0.531	1.778	0.243	0.433
2	1.192	1.348	0.750	1.307	0.379	0.586
3	1.347	1.706	0.836	1.342	0.496	0.798
5	1.484	2.065	1.023	1.787	0.605	1.034
7	1.385	2.024	1.168	2.166	0.746	1.160
15	1.420	2.278	1.328	2.018	0.856	1.210
29	1.152	2.465	1.248	2.067	0.908	1.515
Biotin	mµg/ml	mµg/ml	mµg/ml	mµg/ml	mµg/ml	mµg/ml
0 hr.	0.310	0.090	***	***	***	***
1	0.648	0.343	0.064	0.094	***	***
3	2.960	0.938	0.653	0.460	0.037	0.034
6	4.910	2.170	1.554	0.890	0.148	0.138
12	6.850	2.790	2.580	1.825	0.473	0.380
1 day	8.350	2.320	3.935	2.525	0.774	1.125
2	10.045	13.800	5.490	6.700	1.510	2.090
3	9.770	13.060	5.980	6.360	1.665	2.005
5	11.180	12.900	6.525	6.860	2.495	3.085
7	10.880	7.310	6.600	12.740	2.595	3.235
15	11.025	12.670	6.835	6.560	3.145	3.235
29	11.100	10.470	6.465	5.770	3.240	3.585

1/ The C series were the non-fermented control samples and the FC series the laboratory fermentations.

* = Less than 0.05 µg/ml of niacin.

** = Less than 0.02 µg/ml of pantothenic acid.

*** = Less than 0.025 mµg/ml of biotin.

\- = No determination made.

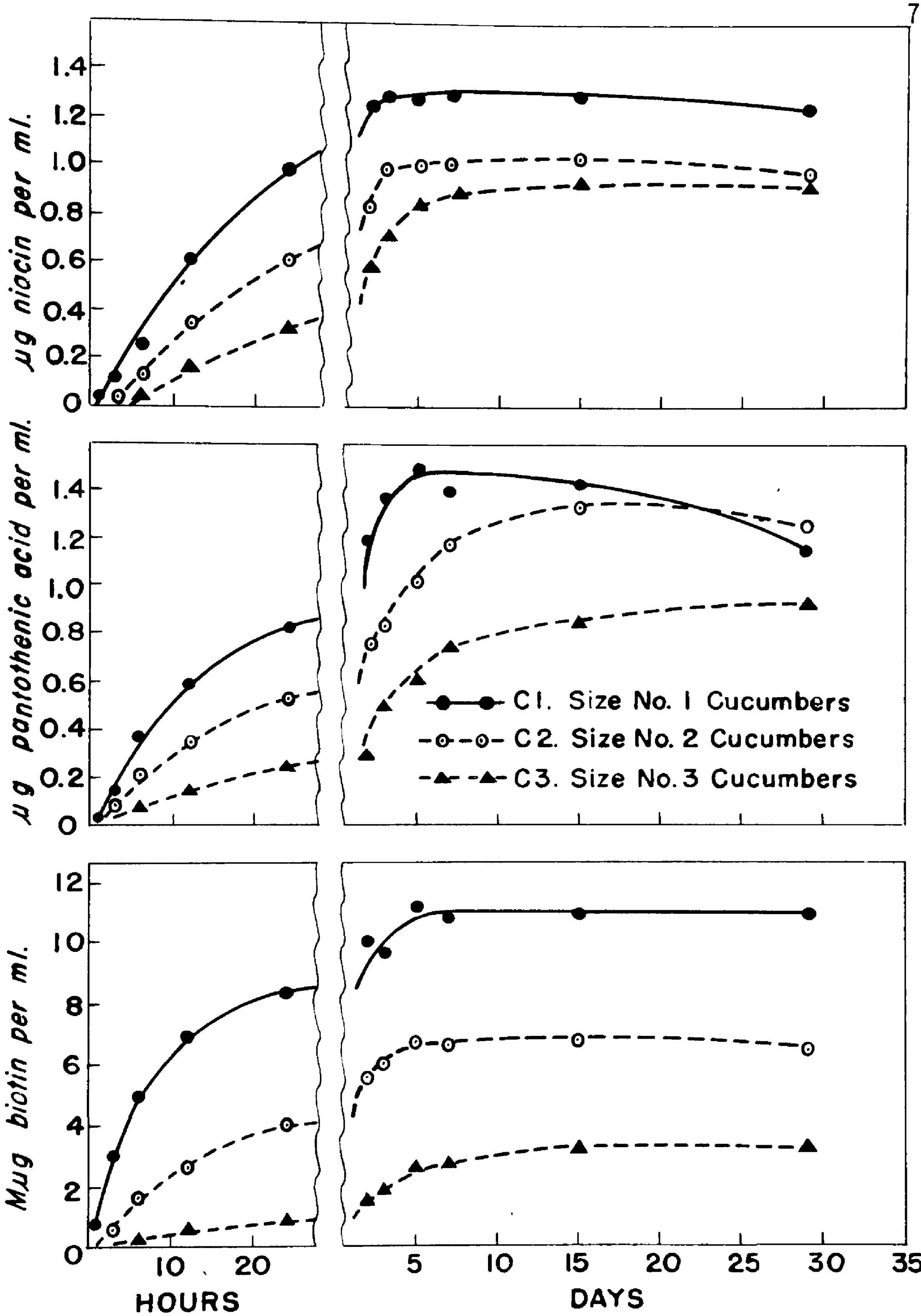

Fig. 11. Comparison of the rates of diffusion and the maximum concentrations attained of niacin, pantothenic acid, and biotin from three different sizes of cucumbers in non-fermented brines.

Cl brine, and C 3 only about one-fourth that of the C 1 brine.

The maximum concentrations of all the vitamins studied were in most instances attained in from 5 to 7 days after brining. The lots with the larger sizes required a longer time than those with smaller sizes of cucumbers. This would be expected due to the ratio of the surface area to volume in the different sizes.

The ratio of cucumbers to brine in the fermented lots was 1:1 while that in the non-fermented lots was 1:1.8. Therefore, the nutrients in the brine would be expected to be more concentrated in fermented brines than in the non-fermented unless these nutrients were utilized or destroyed by the brine organisms or the products of their metabolism. The relative concentrations of niacin and pantothenic acid in the two groups of controls were about what might be predicted from the cucumber-brine ratios. However, the biotin content of two of the fermented brines was apparently influenced by some other factors, particularly during the first day. The biotin level in the FC 1 lot was only about one-fourth that of the C 1 lot and in the FC 2 lot only slightly over one-half that of the C 2 lot after one day. The biotin content of the FC 3 lot was slightly lower than that in C 3 for 12 hours, but was higher than that in the C 3 at one day. However, two days after brining the biotin levels in all three fermenting brines were significantly higher than in the non-fermenting.

