THE EFFECTS OF VARIOUS FRUIT JUICES ON URINARY pH AND BACTERIOSTASIS AND THEIR POTENTIAL USE IN THE TREATMENT OF URINARY TRACT INFECTIONS

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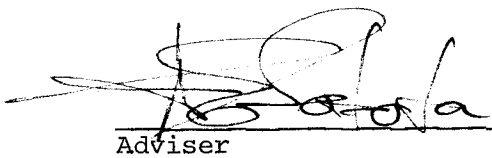

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ABSTRACT

THE EFFECTS OF VARIOUS FRUIT JUICES ON URINARY pH AND BACTERIOSTASIS AND THEIR POTENTIAL USE IN THE TREATMENT OF URINARY TRACT INFECTIONS

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Master of Science

Youngstown State University, 1984

Many believe that cranberry juice can be ingested to prevent and treat urinary tract infections (UTIs). Investigations into the mechanism of action of cranberry juice has revealed that daily ingestion of even large amounts of cranberry juice can only rarely increase the amount of urinary hippuric acid and decrease the urinary pH enough to be bacteriostatic. A more recent investigation, however, has demonstrated that bacterial adherence can be prevented by urine collected 1-3 hours after ingestion of cranberry juice, but that urine samples collected later, or even morning samples collected following late night ingestion, proved inactive. For this study, 3 adult males ingested 24 ozs. of either grape, cranberry, or orange juice, on separate mornings. Urinary volume, pH, and bacteriostatic effects upon Escherichia coli were monitored hourly for a period of 6 hours after ingestion. It was concluded that none of these three juices were immediately effective bacteriostats, and they did not differ significantly from each other in their effects upon bacterial growth. However, they did differ significantly in their effects upon urinary pH. Grape produced a more acidic urine than cranberry juice, and orange juice ingestion produced a more alkaline urine.

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TABLE OF CONTENTS

PAGE

ABSTRACT ii

ACKNOWLEDGEMENTS iii

TABLE OF CONTENTS iv

LIST OF FIGURES v

LIST OF TABLES vii

CHAPTER

I. INTRODUCTION 1

II. MATERIALS AND METHODS 9

Bacteria 9

Human Subjects 9

Juices and Diet 9

Urine 10

Bacterial Dilution and Culture Media 11

Bacterial Growth in Urine 12

Dilutions 12

Plating 12

Colony Counts 13

III. RESULTS 14

Effects Upon Bacterial Growth 15

Effects Upon pH 17

Correlation Studies 19

IV. DISCUSSION 21

APPENDIX A. Tables 29

APPENDIX B. Figures 40

BIBLIOGRAPHY 58

LIST OF FIGURES

FIGURE		PAGE
1.	Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Grape Juice	41
2.	Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Grape Juice (A is Advanced 1 Hour and B is Delayed 1 Hour	42
3.	Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Cranberry Juice	43
4.	Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Cranberry Juice (A is Advanced 1 Hour)	44
5.	Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Orange Juice	45
6.	Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Orange Juice (A is Delayed 1 Hour and B is Advanced 1 Hour)	46
7.	Bacterial Growth in Urine Media as a Function of Time After Ingestion of Grape Juice	47
8.	Bacterial Growth in Urine Media as a Function of Time After Ingestion of Cranberry Juice	48
9.	Bacterial Growth in Urine Media as a Function of Time After Ingestion of Orange Juice	49
10.	pH of Urine as a Function of Time After Ingestion of Grape Juice	50
11.	pH of Urine as a Function of Time After Ingestion of Grape Juice (B is Advanced 2 Hours)	51
12.	pH of Urine as a Function of Time After Ingestion of Grape Juice (From Experiments in Which No Growth Data were Obtained)	52
13.	pH of Urine as a Function of Time After Ingestion of Cranberry Juice	53
14.	pH of Urine as a Function of Time After Ingestion of Cranberry Juice (A is Delayed 1 Hour)	54
15.	pH of Urine as a Function of Time After Ingestion of Cranberry Juice (From Experiments in Which No Growth Data were Obtained)	55

LIST OF FIGURES (CONT.)

FIGURE PAGE

16. pH of Urine as a Function of Time After Ingestion of Orange Juice 56

17. pH of Urine as a Function of Time After Ingestion of Orange Juice (From Experiments in Which No Growth Data Were Obtained 57

LIST OF TABLES

TABLE		PAGE
1.	Data Obtained from Ingestion of Grape Juice	30
2.	Data Obtained from Ingestion of Cranberry Juice	30
3.	Data Obtained from Ingestion of Orange Juice	31
4.	Additional Volume and pH Data for All Three Juices	32
5.	Results of Randomized Complete Block ANOV by Ranks (Friedman's Test) to Test Each Ho: There is No Difference in Bacterial Growth in Urine/BHI Media at (Time Specified) Due to the Three Different Juices	33
6.	Results of Randomized Complete Block ANOV by Ranks (Friedman's Test) to Test Each Ho: There is No Difference in Bacterial Growth in Urine Media at (Time Specified) Due to the Three Different Juices	33
7.	Results of Randomized Complete ANOV by Ranks (Friedman's Test) to Test Each Ho: There is No Difference in Bacterial Growth Potential in Urine at (Time Specified) Due to the Three Different Juices	34
8.	Results of Model I Randomized Complete Block Design to Test Each Ho: There is No Difference in pH at (Time Specified) Due to the Three Different Juices	35
9.	Results of Newman-Keuls Multiple Range Test to Test Each Ho: $\mu_B = \mu_A$ for Those Times in Which Differences in pH Due to the Different Juices Were Detected in Table 8	35
10.	Results of Nested ANOV for pH Data of Tables 1,2 & 3 . . .	36
11.	Results of Newman-Keuls Multiple Range Test to Test Each Ho: $\mu_B = \mu_A$ to Determine Between Which Juices Population Means Differences Exist for Table 10	36
12.	Results of Model I ANOV to Test Ho: μ pH of Control (Time Zero) Urine = μ pH of Grape Juice Urine = μ pH of Cranberry Juice Urine = μ pH of Orange Juice Urine	37
13.	Results of Dunnett's Test for Comparing a Control Mean pH (Time Zero) to Each Other Group Mean pH (i.e., Grape, Cranberry, and Orange) Based on the Results of Table 12 .	37
14.	Results of Studies of Correlation Between Urinary pH and Bacterial Growth in Urine	38

LIST OF TABLES (CONT.)

TABLE PAGE

15. Results of Studies of Correlation Between Urinary Volume and Bacterial Growth in Urine 39

16. Results of Studies of Correlation Between Urine Volume and pH 39

CHAPTER I

INTRODUCTION

Urinary tract infections (UTIs) are commonly encountered health disturbances in primary care medicine. Cystitis alone accounted for more than 5 million yearly patient visits in the mid 1970s (National Ambulatory Medical Care Survey). Prostatitis, asymptomatic bacteriuria, pyelonephritis, and urethritis are also included under the heading of UTI (Froom, 1980).

There is a much greater prevalence of UTI in women than in men, with nearly 5% of all adolescent women having a history and 1.2% being infected at any given time (Kunin, 1970). The incidence of UTI in pregnant women is estimated at 2-10%, and it increases with decreasing socioeconomic status (Marchant, 1978). Women may be more susceptible than men because of the shortness of the female urethra, the shorter distance between the urethral orifice and the anus, abrasion and inoculation during coitus, changes in the ability of bacteria to adhere to uroepithelial surfaces, and other reasons (Froom, 1980).

Escherichia coli is the most frequently isolated pathogen in UTI, followed by Proteus, Pseudomonas, Klebsiella, and others (Brooks et al., 1971; Nahata et al., 1981). First-time infection generally involves E. coli, whereas the other types are more common in recurrent infections (Froom, 1980). Brooks' group looked at several uropathogenic properties of E. coli in recurrent UTI, including the ability to colonize uroepithelial surfaces, relative resistance to low pH and other inhibitors, resistance to phagocytosis and serum bacteriocides, and the potential to

release toxins (Brooks, 1980). The study concluded that strains with a combination of uropathogenic characteristics occurred more frequently in upper tract infections and only rarely in periurethral areas of control subjects, but also that E. coli with relatively few pathogenic properties were also often isolated in UTI.

Accurate diagnosis of UTI is based upon the demonstration of at least 10^5 bacteria or colony-forming units (CFU) per ml. of urine (Washington et al., 1981).

Only about 10% of all UTIs involve the upper tract, and these infections are indicated when bacteria coated with antibodies are found in the patient's urine (Froom, 1980). Probably the most dangerous UTIs are those of the upper tract, which can cause pyelonephritis, renal scarring, and other serious pathologies, and those occurring during pregnancy. Decreased infant birth weights, two-fold increases in perinatal mortality, and increased incidence of eye infections and impaired motor activity have been associated with UTI during pregnancy (Froom, 1980).

Urinary tract infections are generally treated by oral administration of antibacterial agents, or a combination of antibacterial agents and urinary acidifiers. For example, methenamine therapy is often used to treat chronic bacteriuria in geriatric patients, and is often given in conjunction with ascorbic acid or cranberry juice (Nahata et al., 1981).

For years, cranberry juice has played a dual role in combating UTI: sometimes it is ingested as the sole prophylactic measure or treatment in providing symptomatic relief, and sometimes it is recommended as a urinary acidifier to increase the effectiveness of prescribed antibiotics. In some hospitals and nursing homes, cranberry juice is

regulary administered to catheterized patients and to others who are highly susceptible or chronically affected by UTIs (Nahata et al., 1981). More importantly, researchers, in controlled experiments, have reported its successful use, both as a treatment and prophylactic measure (Prodromos et al., 1968). In short, cranberry juice is widely used, and in regards to its effectiveness as a treatment or prophylactic measure, some supportive evidence does exist. There are, however, great controversies concerning the juice's ability to acidify urine, and its mechanism of action as a UTI treatment.

Studies of the ability of cranberry juice to acidify the urine have often involved close examination of its constituent compounds and monitoring of their metabolic end-products. The complete composition of cranberry juice has not been determined, but it is approximately 13.5g/100 ml sugar (Li and Shuhmann, 1983) and most of its pigments and organic acids have been identified. Four anthocyanins, namely, the monoarabinosides and monogolactosides of cyaniden and peonidin (Chiriboga and Francis, 1970), and eight yellow and brown derivatives of myricetin and quercetin flavonols (Lees and Francis, 1971) are the predominant pigments. Cranberry juice is about 1.32% quinic acid, 0.92% malic acid, and 1.08% citric acids (Coppola et al., 1978).

The quinic acid constituent of cranberry juice has been the greatest center of attention in urinary pH work. It was demonstrated as far back as the 1920s and 1930s that ingestion of cranberries caused increased urinary hippuric acid content (Blatherwick and Long, 1923; Fellers et al., 1933). Ingestion of quinic acid was also found to increase the urinary concentration of hippuric acid. On this basis some researchers have hypothesized that the quinic acid in cranberry juice is

converted to and excreted as hippuric acid in the urine (Bodel et al., 1959). Bodel and associates were able to demonstrate increased urinary hippuric acid concentration following ingestion of large volumes of cranberry juice cocktail. Their in vitro studies indicated that a minimum concentration of hippuric acid of 0.02-0.04M, and a pH of 5.0 or less, were, in combination, bacteriostatic. However, they found that these values were rarely simultaneously detected in urine samples even after ingestion of maximal tolerable amounts of the cocktail.

In another study, four subjects ingested up to 4000 mls. of cranberry cocktail daily. Daily monitoring of their urine revealed only transient decreases in urinary pH (Kahn et al., 1967).

Nahata has noted that a pH of 5.5 or less is optimal for the conversion of methenamines to formaldehyde, a urinary antiseptic (Nahata et al., 1977). Ascorbic acid, which is either a normal constituent of or added nutrient to many juices and fruit drinks, has often been administered orally as a urinary acidifier. In the mid-1970s, Muiznieks tested the effects of daily doses of 2.0-8.0 grams of vitamin C on 16 people (Muiznieks, 1978). Urine was collected 5 times daily for 3 days, and the effectiveness of ascorbic acid and sodium ascorbate were compared at the 6.0 g/day dosage level. Muiznieks concluded that doses of 2.0-4.0 g/day did not, on the average, significantly lower urinary pH. He also found that ascorbic acid is more effective than sodium ascorbate as a urinary acidifier, and that vitamin C's effect upon urinary pH is highly variable.

Other researchers have reached similar conclusions. In one study, ten men were each subjected to 4.0 and 6.0 gram daily doses of ascorbic acid, and the total quantity of urine voided by each in a day

was pHed (Nahata et al., 1977). Nahata concluded that the use of ascorbic acid as a urinary acidifier was not warranted. More recent work by Nahata involved daily oral doses of methenamine mandelate (MM), MM plus ascorbic acid, and MM plus ascorbic acid, and cranberry juice cocktail (Nahata et al., 1981). The hypothesis being tested was that knowledge of the identity of the infecting pathogen and the patient's urinary pH could be used to predict the effectiveness of methenamine therapy. The pH and type of pathogen were concluded to be unreliable predictors of MM effectiveness, irregardless of the simultaneous administration of the commonly enployed urinary acidifiers.

In short, cranberry juice and vitamin C (which is sometimes administered with or without cranberry juice, or together with cranberry juice in fortified cranberry juice cocktail) are not often capable of significant urinary acidification. Also, cranberry juice ingestion does not induce enough hippuric acid excretion to be bacteriostatic. However, because many undocumented testimonials and the work of Prodromos and others (Prodromos et al., 1968) support the validity of cranberry juice as a UTI treatment, other attempts have been made to explain the mechanism of action of the juice.

Early work carried out with rabbit bladder demonstrated that the bladder's "primary antibacterial defense mechanism" is the ability to resist bacterial attachment (Parsons et al., 1975). Parsons and his associates compared bacterial adherence to rabbit bladder mucosa before and after treatment with HCl, and discovered that HCl treatment caused bladder to be much more receptive to adherence to by E. coli. He contended that, because the effects of HCl treatment disappeared after 24 hours, a secretory mechanism was implicated. Later, Parsons used

histochemical staining techniques to demonstrate that HCl was destroying a mucopolypaccharide (mucin) layer on the bladder's surface (Parsons et al., 1977), and that this "anti-adherence factor" resisted adhesion of not only E. coli, but also Staphylococcus and Klebsiella species (Parsons et al., 1978). The mechanism of the mucin's anti-adherence effect was dependent upon pH, which suggested that the mucin served as an electrochemical coat to which bacteria have great difficulty in adhering (Parsons et al., 1978). In the same report, Parson's data suggests that neither a chelating agent, nor IgA act as anti-adherence factors.