The principal differences observed in the microbiological activity in the three laboratory fermentations during the first day were in the yeast and coliform populations. The populations of these two groups of organisms were noted to be much higher over this period in the FC 1 and

FC 2 brines than in that from the FC 3 lot. Both groups were observed to decrease in numbers in the FC 1 and FC 2 brines after one day. According to the work of Rosen and Fabian (51), either one or both of these groups of brine organisms may utilize biotin and, thus, might be responsible for the relatively low levels noted in the FC 1 and FC 2 brines during the first day after brining.

Amino acids in non-fermenting and fermenting control brines. The results of the study of the amino acids concentrations in the non-fermented controls and fermented laboratory brines are given in Tables 28 and 29. Since these were only a few instances where the results obtained with the fermented brines were noted to be very dissimilar to those of the non-fermented brines, only the concentrations of the amino acids in the latter lots are presented in Figs. 12 and 13.

A definite relationship was evident between the size of the cucumbers and the concentration of all six of the amino acids in the brines. Thus, fermentations of small sized cucumbers would have a higher potential of both the vitamins and amino acids which are essential for *L. plantarum* than would those of large sized cucumbers.

The time required for the brines to attain the maximum concentrations of the amino acids was much longer than it was with the vitamins. Fifteen to 29 days were required for the amino acid levels to reach their maximum. The cystine content of the brines of all 6 lots was noted to continue to show a significant increase at 29 days.

The concentrations of leucine, isoleucine, valine, glutamic acid, and cystine observed in the three fermented brines as compared to those

TABLE 28

A comparison of the leucine, isoleucine, and valine concentrations in non-fermented and fermented brines covering cucumbers of three different sizes

Time from brining	Size No. 1		Size No. 2		Size No. 3	
	C 1	FC 1	C 2	FC 2	C 3	FC 3 1/
L-leucine	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
0 hr.	*	*	*	*	*	*
1	*	*	*	*	*	*
3	5.55	*	*	*	*	*
6	11.50	8.60	6.65	5.65	5.20	*
12	29.90	21.70	17.65	15.75	10.85	5.50
1 day	65.60	68.90	40.77	57.10	20.80	23.20
2	120.10	167.40	81.13	126.20	43.10	72.70
3	152.33	200.40	110.27	165.00	58.60	79.30
5	178.00	235.60	130.67	205.40	82.30	122.80
7	185.10	213.30	142.33	265.70	95.00	135.60
15	207.00	275.30	155.50	233.70	120.00	167.80
29	214.00	274.50	167.50	230.20	130.87	187.50
L-isoleucine						
0 hr.	**	**	**	**	**	**
1	**	**	**	**	**	**
3	**	**	**	**	**	**
6	5.60	4.60	3.25	**	**	**
12	18.05	11.43	10.60	7.98	5.50	**
1 day	42.80	39.75	25.38	33.00	11.52	12.48
2	78.50	88.95	51.67	64.13	23.70	40.00
3	97.00	110.50	69.64	85.50	32.30	46.95
5	104.0	130.75	77.57	108.00	47.37	68.05
7	106.10	117.30	81.04	142.50	59.54	74.55
15	122.45	158.55	93.20	125.75	71.37	96.25
29	126.10	158.45	93.75	128.25	79.00	108.35
L-valine						
0 hr.	**	**	**	**	**	**
1	**	**	**	**	**	**
3	**	**	**	**	**	**
6	6.88	5.45	3.08	2.68	**	**
12	21.75	14.08	11.80	10.08	4.93	**
1 day	50.25	46.80	29.23	39.50	13.25	16.45
2	89.20	111.70	57.60	81.35	29.50	52.00
3	107.00	136.80	73.80	107.50	39.30	62.60
5	116.20	155.75	85.63	131.60	54.00	84.00
7	122.50	140.30	92.25	172.50	61.10	92.00
15	131.60	181.40	101.50	150.35	73.00	116.50
29	137.10	177.75	103.05	153.25	79.70	134.40

1/ The C series were the non-fermented control samples and the FC series the laboratory fermentations.

* = Less than 5.0 µg/ml.

** = Less than 2.5 µg/ml.

TABLE 29

A comparison of the tryptophane, glutamic acid, and cystine concentrations in non-fermented and fermented brines covering cucumbers of three different sizes

Time from brining	Size No. 1		Size No. 2		Size No. 3	
	C 1	FC 1	C 2	FC 2	C 3	FC 3[1/]
Tryptophane	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
0 hr.	*	*	*	*	*	*
1	*	*	*	*	*	*
3	0.52	*	*	*	*	*
6	1.69	1.02	0.85	0.55	*	*
12	4.78	3.69	2.76	2.51	1.35	0.61
1 day	11.71	11.75	6.06	9.75	3.22	4.17
2	18.18	19.54	10.61	13.40	6.08	11.56
3	25.30	28.78	15.20	14.48	8.54	10.74
5	30.02	28.74	19.92	16.26	12.15	7.73
7	31.30	8.28	21.24	24.92	13.47	3.65
15	33.72	16.91	23.87	9.05	16.00	5.08
29	31.82	11.26	24.60	7.38	17.05	6.57
Glutamic acid						
0 hr.	**	**	**	**	**	**
1	**	**	**	**	**	**
3	**	**	**	**	**	**
6	10.37	13.35	5.15	6.10	**	**
12	30.10	20.60	16.30	12.95	10.80	6.20
1 day	63.50	55.70	36.55	36.10	21.65	22.10
2	104.00	116.70	61.50	92.20	39.20	47.90
3	115.80	146.40	81.10	116.20	48.50	65.20
5	132.50	164.00	95.60	134.00	64.20	105.50
7	149.60	140.30	107.10	168.00	75.50	109.00
15	182.60	130.20	129.20	138.40	101.90	128.20
29	203.00	118.90	137.00	136.00	111.80	136.10
Cystine						
0 hr.	*	*	*	*	*	*
1	*	*	*	*	*	*
3	*	*	*	*	*	*
6	0.76	0.65	*	0.55	*	*
12	1.84	1.80	1.19	1.89	0.68	0.70
1 day	3.47	4.08	2.86	5.37	1.56	2.38
2	10.35	13.61	7.60	9.04	3.50	5.85
3	13.16	19.52	10.20	14.07	5.53	7.06
5	14.93	20.56	13.44	12.92	8.96	11.56
7	17.79	19.89	14.69	19.02	11.14	12.91
15	19.22	17.74	18.15	14.73	16.10	13.65
29	22.80	18.84	20.05	16.32	17.49	14.70

1/ The C series were the non-fermented control samples and the FC series the laboratory fermentations.

* = Less than 0.5 µg/ml.