Other investigators did studies of E. coli adhesion to human uroepithelial cells. Uroepithelial cells were isolated from morning urine, and the ability of E. coli to adhere to these was found to be dependent upon incubation time, temperature, osmolarity, and pH (Svanborg-Eden et al., 1977). Using similar methods, Schaeffer's group also noted a pH effect on adherence (optimum pH was 4.0 to 5.0), and discovered that adherence takes place within 60 seconds, and involves a limited number of epithelial receptors (Schaeffer et al., 1979). The uroepithelial receptors are believed to be composed of 2-D-mannosepyranoside residues (Ofek et al., 1977; Aronson et al., 1979; Schaeffer et al., 1980). Schaeffer also detected an association between bacterial adhesion and pathology in UTI. The ability of E. coli cells to adhere to uroepithelial cells from uninfected individuals was significantly different from their ability to adhere to the cells of infected persons (Schaeffer et al., 1981). Also, differences in adhering ability were detected between strains of E. coli isolated from cases involving different manifestations of UTI, such as pyelonephritis, cystitis, and asymptomatic bacteriuria (Svanborg-Eden et al., 1978).

Sobota tested the ability of cranberry juice urine to inhibit adhesion of E. coli to uroepithelial cells (Sobota, 1984). The ability of E. coli to adhere was inhibited by 80% when urine from mice on a cranberry juice cocktail diet was compared to urine of control mice. More importantly, the ability to significantly inhibit adherence was demonstrated 1 to 3 hours after ingestion of cranberry juice cocktail for the urine of two-thirds of human volunteers. This work suggests that the ingestion of cranberry juice may aid in the treatment of UTI by preventing bacteria from adhering to uroepithelial surfaces.

It is important to note that anti-adherence was demonstrated 1 to 3 hours after ingestion of the cocktail; this report by Sobota notes that pre-retirement ingestion led to the production of morning urine that was inconsistent in its antiadherence effects (Sobota, 1984). Also, researchers who tested cranberry juice and ascorbic acid for bacteriostatic and pH effects did not design their experiments to study immediate and initial effects so much as for continuous regimen effects (Bodel et al., 1959; Kahn et al., 1967; Muiznieks, 1978; Nahata et al., 1981). The assertion here is that the ability of ingestion of cranberry juice to immediately or initially acidify urine or to cause the production of bacteriostatic urine may have been overlooked.

The present study was specifically designed to look at the immediate and initial ability of ingestion of cranberry juice cocktail to produce acidic or bacteriostatic urine. The design also allows for the detection of any correlations between hourly pH and hourly growth, hourly pH and hourly volume, and hourly growth and hourly volume. The effects of grape juice and orange juice were similarly examined.

Grape juice and orange juice were included in this report for several reasons. Grape juice was selected because, like cranberry juice, it contains a substantial amount of anthocyanins (Smith and Luh, 1965; Konowalchuk and Spiera, 1976). Orange juice was selected because it has a high concentration of organic acids (especially tartaric, benzoic, succinic, malic, and citric) (Sinclair, 1968) and because of its naturally high ascorbic acid content. Urinary acidification is often beneficial in conjunction with antibiotic treatment of UTI. Additionally, the juices would be useful since drug treatment is not always adviseable for treatment of UTIs during pregnancy, nor beneficial in cases of chronic UTI (Froom, 1980); the discovery of natural foods with the ability to produce an acidic or bacteriostatic urine would be beneficial. Finally, grape and orange juice were included to serve as a comparison with cranberry juice as urine acidifers or bacteriostats.

CHAPTER II

MATERIALS AND METHODS

Bacteria

The bacterium used in this study was Esherichia coli isolate CI-18, provided by Dr. Anthony Sobota of Youngstown State University. This bacterium was isolated from a patient with clinically diagnosed bacteriuria and was obtained from the clinical microbiology laboratory of the Youngstown Hospital Association. Stock cultures were obtained by growing the isolate in BBL #11059 brain heart infusion (BHI) for 48 hours at 37°C. These cultures were then stored at 0 to 4°C for a maximum of two weeks before being replaced. For growth determinations, 10 mls. of sterile urine were inoculated from the stock, and cultured statically for 48 hours at 37°C, and then diluted and plated as outlined below.

Human subjects

Three 24 year old male volunteers (subjects A, B, and C) took part in this work. All three were healthy, showing no signs of current bacteriuria. They weighed between 150 and 160 lbs. Only subject C had a prior history of UTI. None of the three was on antibiotics.

Juices and diet

Each subject drank 24 ozs. of either Ocean Spray cranberry juice cocktail, Welch's unsweetened grape juice, or Minute Maid orange juice at 8:00 a.m. on the mornings of the test. Both the cranberry cocktail and the grape juice were fortified with 100% of the U.S. RDA of vitamin C by the manufacturers.

Each subject ate one McDonald's "sausage McMuffin with egg" approximately 15 minutes prior to drinking the juice. No other food or drink was taken on the mornings of the experiments, and fasting was observed for a period of 6 hours after ingestion of juice.

Urine

Urine samples were collected immediately prior to ingestion of juice. These samples served as controls, and are referred to as "time zero" samples. The juice was then ingested and for 6 hours samples were collected every 60 minutes (samples "60, 120, 180, 240, 300, and 360"). The volume of each fresh sample was determined with a Pyrex 100 ml. graduated cylinder, and pH determinations were performed with a Sargeant Model DR pH meter. For use as bacterial growth media, urine samples were processed in two ways: 1) portions of each fresh sample were mixed with BHI and used immediately as a growth medium and 2) the remainders of each sample were frozen to be used as thawed urine media at a later date.

For the immediate growth determinations, 7 mls. of each fresh urine sample (i.e., samples zero through 360) were filtered through Whatman No. 5 qualitative paper, drawn-up into Beton-Dickinson #5625 tuberculin syringes, and pushed through Gelman Acrodisc 0.2μ disposable filter assemblies (#4197) to remove any bacteria that may have contaminated the sample during the collection process. These twice-filtered samples were directly deposited into Pyrex #9825 glass culture tubes containing 3 mls. of sterile BBL #11059 BHI solution and capped. All urine/BHI media were inoculated with E. coli on the same day that they were prepared.

Frozen samples were thawed by shaking at room temperature on an Eberbach shaker, and then filtered through Whatman No. 5 qualitative paper. The pH of each filtered sample was recorded, and then 10 mls. of each of these samples were processed in the same manner as the fresh samples.

Bacterial dilution and culture media

Pyrex #9825 glass test tubes were each filled with 10 mls. of one-tenth of the recommended concentration of BBL #11059 BHI in glass distilled water (prepared with the Corning AG-1B glass water distiller). The tubes were then capped, sterilized by autoclaving (120^{o}C for 15 minutes) and used to serially dilute E. coli grown in urine or urine/BHI media. Dilution tubes were prepared one week before their use, and stored at room temperature.

For cultivation of the bacteria, 3 ml. aliquots of BHI were prepared and stored at room temperature. Any of the tubes displaying contamination were discarded prior to use.

Bacterial growth plates

BBL #11777 trypticase soy broth (TSB) and BBL #11853 agar were combined in glass distilled water in concentrations recommended by the manufacturer. The solution was then stirred with heat, autoclaved at 120^{o}C for 15 minutes, and 10 ml. aliquots were aseptically dispensed into sterile Curtin Matheson #078-964 petri dishes. These TSB plates were then sealed in plastic bags and refrigerated. Fresh plates were prepared every 2-3 weeks.

Bacterial growth in urine

A "series" consists of the zero sample and 6 subsequent hourly urine samples collected from one individual after ingestion of one of the juices. Typically, two series were run at a time. The 10 ml. samples of urine, or urine/BHI, were inoculated with the E. coli and grown statically for 48 hours in a Lab-Line #400 incubator at 37°C. Upon removal from the incubator, the zero, 60, 120, 180, 240, 300, and 360 samples for each series were grouped together and placed in a test tube rack. One sample at a time was diluted and then immediately plated.

Dilutions

A sample was first vortexed for 5 seconds on a Vortex Junior Mixer, and 0.1 ml. of it was transferred with a 1.0 ml. serological pipette to a sterile BHI dilution tube. The dilution tube was vortexed for 5 seconds, and 0.1 ml. of its contents were transferred with a new pipette to another dilution tube, etc..., until a total of 5 serial dilutions were performed for the sample. The dilutions of the sample were then immediately plated.

Plating

One-tenth ml. from each of the 5 serial dilutions was transferred to 5 TSB plates, and evenly distributed upon the surfaces of the plates with glass spreaders. The glass "hockey sticks" were sterilized by dipping into 95% ethanol, flaming, and cooled at room temperature. All plates were then incubated overnight at 37°C, and colony counts were done.

Colony counts

Typically, the plate of the 3rd dilution of a 48 hour growth sample contained 10 to 300 colonies, and this number X 10^7 was recorded as the number of bacteria that were present per ml. of the undiluted growth sample. When the number of colonies on the third plate either exceeded 300 or was less than 10, plates of greater or lesser dilutions were counted, respectively.

CHAPTER III

RESULTS

Results are expressed in Tables (Appendix A) and Figures (Appendix B). All statistical analyses were performed as outlined in Zar (Zar, 1974).

The effects of each juice on urine volume, pH, and bacterial growth are presented in Tables 1, 2, and 3. When this project was first begun, several trials were spent in trying to standardize the diluting and plating techniques for bacterial growth determinations. Although the growth data from these earlier efforts were discarded, the pH and volume records were saved and are presented in Table 4.

The major problem first encountered in the growth trials, was that diluting and plating 48 hour bacterial cultures gave growth on plates for only the plates of the first dilutions of a series. It was later discovered that the E. coli cultured in urine or urine/BHI were too frail to withstand much time in a dilution, even when BHI was used as a dilution medium. Satisfactory growth results were obtained when each sample was streaked onto TSB plates immediately after it was serially diluted. The original practice was to serially dilute every sample, and then to plate each.

Bacterial growth and urinary pH are expressed in several ways in Tables 1, 2, and 3. Fresh urine was immediately pHed, and the volume was recorded. These fresh urine samples were then immediately mixed with BHI and inoculated for bacterial growth determinations. Thus the "volume," "pH," and "growth/ml in urine/BHI" rows in Tables 1, 2, and 3 refer to freshly obtained urine.

About 10 mls. of each fresh sample was then frozen for use at a later date. In Tables 1, 2, and 3, "pH (thawed)" refers to the pH of thawed urine after crystals of urates, uric acid, carbonates, oxalates, and phosphates were filtered out (Haber, 1978). After the urine was thawed, filtered, pHed, and re-filtered to sterility, 10 mls. of it were used to grow E. coli without BHI ("growth/ml in urine" in Tables 1, 2, and 3). Finally, growth is expressed in the Tables as "growth potential:" the product of the volume of urine collected for that hour and the number of E. coli (CFUs) that grew per ml. of urine.

Effects upon bacterial growth

The number of CFUs per ml. in urine/BHI media are presented graphically as a function of time for each juice in Figs. 1-6. Figure 1 shows that, for grape juice, growth values for the 3 subjects were within 200-300 CFUs/ml of each other following time zero values that differed as much as nearly 500 CFUs/ml. Overall, the curves in Fig. 1 show a decreasing trend from two or three hours after ingestion through the remainder of the experiment. Figure 2 shows how similar the curves of Fig. 1 appear when corrected for time; all 3 curves demonstrate a large initial peak, then two more points of inflection and a generally decreasing growth with time.

The growth of E. coli in cranberry juice urine/BHI media is depicted in Figs. 3 and 4. Maximal growth occurred with samples of urine collected at times later in the experiments than for the grape juice samples, i.e., at the fourth and fifth hours. Figure 4 shows the effects of correcting for time; if not for the poor growth in subject C's urine at time 180, the curves would be similar. More specifically, values would be compacted into a tight range of about 100 CFUs/ml at times 120

and 180, and then each subject would express a peak (of varying magnitude) in the fourth and fifth hours.

Figures 5 and 6 illustrate a tendency for ingestion of orange juice to cause dual growth peaks in orange juice urine/BHI media. These are of greater magnitude and more centrally located than the subjects growth peaks after ingestion of grape or cranberry juices. Figure 6 illustrates one possible correction for time for the curves in Fig. 5.

Bacterial growth in thawed urine (no BHI added) as a function of time after ingestion of juice is depicted in Figs. 7, 8, and 9. Orange juice urine (Fig. 9) gives two growth peaks, as it did when mixed with BHI. Cranberry juice urine (Fig. 8) gave single peaks earlier in the experiment than it gave when mixed with BHI. Figure 7 illustrates the effects of grape juice urine on bacterial growth as a function of time. The curves in Fig. 7 are similar for all three subjects in that each establishes a single strong peak, albeit at different times. It can be observed that Fig. 7 is, however, quite different from Fig. 1 of growth in grape juice urine/BHI media.

Bacterial growth was also analyzed statistically, and the results are presented in Tables 5, 6, and 7. The effects of the three juices were compared for each hour. No significant differences in growth due to the different juices were detected when growth was expressed as either growth in urine/BHI media (Table 5), growth in urine (Table 6), or growth potential (Table 7).

Remarkably low growth numbers were obtained for subject B. Specifically, these are the time zero samples obtained on the day subject B drank grape juice (Table 1) and the zero and 60 minute samples collected on the day he drank orange juice (Table 3). A one-thousand to ten-

thousand fold decrease in growth in urine/BHI media was observed, and up to a one-thousand fold decrease in growth in urine was observed.

Effects upon pH

The effects of the ingestion of the juices on pH were analyzed. Figures 10-17 illustrate graphically the changes in pH as a function of time. Figure 10 represents changes due to ingestion of grape juice and is based upon values taken from Table 1. The curves for subjects A and C appear similar. Figure 11 reveals what the curves look like when the effects upon Subject B are advanced 2 hours. In general, the graph demonstrates that grape juice ingestion at first lowers urine pH, and then restores it to normal or above normal values 3 or 4 hours after ingestion. Only for subject B (Fig. 12) is the tendency reversed.

Figure 13 demonstrates the effects of cranberry juice upon pH. In Fig. 14, A is delayed 1 hour and, in Fig. 15, the pH data for cranberry juice urine from Table 4 are graphed. As with grape juice, the higher pH values are found several hours after ingestion, and B is again the atypical subject, giving no peaks in Fig. 13, and a very great, early peak in Fig. 15.

Orange juice is the most consistent and predominating in its effects upon urinary pH, as can be seen in Figs. 16 and 17. For all 5 curves, the highest pH readings were recorded at 180 minutes. Also, the pH obtained for an individual at 180 minutes was unsurpassed by any other juice at any time after ingestion.

Changes in pH due to orange juice show a strong correlation with time. The pH values from Tables 3 and 4 were pooled for orange juice, and the Spearman correlation coefficient was determined. A significant positive correlation ($r_s = 0.786$, $N = 15$, $\alpha = 0.05$) was calculated for

samples 60 through 180, and a significant negative correlation (r_s = -0.718, N = 20, α = 0.05), was obtained for samples 180 through 360.

The pH effects of grape juice ingestion on subjects A, B, and C, the pH effects of cranberry juice ingestion on A, B, and C, and the pH effects of orange juice ingestion on the 3 subjects were statistically compared for each hour. A model I randomized complete block design was employed, and the results are presented in Table 8. Differences were detected at times 120 and 180, and Table 9 shows the results of a multiple range test. At 120 minutes, the mean pH of grape juice urine was significantly lower than the mean pH of cranberry juice urine, which was in turn lower than orange juice urine. At 180 minutes, the pH of grape juice urine was significantly lower than that of orange juice urine, but the test is not strong enough to detect a difference between cranberry juice and the other two.