** = Less than 5.0 µg/ml.

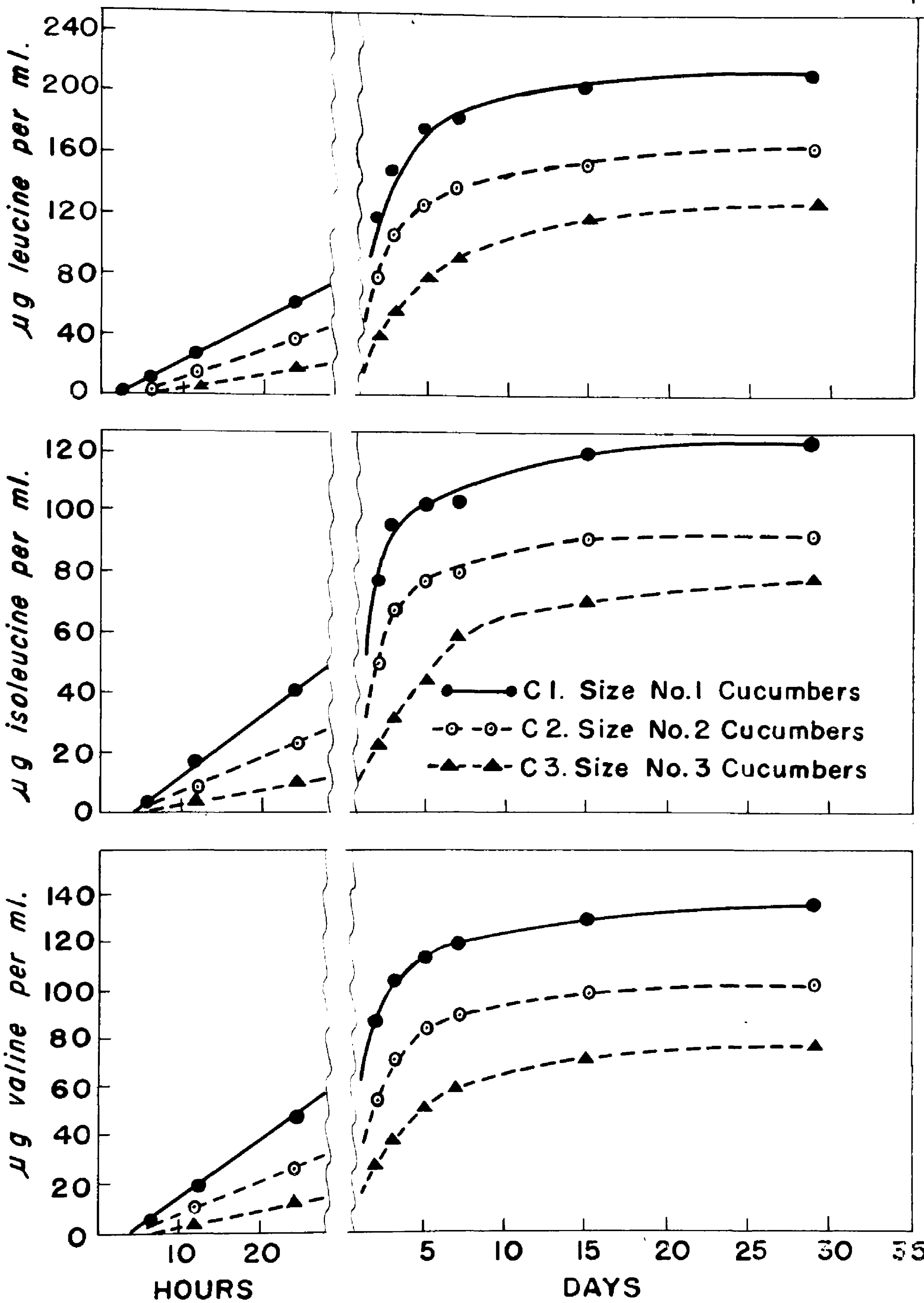

Fig. 12. Comparison of the rates of diffusion and the maximum concentrations attained of leucine, isoleucine, and valine from three different sizes of cucumbers in non-fermented brines.