Using the pH values from Table 1, 2, and 3, a nested analysis of variance (ANOVA) was performed (Table 10). The groups were the 3 juices, and the subgroups were subjects A, B, and C. A difference in pH owing to the three different juices was detected (F = 8.353) and a difference between individuals in affecting pH was detected (F = 2.832). To detect where the differences between the juices existed, pH means for grape, cranberry, and orange were compared using the Newman-Keuls multiple range test (Table 11). At the α = 0.05 significance level, differences between orange and grape, orange and cranberry, and cranberry and grape were detected. Grape juice caused, on the average, the most acidic urine samples, followed by cranberry, and then followed by orange.

A one-way model I ANOVA comparing the time zero pH values from Tables 1, 2, 3, and 4 (n_i = 16) to the 60 through 360 grape, cranberry,

and orange values from Tables 1, 2, and 3 was also performed (Table 12). A significant F value was calculated, and Dunnett's test for comparing a control mean to each other group mean (Table 13) was employed to compare the zero urine pH mean to the means of grape, cranberry, and orange urines. The zero pH mean was significantly greater than the grape juice urine pH mean, equal to the cranberry mean, and less than the orange juice mean.

Correlation studies

Because volume, pH, and growth data were collected for each hourly urine sample, correlation studies between these variables were made possible.

Studies of correlation between pH and growth were manifested in 3 ways: 1) the pH of each fresh urine sample was tested for correlation with the growth/ml in urine/BHI media for that hour; 2) the pH of each thawed urine sample was tested for correlation with the growth/ml in urine for that hour; and 3) the pH of each thawed urine sample was tested for correlation with the growth potential for that hour. The results of these correlation studies are presented in Table 14.

In part I of Table 14, it can be seen that a negative correlation was shown for cranberry juice urine pH and growth/ml in cranberry juice urine/BHI media. Neither of the other two juices demonstrated a correlation under similar testing. In parts II and III of Table 14, only subject A showed a (positive) correlation between pH and growth in urine, and between pH and growth potential.

The results of studies of correlation between urine volume and growth in urine, and urine volume and pH, are tabulated in Tables 15 and 16. A positive correlation for the former resulted when orange juice was

considered. Neither of the other two juices showed a correlation between volume and growth in urine, and none of the juices or individuals showed any type of correlation between urine volume and urine pH.

CHAPTER IV

DISCUSSION

Attempts to reveal the mechanism of action by which cranberry juice exerts its effects have, until recently, met with little success. Although regular ingestion of cranberry juice does increase urinary hippuric acid excretion, rarely enough of this compound is excreted to make the urine bacteriostatic or more acidic than control values (Bodel et al., 1959; Kahn et al., 1967). The most recent study suggests that ingestion of cranberry juice can cause production of a urine that inhibits bacterial adherence to uroepithelial surfaces, and this is the mechanism of action by which cranberry juice aids in the treatment of UTIs (Sobota, 1984).

Sobota noted that anti-adherence activity was manifested in the urine produced 1-3 hours after ingestion of the juice. When experiments were performed in which cranberry juice was ingested before retiring, his report noted that morning urine samples were inconsistent in their anti-adherence activity.

The present study examined the immediate effects of ingestion of cranberry juice upon urinary pH and bacteriostasis. Grape juice and orange juice were also included. The initial and immediate effects were of interest because urine active in anti-adherence ability was produced within three hours after ingestion of the juice (Sobota, 1984), and studies that had previously looked at the ability of cranberry juice to produce an acidic or bacteriostatic urine were based upon the effects of

daily ingestion of the juice, and not on the initial or immediate effects (Bodel et al., 1959; Kahn et al., 1967).

The results of this report do not support the assertion that cranberry juice will, within 6 hours of its ingestion, produce a bacteriostatic or significantly acidic urine.

Urine collected at 6 hourly intervals after ingestion of juice always allowed bacterial growth for all 3 men tested. Growth tests typically produced 10^7 to 10^8 CFUs/ml of urine for all 6 hours. Control (time zero) samples typically produced numbers of the same magnitude or lower. In short, nothing more than a 10-fold reduction in bacterial growth was ever noted for urine samples collected after ingestion of the juice when compared to the control of the same series.

The lowest growth numbers were obtained from subject B, and these were generally before any juice was ingested. For subject B, the six hourly samples after ingestion of grape juice grew 30 to 50 times more CFUs/ml than did the zero time sample for the growth in urine/BHI tests. When urine from this subject was used as growth medium (i.e., no BHI added), the zero time sample only grew about one-hundreth as many CFUs/ml as the following 60 through 360 samples. Similar results were obtained for B for the zero and 60 minute samples he produced on the morning on which he drank orange juice; the zero and 60 samples grew as little as one-hundreth as many CFUs/ml as the other samples when urine/BHI growth medium was used, and growth tests done with urine produced 10^3 to 10^4 less CFUs/ml at times zero and 60.

Possible explanations for the low growth for subject B include: 1) human errors performed during the laboratory techniques; 2) morning urine for subject B has poor nutritional value; and 3) B's morning urine

contains something that inhibits bacterial growth. Poor technique can be ruled out because the chances are slim that poor experimental technique was responsible 3 times, all for the same one-of-three subjects, twice for zero samples and in a 60 minute sample in the same series as one of the zero samples, and resulted in poor growth for both the urine/BHI and straight urine growth tests (i.e., tests done separately and on different days).

Because growth was reduced in both urine and urine/BHI media, the possibility that a bacterial nutrient was lacking in subject B's morning urine and was replaced after eating the egg McMuffin and drinking the juice is doubtful. The zero and 60 samples for B that gave the low growth as a urine media should have given normal growth when the urine was mixed 7:3 with BHI. The urine/BHI media only grew about 3% of the number of CFUs/ml expected of a 7:3 dilution of BHI. An inhibitor may have been present in B's morning urine, and was cleared from his urinary tract when he voided the zero sample, or, in the case of the orange juice day, the factor was not completely cleared until he voided the 60 sample. Whether poor growth in B's morning urine is due to the urine's poor nutritional value, or the presence of an inhibitor (or both) is not clear. It is clear, however, that no urine collected after ingestion of any of the three juices, from any of the 3 subjects, could match the low growth values of subject B's morning urine.

Grape juice and orange juice were also tested for their ability to grow bacteria. Graphs of the effects of ingestion of grape juice reveal that growth is slightly reduced at some points during the experiment, but never is below the range of the zero samples. Ingestion of orange juice generally increases the ability of urine to grow E. coli.

The effects of adding BHI to urine for growth determinations were dramatic. For grape juice urine, a characteristic decrease in growth over time occurred. When grape juice urine without BHI was used, single peak maxima resulted at different times of the experiment for the different subjects. For cranberry juice, maximal growth occurred during the earlier hours of the experiment when urine alone was used as medium, and growth maxima occurred toward the later hours for urine/BHI media. Orange juice urine was little affected by the addition of BHI.

Because the addition of BHI increased some areas of the juice's urine growth curves more than others, a simple added effect between urine and BHI does not seem to be the case. A more complex interaction effect is indicated.

Due to the variability among the values recorded for the bacterial growth determinations, parametric ANOVA tests were not employed. The sample sizes would have to be much larger than they are for the parametric ANOVA to be strong enough to detect even large differences between the effects of the juice treatments (Cochran and Cox, 1957). The results of the nonparametric statistical analyses showed no differences in bacterial growth due to the different juices for each hour tested. The total number of bacteria that could grow in an hourly sample is equal to the product of the volume (ml) of that sample and the concentration of bacteria per ml. that that urine sample can sustain. This expression of growth, called "growth potential," was also included in the tests, but differences between juices for the same hour were not detected when this expression of growth was used.

In short, interpretation of the graphical data revealed that none of the three juices caused much less bacterial growth in urine than did

the control urines, within the 6 hour test period. These findings agree with the results of others who tested the effects of regular, daily ingestion of cranberry juice (Bodel, et al., 1959; Kahn et al., 1967). The nonparametric tests indicated no significant differences in bacterial growth due to the different juices for each hour tested; cranberry juice was no better or worse than grape or orange juices in permitting growth.

The ability of these three juices to cause production of an acidic urine was also tested. The pH values of fresh urine were used in tests determining the effects of ingestion of juice upon urine pH, and when correlations between urine pH and urine volume, and between urine pH and growth in urine/BHI were studied. The pH values of thawed urine were used when correlations between urine pH and bacterial growth in urine were studied.

The effects of the juices upon urinary pH was first analyzed graphically. Grape juice produced, within the first two hours after its ingestion, and again during the 5th and 6th hours, a urine slightly more acidic than the zero time urine. Cranberry juice consistently produced its lowest pH values at times 300 and 360, and these readings were close to the lowest readings produced by grape juice. Orange juice consistently produced the highest urinary pH readings. These readings always occurred at 180 minutes after ingestion, and pH returned to normal control values toward the 5th and 6th hours.

The pH results were statistically analyzed, and differences in the effectiveness of the juices were detected at 120 and 180 minutes. Orange juice produced urine that was more alkaline at both times. Grape and cranberry juices were distinguished at time 120, when grape was found to produce a significantly more acidic urine.

A one-way ANOVA followed by a multiple range test showed that, for the 6 hour duration of the experiment (i.e., pH values of samples 60 through 360 pooled for all 3 subjects for each juice) grape juice produced significantly lower pH values than cranberry, and both juices produced lower pH reading than orange juice. When the zero time samples were pooled and compared with the values produced by each of the three juice treatments, grape juice urine was significantly lower, and orange juice urine pH was significantly higher than the pH of control (time zero) samples. It should be noted that, because urine pH was dependent upon time after ingestion of juice (for all three juices), pooling the six 60 through 360 values of a series is reflected in an increased error mean square when an ANOVA is performed. The chance of committing a Type II error are increased, but conclusions based upon detection of differences (i.e., rejection of the null hypothesis) can be made with confidence (Zar, 1974).

Taken in their totality, the graphical analysis of pH effects, and the non-parametric and parametric statistical analysis of pH clearly indicate that ingestion of either grape or cranberry juice will produce a more acidic urine than orange juice. Grape is slightly more effective than cranberry, especially during the second hour after ingestion.

It is interesting to note that the pH values recorded in the early work in which no growth data were recorded, are generally higher than the pH values recorded later (Tables 1, 2, and 3) for the corresponding juice-produced urine. The early data were collected in the summer, and the later work was performed in the middle of winter. Because a correlation between urine pH and time of year may exist, the pH data of the early and later work was not pooled for statistical analyses.

It was also the purpose of this report to examine the possibility of correlations between urine pH, volume, and bacterial growth in urine for the three juices. Only for cranberry juice was a (negative) correlation between pH of urine and growth in urine established, and the correlation was established only for bacterial growth expressed as growth in urine/BHI.

Why cranberry juice and not grape or orange juice shows a correlation between the pH of the urine and growth in urine/BHI is difficult to explain, especially since the interaction of urine and BHI is not well-established or well-characterized. It may be that cranberry juice urine and BHI form a growth medium whose value as a nutritional source is dependent upon pH.

Only for subject A was there a significant (positive) correlation between bacterial growth in urine and the pH of the urine. For A, changes in pH may reflect changes in the concentration of a given nutrient, or may even reflect the presence or absence of various nutrients or other compounds that inhibit or promote bacterial growth.

Studies of correlation between urine volume and bacterial growth in urine, and between urine volume and urine pH were also included in this report. Previous studies revealed that increased urine output resulted in decreased titrateable acidity, but no change in urinary pH, following ingestion of cranberry juice (Bodel et al., 1959). These results were confirmed in this study. No correlation between urine volume and pH was demonstrated for any juice or any individual. A positive correlation between urinary volume and bacterial growth per ml was, however, detected for orange juice. The simplest explanation for this would be

that, as urine volume increased, something in orange juice urine that inhibits bacterial growth becomes diluted.

It is sometimes beneficial to keep urinary pH within a certain range of values during drug treatment for UTI. For example, methenamine is most readily converted to formaldehyde, a urinary antiseptic, at a pH of 5.0 to 5.5 (Nahata et al., 1977; Nahata et al., 1981). The present study suggests that cranberry juice and grape juice can be ingested to produce urine with predictable pH values at certain times after their ingestion. Grape juice produces a more acidic urine than cranberry juice, which did not produce a more acidic urine than controls, and exerts its effects 1 or 2 hours after ingestion.

Orange juice ingestion raises urinary pH to high values 3 hours after its ingestion. Ingestion of orange juice has no real value during UTI when methenamine and other drugs which require much lower urinary pH values are being used. However, orange juice ingestion may be of value in drug treatments of other pathologies where consistent, predictable urinary alkalation is beneficial.

APPENDIX A

Tables

TABLE 1

DATA OBTAINED FROM INGESTION OF GRAPE JUICE

		ZERO	60	120	180	240	300	360
A	volume (ml)	37.0	53.0	47.5	56.0	55.5	59.5	157.
	pH	5.80	5.30	5.20	5.37	5.22	5.09	5.01
	growth/ml in urine/BHI[a]	552	406	274	350	246	242	168
	pH (thawed)	5.86	5.45	5.29	5.46	5.29	5.18	5.15
	growth/ml in urine[a]	62	132	44	21	18	3	1
	growth potential[a]	2294	6996	2090	1176	999	179	157
B	volume (ml)	59.0	62.5	55.0	55.0	67.0	69.5	54.0
	pH	5.41	5.39	5.28	5.16	5.17	5.51	5.79
	growth/ml in urine/BHI[a]	5	273	161	172	181	121	125
	pH (thawed)	5.47	5.49	5.38	5.29	5.34	5.61	5.87
	growth/ml in urine[a]	0.03	30	24	32	27	116	18
	growth potential[a]	1.77	1875	1320	1760	1809	8062	972
C	volume (ml)	57.0	50.0	81.0	89.5	49.0	38.0	30.0
	pH	5.25	5.00	4.94	5.34	5.53	5.38	5.38
	growth/ml in urine/BHI[a]	269	195	300	280	149	152	160
	pH (thawed)	5.19	5.01	4.94	5.32	5.44	5.40	5.30
	growth/ml in urine[a]	4	18	109	256	52	25	23
	growth potential[a]	288	900	8829	22912	2548	950	690

[a]Actual data were coded by dividing by 10^7.