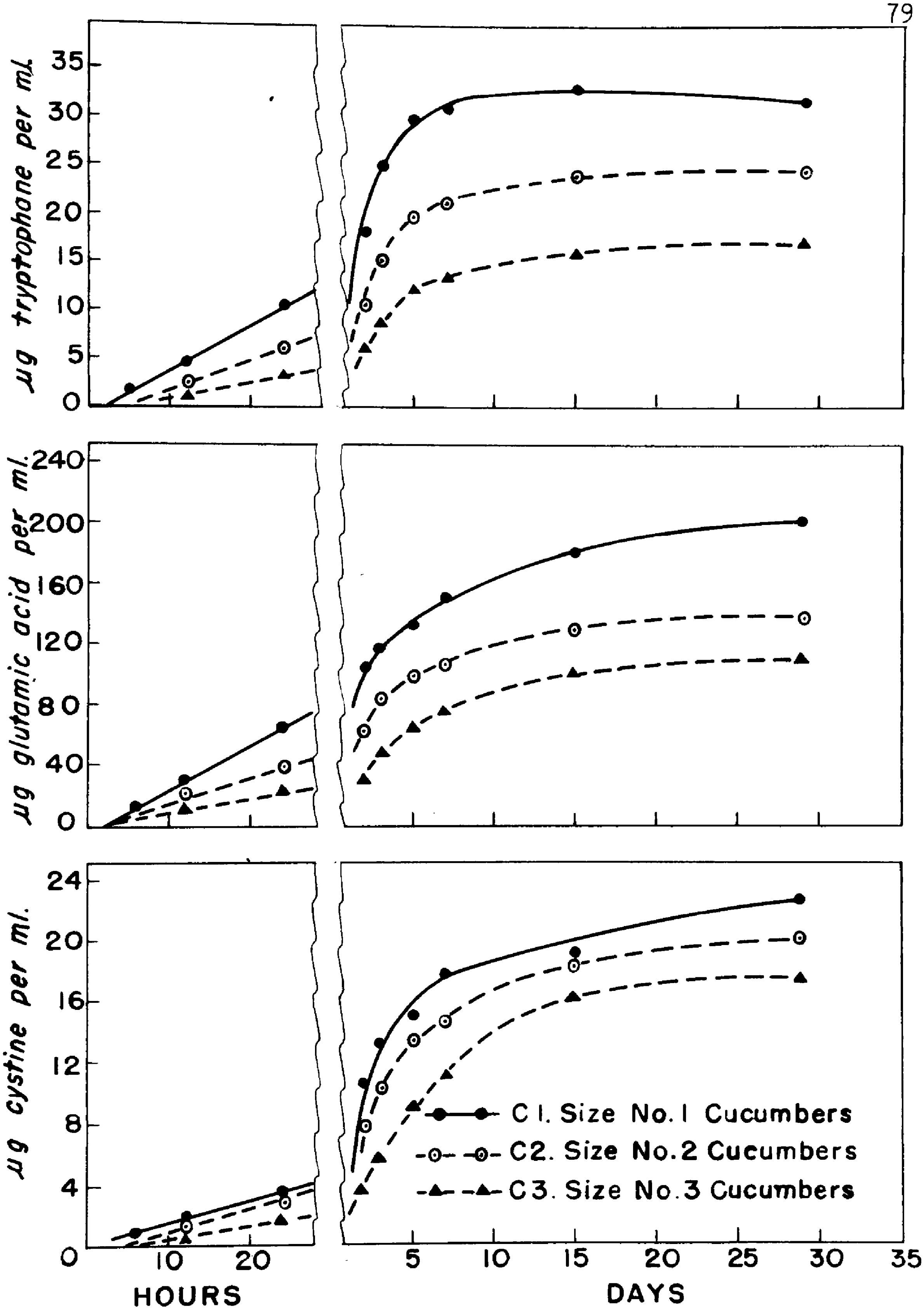

Fig. 13. Comparison of the rates of diffusion and the maximum concentrations attained of tryptophane, glutamic acid, and cystine from three different sizes of cucumbers in non-fermented brines.

in similar lots of the non-fermented brines showed no evident influence of the fermentation except during the first 12 hours or 1 day. After that time the concentrations of these five amino acids in the fermenting brines were higher in every instance than in the comparable lot of non-fermenting brine. Conversely, just the opposite relationship was observed in the samples taken 12 hours after brining. This is the same type of relationship observed in the biotin concentrations of these brines.

The tryptophane content of the brines was markedly influenced by the fermentation. For the first few days the tryptophane levels in the fermenting brines was comparable to those of the non-fermenting brines. However, a marked reduction in the concentration of this amino acid was noted at 5 days in lot FC 3, at 7 days in FC 1, and at 15 days in FC 2.

On comparison of the microbiological activity in these brines with the tryptophane levels, it was noted that yeasts were very active in all 3 lots at the same period of time that the reduction in tryptophane occurred. The trends of yeast populations are compared to the tryptophane levels in the brines in Fig. 14. In the FC 1 lot the tryptophane level dropped at two different times and in both instances the yeast activity was noted to increase. While increases in yeast numbers were noted in both FC 2 and FC 3 lots prior to a decrease in tryptophane, this occurred within the first 7 days after brining when the concentration of the amino acids in the non-fermented control brines were still increasing. This indicates that the yeasts were quite active in the destruction of tryptophane in these three laboratory fermentations.

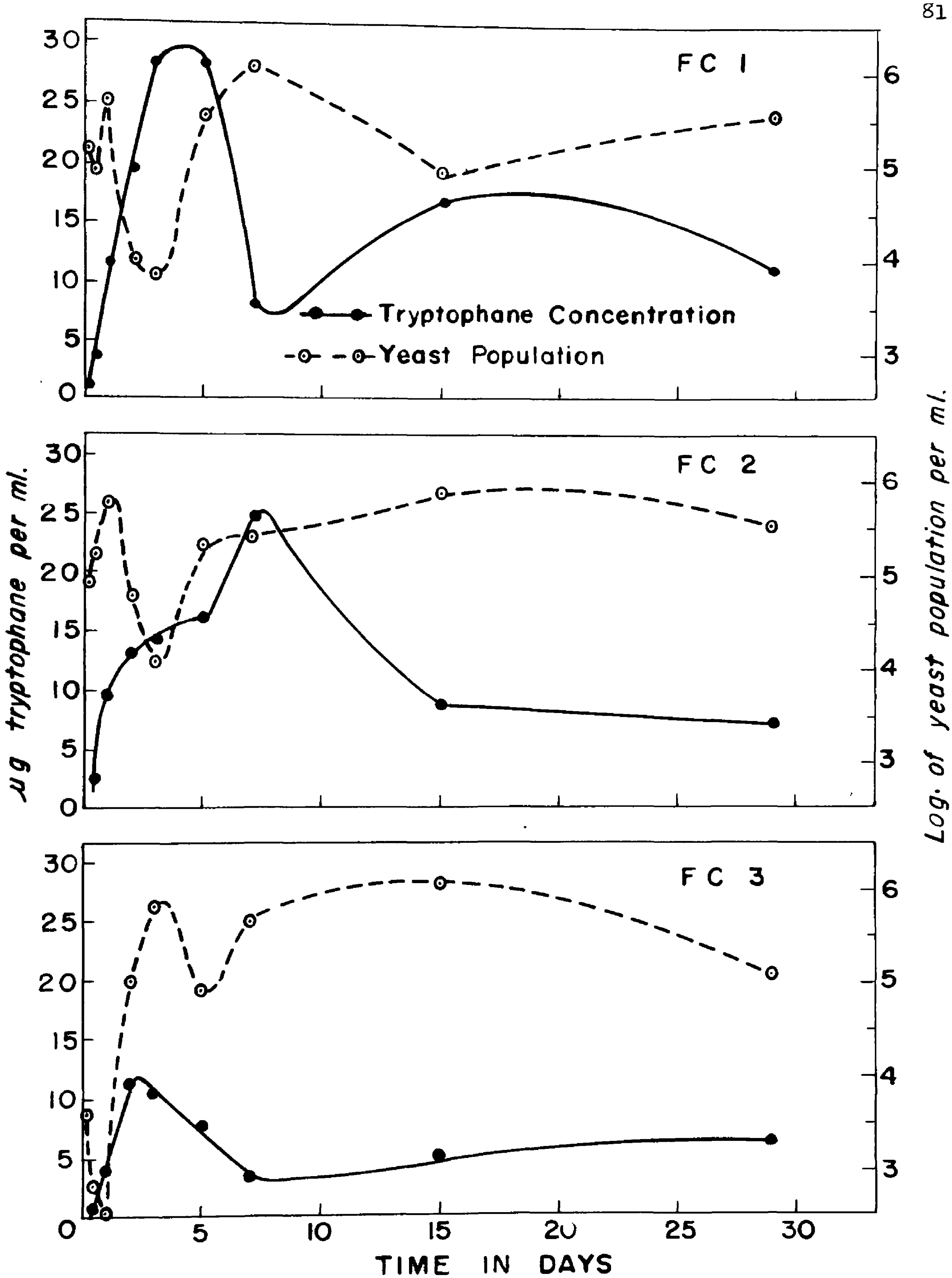

Fig. 14. Comparison of tryptophane concentrations and yeast activity in three laboratory fermentations.

The coliform organisms had completely disappeared from these fermentations by the fifth day and the peak of the acid-forming bacteria populations had been passed in both the FC 1 and FC 2 lots before any reduction in tryptophane was noted. Therefore, these organisms probably did not contribute greatly to the destruction of tryptophane.

The Effect of Various Microorganisms on the Vitamin and Amino Acid Content of Cucumber Brines

The study of commercial and laboratory fermentations indicated that the levels of some of the vitamins and amino acids in the brine may be influenced by the microflora present. Thus, a survey was made of 2 species of bacteria and 4 yeast species that have been found to be most active in cucumber fermentations (*viz*, *L. plantarum*, a coliform organism, *Torulopsis holmii*, *Torulaspora rosei*, *Torulopsis caroliniana*, and *Hansenula subpelliculosa*) as to their effect on the concentrations of these nutrients in brine.

Large lots of the non-fermented brines described in the previous section were obtained when the cucumbers were removed from them. These brines were frozen and held for use in this study. The brine was prepared by filtering through cheese cloth to remove dirt particles. After thorough mixing, it was dispensed in 500 ml Erlenmyer flasks, 250 ml per flask, and sterilized at 15 pounds pressure (121°C) for 20 minutes.

The bacteria and yeast cultures to be tested were grown in micro-inoculum broth (Difco) for 24 hours, centrifuged, and resuspended in five ml of sterile isotonic saline. One drop of this suspension was used to inoculate a flask of brine. Each organism was inoculated into one flask of the sterile brine. One flask was not inoculated and served as the control.

After five days incubation at 30°C, heavy growth was noted in all of the inoculated flasks as evidenced by turbidity and (or) sediment. Since several days were required to run all the microbiological assays, the flasks of brine were autoclaved at 15 pounds pressure for 10 minutes to prevent further activity.

The results of the vitamin and amino acid studies on these lots may be noted in Figs. 15, 16, and 17, and Table 30. It is readily apparent that the bacteria and yeasts tested had little or no effect on the niacin content of the brine. The niacin concentrations of the various inoculated lots of brine varied less than 5 percent from that of the uninoculated control. Thus, they were not believed to be significant.

However, *L. plantarum* produced a significant reduction in both the pantothenic acid and biotin content of the brine. The coliform organism and the four yeasts failed to lower the pantothenic acid level, but all of these organisms except *Hansenula subpelliculosa* reduced the biotin content by considerably more than 10 percent. The *Hansenula* species lowered the biotin level by almost 10 percent.

A synthesis of pantothenic acid was indicated in the brines inoculated with *Hansenula subpelliculosa* and *Torulaspora rosei*.

These results were in general agreement with the studies made by Rosen and Fabian (51) on the effect of these microorganisms on the niacin, pantothenic acid, and biotin content of cucumber juice. However,

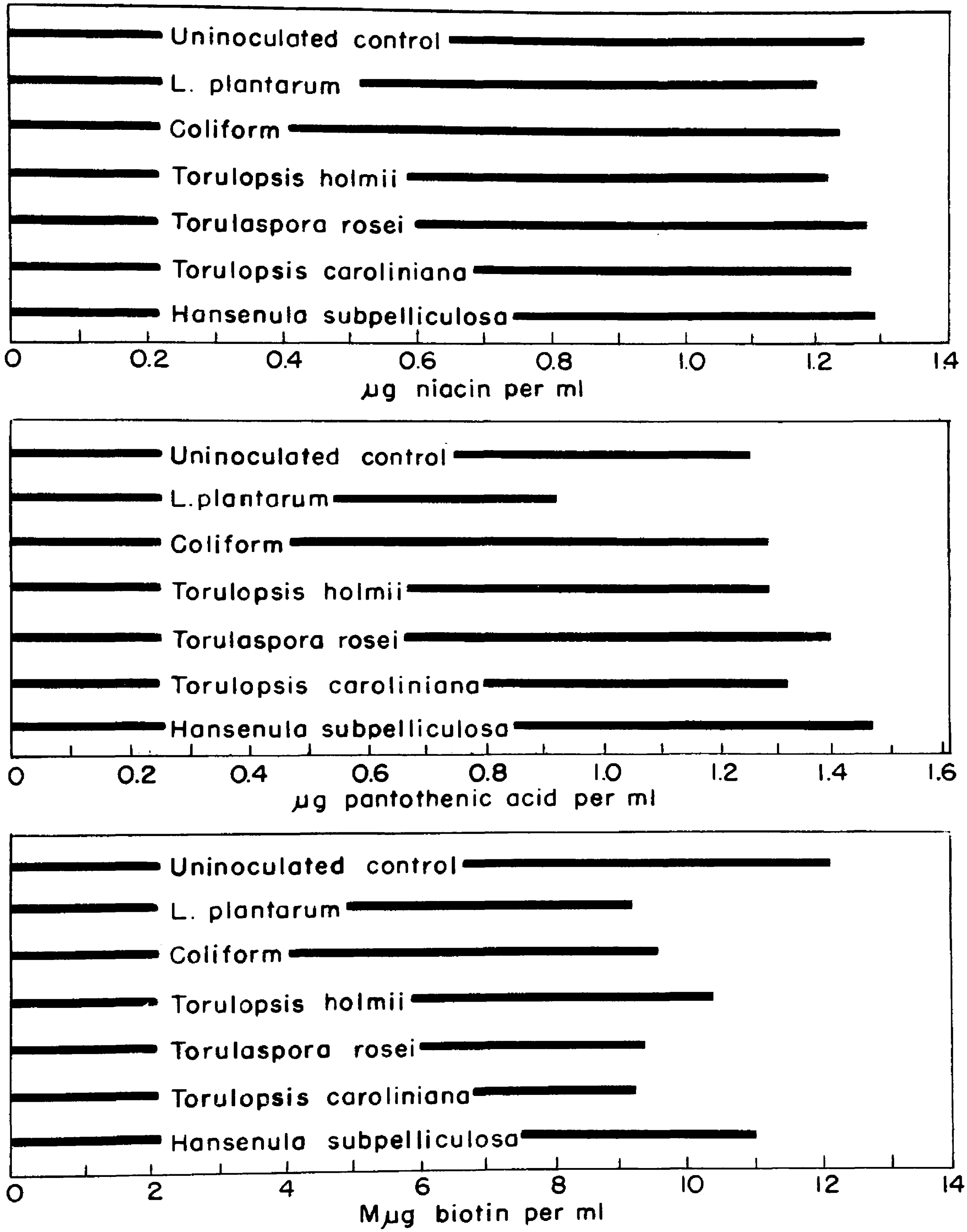

Fig. 15. The effect of various microorganisms on the available niacin, pantothenic acid, and biotin content of cucumber brines.