TABLE 2

DATA OBTAINED FROM INGESTION OF CRANBERRY JUICE

		ZERO	60	120	180	240	300	360
A	volume (ml)	73.0	47.0	35.0	28.0	41.0	38.0	29.0
	pH	6.64	5.99	6.25	5.52	5.34	5.29	5.28
	growth/ml in urine/BHI[a]	116	149	121	193	200	253	240
	pH (thawed)	6.78	5.87	6.07	5.61	5.38	5.30	5.27
	growth/ml in urine[a]	17	7	18	16	8	15	16
	growth potential[a]	1241	329	630	448	328	570	464
B	volume (ml)	124.5	174.0	96.5	55.5	36.0	38.0	48.0
	pH	5.84	5.67	5.49	5.51	5.49	5.49	5.50
	growth/ml in urine/BHI[a]	370	273	207	211	420	435	230
	pH (thawed)	5.73	5.55	5.32	5.29	5.38	5.40	5.48
	growth/ml in urine[a]	22	166	139	16	14	14	25
	growth potential[a]	2739	28884	13413	888	504	532	1200
C	volume (ml)	63.0	33.5	38.0	39.5	44.0	35.5	24.0
	pH	6.57	6.26	5.52	6.83	6.58	5.69	5.40
	growth/ml in urine/BHI[a]	600	3	146	3	232	140	145
	pH (thawed)	6.70	6.10	5.57	7.07	6.84	5.71	5.41
	growth/ml in urine[a]	26	9	19	80	16	17	11
	growth potential[a]	1608	302	722	3160	704	604	264

[a]Actual data were coded by dividing by 10^7.

TABLES (CONT.)

TABLE 3

DATA OBTAINED FROM INGESTION OF ORANGE JUICE

		ZERO	60	120	180	240	300	360
A	volume (ml)	46.0	46.5	44.0	46.0	50.0	53.0	121.
	pH	6.09	5.84	6.98	7.01	6.87	6.67	6.57
	growth/ml in urine/BHI[a]	237	356	307	370	403	245	153
	pH (thawed)	6.04	6.04	7.58	7.69	7.30	6.95	6.75
	growth/ml in urine[a]	28	45	41	32	62	95	55
	growth potential[a]	1288	2093	1804	1472	3100	5035	6655
B	volume (ml)	43.0	29.0	31.0	37.0	45.5	46.0	39.0
	pH	5.56	5.73	6.50	6.63	6.06	5.97	5.54
	growth/ml in urine/BHI[a]	7	5	88	592	350	324	370
	pH (thawed)	5.65	5.81	6.69	6.87	6.15	6.13	5.70
	growth/ml in urine[a]	0.003	0.004	6	8	15	40	1
	growth potential[a]	0.129	0.116	186	296	683	1840	39
C	volume (ml)	95.5	79.0	122.0	97.5	61.5	69.0	32.0
	pH	5.37	5.03	6.16	6.82	6.37	5.98	5.20
	growth/ml in urine/BHI[a]	89	105	571	394	300	383	319
	pH (thawed)	5.45	5.14	6.32	7.25	6.48	6.07	5.28
	growth/ml in urine[a]	4	30	167	8	136	26	4
	growth potential[a]	382	2370	20375	780	8364	1794	128.

[a]Actual data were coded by dividing by 10^7.

TABLES (CONT.)

TABLE 4

ADDITIONAL VOLUME AND pH DATA FOR ALL THREE JUICES

Grape Juice

		ZERO	60	120	180	240	300	360
C	volume (ml)	15.0	22.0	30.0	44.5	50.5	37.0	21.5
	pH	5.30	5.30	5.10	5.55	6.61	5.87	5.55
B	volume (ml)	61.0	73.5	44.5	58.0	52.5	55.0	21.0
	pH	6.01	6.30	5.54	5.35	5.50	5.40	5.62

Cranberry Juice

		ZERO	60	120	180	240	300	360
C	volume (ml)	63.0	41.5	49.0	44.0	48.5	33.5	29.5
	pH	5.30	5.15	5.25	5.80	5.30	5.20	5.25
C	volume (ml)	41.0	42.0	41.5	35.5	39.5	32.5	21.5
	pH	5.30	5.40	5.60	6.30	6.35	5.50	5.50
B	volume (ml)	47.5	61.0	73.5	42.0	42.5	60.0	70.0
	pH	6.00	6.45	5.55	5.60	5.50	5.25	5.25

Orange Juice

		ZERO	60	120	180	240	300	360
B	volume (ml)	61.5	49.5	63.0	72.0	26.5	36.5	46.5
	pH	6.00	5.71	7.51	7.80	6.79	5.88	5.50
C	volume (ml)	40.0	42.0	63.5	88.0	41.0	50.5	34.5
	pH	6.21	6.15	7.15	7.50	6.28	6.71	6.25

TABLES (CONT.)

TABLE 5

RESULTS OF RANDOMIZED COMPLETE BLOCK ANOV BY RANKS (FRIEDMAN'S TEST)[a] TO TEST EACH Ho: THERE IS NO DIFFERENCE IN BACTERIAL GROWTH IN URINE/BHI MEDIA AT (TIME SPECIFIED) DUE TO THE THREE DIFFERENT JUICES

Time	X_r^2	Probability	Conclusions
60	3.128	0.10<P<0.25	Accept Ho
120	0.630	0.75<P<0.90	Accept Ho
180	4.626	0.05<P<0.10	Accept Ho
240	2.628	0.10<P<0.25	Accept Ho
300	1.962	0.25<P<0.50	Accept Ho
360	0.630	0.75<P<0.90	Accept Ho

[a] α set at 0.05; a=3 and b=3; data from TABLES 1,2 & 3 were tested.

TABLE 6

RESULTS OF RANDOMIZED COMPLETE BLOCK ANOV BY RANKS (FRIEDMAN'S TEST)[a] TO TEST EACH Ho: THERE IS NO DIFFERENCE IN BACTERIAL GROWTH IN URINE MEDIA AT (TIME SPECIFIED) DUE TO THE THREE DIFFERENT JUICES

Time	X_r^2	Probability	Conclusions
60	0.630	0.75<P<0.90	Accept Ho
120	0.630	0.75<P<0.90	Accept Ho
180	1.962	0.25<P<0.50	Accept Ho
240	4.626	0.05<P<0.10	Accept Ho
300	2.628	0.10<P<0.25	Accept Ho
360	0.630	0.75<P<0.90	Accept Ho

[a] α set at 0.05; a=3 and b=3; data from TABLES 1,2 & 3 were tested.

TABLES (CONT.)

TABLE 7

RESULTS OF RANDOMIZED COMPLETE BLOCK ANOV BY RANKS (FRIEDMAN'S TEST)[a] TO TEST EACH Ho: THERE IS NO DIFFERENCE IN BACTERIAL GROWTH POTENTIAL IN URINE AT (TIME SPECIFIED) DUE TO THE THREE DIFFERENT JUICES

Time	X_r^2	Probability	Conclusions
60	0.630	0.75<P<0.90	Accept Ho
120	2.628	0.10<P<0.25	Accept Ho
180	1.962	0.25<P<0.50	Accept Ho
240	4.626	0.05<P<0.10	Accept Ho
300	2.628	0.10<P<0.25	Accept Ho
360	0.630	0.75<P<0.90	Accept Ho

[a] α set at 0.05; a=3 and b=3; data from TABLES 1,2 & 3 were tested.

TABLES (CONT.)