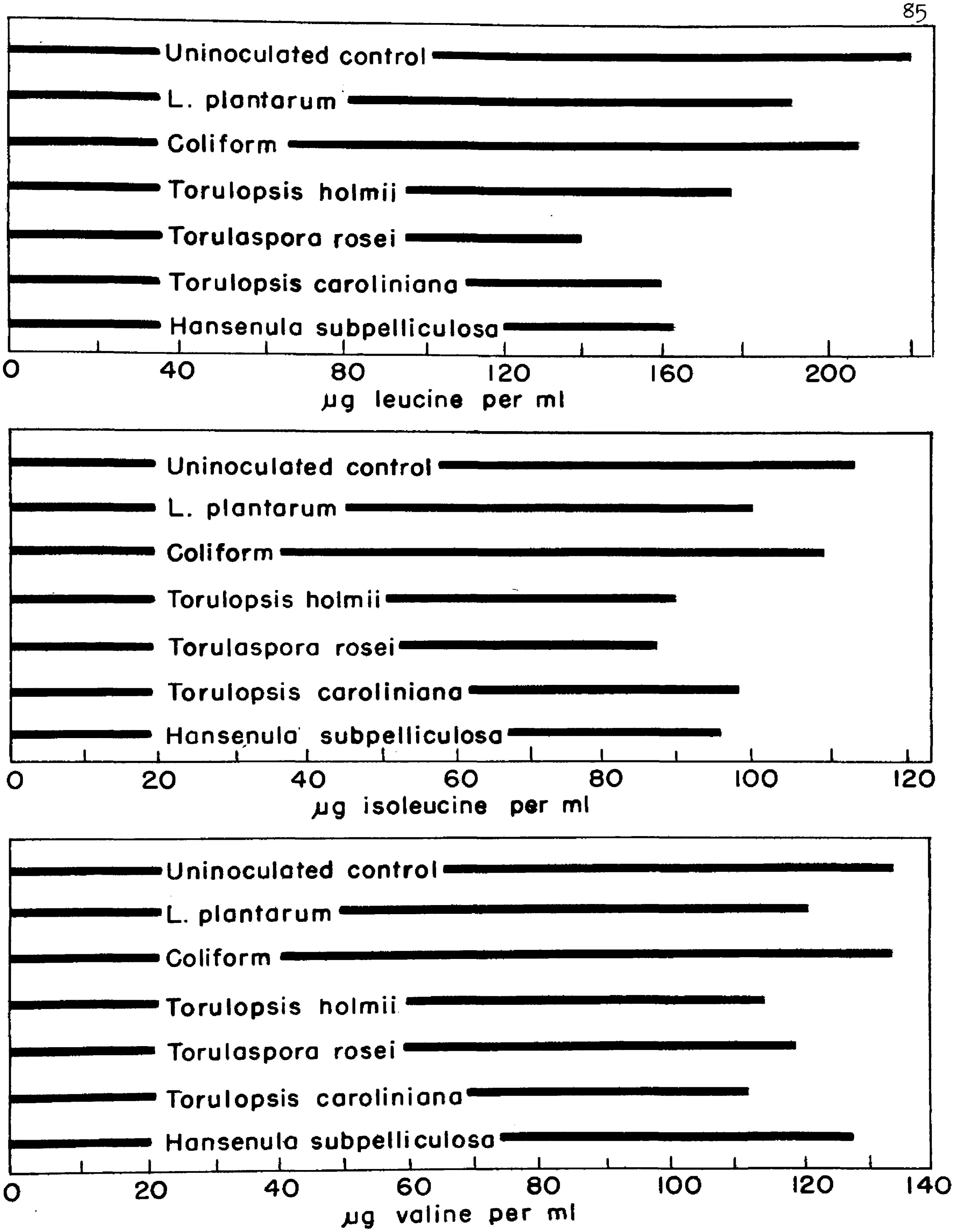

Fig. 16. The effect of various microorganisms on the available leucine, isoleucine, and valine content of cucumber brines.

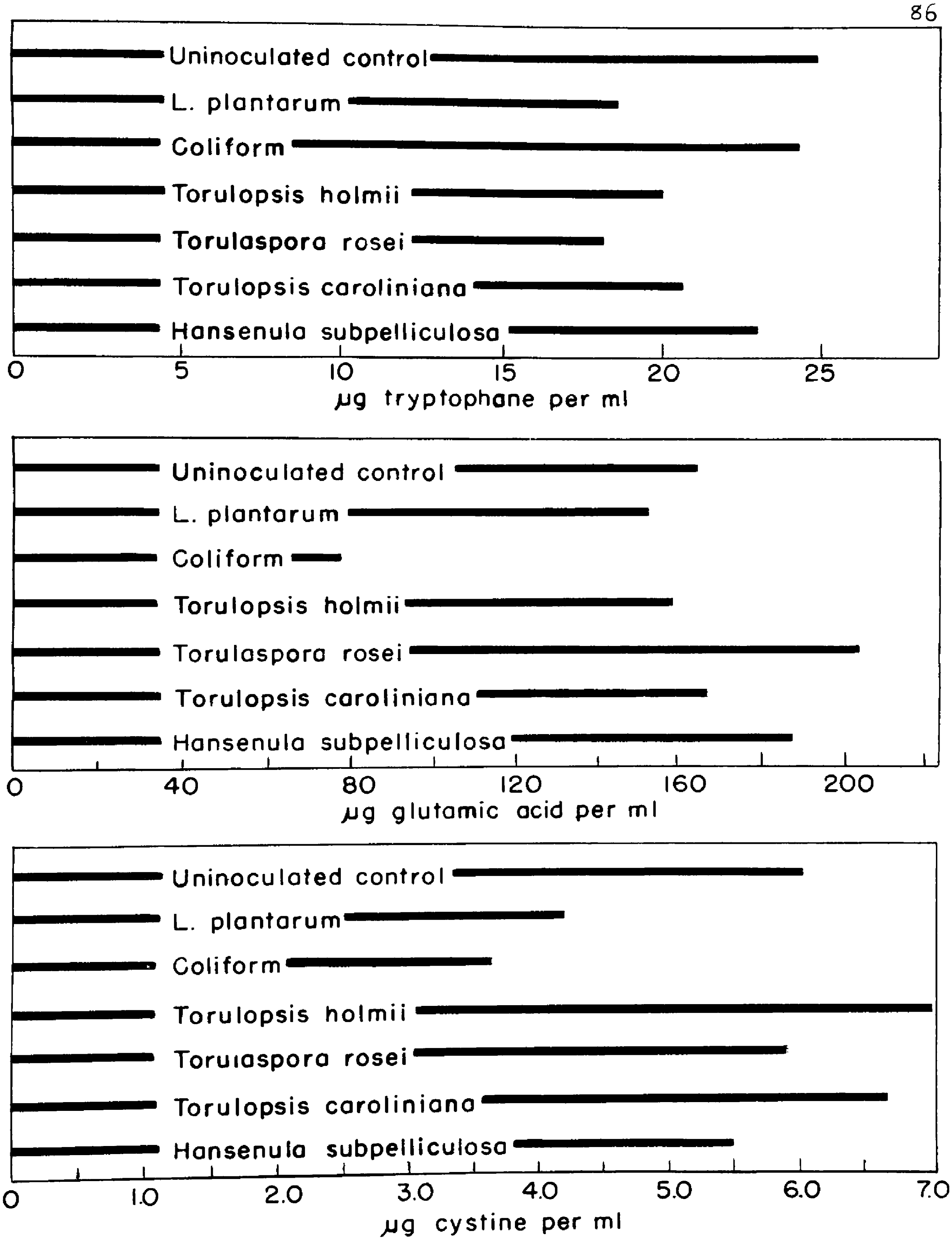

Fig. 17. The effect of various microorganisms on the available tryptophane, glutamic acid, and cystine content of cucumber brines.

TABLE 30

Effect of various microorganisms on the vitamin and amino acid content of non-fermented cucumber brine

Inoculated Organism 1/	Concentrations of vitamins and amino acids after five days incubation								
	Niacin µg/ml	Pantothenic acid µg/ml	Biotin mµg/ml	Leucine µg/ml	Iso-leucine µg/ml	Valine µg/ml	Trypto-phane µg/ml	Glutamic acid µg/ml	Cystine µg/ml
Uninoculated control	1.272	1.251	12.175	220.4	114.7	134.0	24.70	166.0	5.43 (a) 2/ 6.61 (b)
L. plantarum (L-15)	1.207	0.923	9.200	193.1	100.3	121.9	18.55	153.6	4.05 (a) 4.35 (b)
Coliform (C-10)	1.241	1.285	9.700	209.0	107.3	133.9	24.20	76.8	2.24 (a) 3.03 (b)
Torulopsis holmii (FBY-309)	1.220	1.285	10.400	176.5	89.0	115.3	18.94	158.4	6.68 (a) 7.24 (b)
Torulaspora rosei (FBY-287)	1.280	1.388	9.425	139.4	86.3	118.8	18.10	205.0	5.66 (a) 6.07 (b)
Torulopsis caroliniana (RY-200)	1.256	1.318	9.230	159.6	96.5	112.6	20.54	169.4	6.52 (a) 6.80 (b)
Hansenula subpelliculosa (FBY-72)	1.285	1.473	11.050	162.1	94.6	127.6	22.75	190.0	5.10 (a) 5.82 (b)
								Unheated Control	11.93 (b)

1/ All of the isolates tested except *Torulopsis caroliniana* were isolated from the commercial fermentations studied. *Torulopsis caroliniana* was originally isolated by Etchells and Bell from cucumber fermentations, and was supplied to this laboratory by the Northern Regional Research Laboratory, Peoria, Ill.

2/ The (a) values were obtained in the same experiment with the vitamins and other amino acids. The (b) values are the results of a different experiment using a different lot of brine.

a much greater reduction in the biotin content of the brine used in these experiments was noted with L. plantarum than in the diluted cucumber juice as tested by these workers. The extent of growth and acid production in the two different media as well as the difference in the strains tested are variable factors which may account for this significant difference in biotin utilization by L. plantarum.

The results of this experiment were quite similar with regard to the influence of the various microorganisms on the leucine, isoleucine, valine and tryptophane content of the cucumber brine (Figs. 16 and 17 and Table 30). Thus, L. plantarum and the four yeasts tested were noted to lower the levels of all these amino acids, although the decreases in valine and tryptophane contents due to Hansenula subpelliculosa were too small to be significant. The coliform isolate failed to affect the concentrations of these four amino acids to a significant extent.

In the case of glutamic acid, about the opposite relationship was noted. The coliform isolate lowered the glutamic acid content by more than 50 percent while the yeasts failed to decrease the level significantly. In fact, the growth of Torulaspora rosei and Hansenula subpelliculosa resulted in an increase in the available glutamic acid. Only a slight decrease was noted due to L. plantarum.

The concentrations of all of the amino acids except cystine in the uninoculated control lots of brine were approximately what were expected on the basis of the assays made on the non-fermented brine samples in the previous section. The cystine content, however, was considerably lower. The only plausable explanation for this was that much of the cystine was destroyed or rendered unavailable for Leuc. mesenteroides P-60 by the sterilizing process. Similar observations have been made by Riesen et al. (49) and Sarkar et al. (53) on the cystine content of basal media. The former workers demonstrated that the effect of sterilization reduced the availability of cystine to Leuc. mesenteroides about 40 percent but did not affect its availability to Lactobacillus casei. Sarkar et al. (53) noted that the loss in test material was proportional to that in the standard and, thus, did not result in inaccuracies in cystine assays.

To be sure that the differences in cystine concentrations noted in the various inoculated lots of brine was not the result of the sterilization process the experiment was repeated for this amino acid. The brine used in this experiment was also from the non-fermented control lots. It was filtered through cheese cloth and dispensed in 16mm tubes, 10 ml per tube. One tube was saved as an unheated control and kept under toluene in the refrigerator. The other tubes were sterilized at 15 pounds pressure for 10 minutes, cooled, and inoculated with the various microorganisms. The preparation of the inoculum and the inoculations were carried out in the same manner as in the foregoing experiment except that the initial cell suspension was diluted 1-25 before making the drop inoculations.

After five days incubation at 30°C, microbiological assays for cystine were made immediately to prevent the necessity of resterilization. The results of this experiment are given as the (b) values for cystine in Table 30.

On comparing the heated with the unheated controls, it was evident that the sterilizing process reduced the available cystine content of the brine markedly. However, the results obtained on the influence of the various isolates on the cystine content were quite consistent in both trials. Thus, it was believed that the reduction in cystine was incidental to this experiment.

The average values for the two trials are shown in Fig. 17. Both *L. plantarum* and the coliform isolate reduced the available cystine content of the brine markedly--by about 30 percent and 50 percent respectively. The growth of *Torulopsis holmii* resulted in an increase in the available cystine, while the other three yeasts failed to influence the concentration of this amino acid to a significant extent.

It should be pointed out, that the utilization of cystine by the coliform group could be quite important in cucumber fermentations. This group of organisms have been noted to be active in some fermentations the first few days after brining and it was at this period that the cystine concentrations in a few of the fermenting brines studied were noted to be relatively low.

DISCUSSION

The acid fermentation of cucumbers for salt stock is dependent on the growth of the common lactic acid organism, *L. plantarum*. These results agree with those of Etchells and Jones (16) and Rosen and Fabian (51). However, cucumber fermentations are not that simple since a number of yeast species have been shown to be active in both commercial and laboratory fermentations. As was noted by Etchells *et al.* (13), *Torulopsis holmii* was the yeast species found to be most prevalent in the early part of most commercial fermentations studied. *Torulaspora rosei*, however, was dominant in one commercial tank and in three laboratory fermentations. This shows that even under similar conditions the yeast flora may be significantly different.

While no significant *Aerobacter* activity was demonstrated in these experiments, Etchells *et al.* (14) and Rosen and Fabian (51) have noted these organisms to be quite active in commercial fermentations in the South and in laboratory fermentations respectively. Therefore, a wider survey of commercial fermentations in the North for *Aerobacter* activity is indicated.

The vitamin and amino acid requirements of *L. plantarum* isolates from cucumber fermentations were found to be similar to those for the well known 17-5 strain. The requirements of this species for biotin, niacin, and pantothenic acid and for leucine, isoleucine, valine, glutamic acid and tryptophane noted in this investigation are in agreement with the reports by other workers (10, 24, 33, 43, 55). Cystine has also been observed to be essential for this organism in this work and in that by other workers (24, 33, 43, 55). Conversely, this amino acid was found by Dunn *et al.* (10) to be non-essential for *L. plantarum* 17-5 in a greatly enriched medium. While threonine was found to be essential for all strains tested including 17-5 in the basal medium used in these experiments, other investigators (43, 64) have definitely established that *L. plantarum* 17-5 does not require threonine in different media. Thus, it appears that the requirements for these two amino acids depends on the basal medium used for testing.

Some variations were noted among the isolates tested in respect to riboflavin and *p*-aminobenzoic acid and to phenylalanine, tyrosine and arginine requirements. Previous reports have also been conflicting as to the requirements of this species for these nutrients. However, since the basal medium was constant in these experiments the variations noted must be due to differences in strains rather than in the test medium. Similar variations between strains of the same species have been demonstrated by Dunn *et al.* (10) and Shankman *et al.* (56).

Since *L. plantarum* from cucumber fermentations requires a number of vitamins and amino acids for growth and acid production these essential nutrients must be available in cucumber brines in sufficient quantities for this organism if a desirable acid fermentation occurs. This has been found to be true in this investigation and in that of Rosen and Fabian (51) in the case of biotin, niacin, and pantothenic acid. These vitamins are extracted rapidly from the cucumbers by the brine and apparently reach a point of equalization in about 5 days.

While yeasts and bacteria which were isolated from the cucumber fermentations markedly reduced the biotin content of the brine when grown in pure culture, similar reductions were not observed in the cucumber fermentations. This is due possibly to a buffer action of the cucumbers with more biotin diffusing out as it is being utilized.

As might be expected due to their relatively low solubility, the amino acids required longer to attain their maximum concentration in the brine. Nevertheless, all six essential amino acids were present in sufficient concentrations in most instances to support the growth of _L. plantarum_ within 24 hours and were not apparently limiting for the growth of this species in any fermentation studied.

It should be noted that very little yeast activity occurred in these brines in the first week and practically no _Aerobacter_ fermentation was evident and this may have greatly influenced the results obtained with at least two of the amino acids. Thus, it was demonstrated that marked reductions occurred consistently in the tryptophane levels of the brine when yeast activity was greatest and that similar reductions were obtained when pure cultures of the predominating yeasts were grown in sterile brine. If such activity occurred early in the fermentation as demonstrated in some instances by Etchells _et al_. (14), this amino acid might be reduced to a critical level for the growth of _L. plantarum_. Also, much activity of coliform organisms could possibly result in critical concentrations of cystine as these bacteria were shown in these studies to reduce the concentration of this amino acid in brine greatly when grown in pure culture. Although, the other essential amino acids were affected by one or more groups of brine microorganisms their concentrations in the brine were relatively high in comparison to tryptophane and cystine and, thus, would not be expected to become limiting.

A definite relationship was evident between the concentrations of all the vitamins and amino acids studied and the size of the cucumbers in the brine. Thus, the brines covering smaller cucumbers contained higher concentrations of these nutrients than those covering comparable cucumbers of larger sizes when the ratio of brine to cucumbers was constant. Therefore, larger cucumbers must contain less of these nutrients per unit weight or there is less complete equalization of the nutrients between the brine and cucumbers with the larger sizes than in the case of the smaller. However, Rosen and Fabian (51) have shown large differences in vitamin concentrations in cucumbers of the same size. A study of these nutrients in cucumbers of various sizes and varieties would be of much interest.

SUMMARY

A study of the vitamin and amino acid requirements of *L. plantarum* isolated from cucumber fermentations and the availability in cucumber brines of those found essential has been presented. Ten commercial and three laboratory fermentations were characterized for microbiological activity as a basis for this nutrition study.

Under commercial conditions the acid-forming bacteria attained their maximum activity in the first 3 to 6 days and declined throughout the remainder of the fermentation period studied. Yeast populations declined during the first few days and then multiplied rapidly reaching their maximum in from 10 to 20 days after brining. The coliform organisms played no significant role in these fermentations. The microbiological activity in laboratory fermentations was similar except for the yeast picture; yeast activity was variable in these brines.

The principal acid-forming organism was *L. plantarum*, and the most active yeast was *Torulopsis holmii*. However, in the laboratory fermentations and in one commercial tank *Torulaspora rosei* was predominant in the yeast flora. *Hansenula subpelliculosa* and *Brettanomyces versatilis* were also present in the fermentations.

The vitamin and amino acid requirements of *L. plantarum* isolates from cucumber fermentations were very similar to those for *L. plantarum* 17-5. Thus, the vitamins--biotin, niacin and pantothenic acid; and the amino acids--leucine, isoleucine, valine, glutamic acid, cystine, tryptophane, and threonine were essential for this species in the basal media

used. Riboflavin was essential for two strains and p-aminobenzoic acid for three strains from cucumber fermentations. Alanine, phenylalanine, arginine, and tyrosine were either stimulatory or essential for one or more of the isolates.

Biotin, niacin, and pantothenic acid became available in cucumber brines very rapidly, reached their maximum concentrations in 5 to 7 days, and the levels remained relatively constant thereafter. No great influence of fermentation on these vitamins was evident. However, the biotin concentration in control brine was lowered considerably by the growth of the various brine organisms in pure culture.

Although the amino acids were considerably slower in reaching their maximum concentrations in the brine than the vitamins, in most fermentations they were present in sufficient concentrations within 24 hours to support the growth of L. plantarum. Cystine was found to diffuse more slowly than the other five amino acids from the cucumbers into the brine.

Tryptophane was the only amino acid studied which was consistently affected by the fermentation. The level of this amino acid was markedly reduced in the brines at the same period when the yeast activity was greatest, and was also reduced by pure cultures of these microorganisms and by L. plantarum in control brine. Leucine, isoleucine, and valine were also rendered less available in the control brine by the growth of brine yeasts but were not measurably affected in the cucumber fermentations studied. Available cystine and glutamic acid concentrations were greatly lowered by the growth of a coliform isolate from cucumber fermentations.

The available concentrations of all of these essential nutrients for L. plantarum were influenced by the size of the cucumbers; brines containing smaller cucumbers were richer.

These results indicate that the essential vitamins and amino acids are usually not limiting to L. plantarum in cucumber fermentations under these conditions. However, in fermentations where Aerobacter activity is prevalent during the first few days, cystine concentrations might well be reduced to a critical level. Also, it is indicated that much yeast activity early in the fermentation might result in very low tryptophane concentrations. These amino acids bear further investigation under different conditions.

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