TABLE 8

RESULTS OF MODEL I RANDOMIZED COMPLETE BLOCK DESIGN[a] USED TO TEST EACH Ho: THERE IS NO DIFFERENCE IN pH AT (TIME SPECIFIED) DUE TO THE THREE DIFFERENT JUICES

Time	Juices MS[b]	Blocks MS[b]	Remainder MS[c]	F	Probability
60	0.419	0.060	0.119	3.520	0.10<P<0.25
120	1.492	0.280	0.053	28.151*	P<0.005
180	1.766	0.240	0.190	9.295*	.025<P<0.05
240	0.956	0.261	0.201	4.756	0.05<P<0.10
300	0.654	0.001	0.124	5.274	0.05<P<0.10
360	0.142	0.084	0.295	0.481	0.25<P

[a]Blocks are subjects A, B, & C; a=3 & b=3, N=9; data from TABLES 1, 2 & 3 were tested.
[b]Two degrees of freedom.
[c]Four degrees of freedom.
*Significant at α =0.05

TABLE 9

RESULTS OF NEWMAN-KEULS MULTIPLE RANGE TEST TO TEST EACH Ho: $\mu_B = \mu_A$ FOR THOSE TIMES IN WHICH DIFFERENCES IN pH DUE TO THE DIFFERENT JUICES WERE DETECTED IN TABLE 8

Time	Comparison (i.e., B vs A)	SE[a]	q	Conclusions
120	orange (μ=6.55) vs grape (μ=5.14)	0.133	10.602*	at Time 120,
	orange (μ=6.55) vs c. b. (μ=5.75)	0.133	6.015*	pH grape pH
	c. b. (μ=5.75) vs grape (μ=5.14)	0.133	4.586*	c.b. pH orange
180	orange (μ=6.82) vs grape (μ=5.29)	0.252	6.070*	at Time 180,
	orange (μ=6.82) vs c. b. (μ=5.95)	0.252	3.452	pH grape pH
	c. b. (μ=5.95) vs grape (μ=5.29)	0.252	2.620	orange

[a]Based upon s^2=remainder MS from TABLE 8.
*Significant at α =0.05

TABLES (CONT.)

TABLE 10

RESULTS OF NESTED ANOV[a,b] FOR pH DATA OF TABLES 1,2 & 3

Source of variation	SS	DF	MS	F	Probability
total	18.272	53			
between all subgroups	10.740	8			
groups (juices)	7.902	2	3.951	8.353*	0.01<P<0.025
subgroups (subjects A,B, & C)	2.838	6	0.473	2.832*	0.01<P<0.025
error	7.532	45	0.167		

[a]Groups are the 3 juices and subgroups are subjects A,B & C
[b]Each $n_{ij}=6$ (i.e., samples 60 through 360)
*Significant at $\alpha=0.05$

TABLE 11

RESULTS OF NEWMAN-KEULS MULTIPLE RANGE TEST TO TEST EACH Ho: $\mu_B=\mu_A$ TO DETERMINE BETWEEN WHICH JUICES' POPULATION MEANS DIFFERENCES EXIST FOR TABLE 10

Comparison (i.e., B vs A)	SE[a]	q	Conclusions
orange (μ=6.22) vs grape (μ=5.28)	0.096	9.792*	pH grape pH
orange (μ=6.22) vs c. b. (μ=5.73)	0.096	5.104*	c. b. pH
c. b. (μ=5.73) vs grape (μ=5.28)	0.096	4.688*	orange

[a]Based upon s^2=error MS from TABLE 10
*Significant at $\alpha=0.05$

TABLES (CONT.)

TABLE 12

RESULTS OF MODEL I ANOV[a] TO TEST Ho: μpH OF CONTROL (TIME ZERO) URINE[b]= μpH OF GRAPE JUICE URINE[c] = μpH OF CRANBERRY JUICE URINE[c] = μpH OF ORANGE JUICE URINE[c]

Source of variation	SS	DF	MS	F	Probability
total	21.424	69			
groups	7.929	3	2.643		
error	3.495	66	0.204	12.956*	0.0005<P<0.001

[a]Groups are Time zero (n_i=16), grape (n_i=18), c.b. (n_i=18) and orange (n_i=18)
[b]The 16 values come from TABLES 1,2,3 & 4
[c]Samples 60 through 360 for subjects A,B, & C from TABLES 1,2 & 3
*Significant at α=0.05

TABLE 13

RESULTS OF DUNNETT'S TEST FOR COMPARING A CONTROL MEAN pH (TIME ZERO) TO EACH OTHER GROUP MEAN pH (i.e., GRAPE, CRANBERRY, AND ORANGE) BASED ON THE RESULTS OF TABLE 12

Comparison	SE[a]	q'	Conclusions
ZERO (μ=5.79) vs orange (μ=6.22)	0.156	2.756*	pH grape pH
ZERO (μ=5.79) vs c. b. (μ=5.73)	0.156	0.385	ZERO=pH c.b
ZERO (μ=5-79) vs grape (μ=5.28)	0.156	3.269*	pH orange

[a]Based upon s^2=error MS from TABLE 12
*Significant at α=0.05

TABLES (CONT.)

TABLE 14

RESULTS OF STUDIES OF CORRELATION[a] BETWEEN URINARY pH AND BACTERIAL GROWTH IN URINE

I. Ho: $p_s=0$ for urinary pH and growth/ml in urine/BHI media for:[b,c]

	n	r_s	Probability	Conclusion
grape juice	18	-0.439	0.05<P<0.10	No Correlation (NC)
c. b. juice	18	-0.535*	0.02<P<0.05	Correlation
orange juice	18	0.279	0.20<P<0.50	NC
subject A	18	0.170	0.50<P	NC
subject B	18	0.090	0.50<P	NC
subject C	18	-0.003	0.50<P	NC

II. Ho: $p_s=0$ for pH (thawed) and growth/ml in urine for:[b,c]

	n	r_s	Probability	Conclusion
grape juice	18	0.267	0.20<P<0.50	NC
c. b. juice	18	0.101	0.50<P	NC
orange juice	18	0.398	0.10<P<0.20	NC
subject A	18	0.596*	0.01<P<0.02	Correlation
subject B	18	0.380	0.10<P<0.20	NC
subject C	18	0.257	0.20<P<0.50	NC

III. Ho: $p_s=0$ for pH (thawed) and growth potential in urine for:[b,c]

	n	r_s	Probability	Conclusion
grape juice	18	0.271	0.20<P<0.50	NC
c. b. juice	18	0.035	0.50<P	NC
orange juice	18	0.267	0.20<P<0.50	NC
subject A	18	0.579*	0.02<P<0.05	Correlation
subject B	18	-0.376	0.10<P<0.20	NC
subject C	18	0.158	0.50<P	NC

[a] Spearman rank correlation test

[b] Data from subjects A,B & C are pooled for each juice tested

[c] Data from all 3 juices are pooled for each subject tested

*Significant at $\alpha=0.05$

TABLES (CONT.)

TABLE 15

RESULTS OF STUDIES OF CORRELATION[a] BETWEEN URINARY VOLUME AND BACTERIAL GROWTH IN URINE

Ho: $p_s=0$ for urine volume and growth/ml in urine for:[b]

	n	r_s	Probability	Conclusion
grape juice	18	0.070	0.50<P	NC
c. b. juice	18	0.380	0.10<P<0.20	NC
orange juice	18	0.682*	.002<P<.005	Correlation

[a]Spearman rank correlation test
[b]Data for subjects A,B & C were pooled for each juice tested
*Significant at α=0.05

TABLE 16

RESULTS OF STUDIES OF CORRELATION[a] BETWEEN URINE VOLUME AND pH

Ho: $\rho_s=0$ for urine volume and pH for:[b,c]

	n	r	Probability	Conclusion
grape juice	18	-0.373	0.10<P<0.20	NC
c. b. juice	18	0.050	0.50<P	NC
orange juice	18	0.117	0.50<P	NC
subject A	18	0.080	0.50<P	NC
subject B	18	-0.230	0.20<P<0.50	NC
subject C	18	0.138	0.50<P	NC

[a]Simple correlation coefficient; α set at 0.05
[b]Data from subjects A,B & C were pooled for each juice tested
[c]Data from all 3 juices were pooled for each subject tested

APPENDIX B

Figures

FIGURES

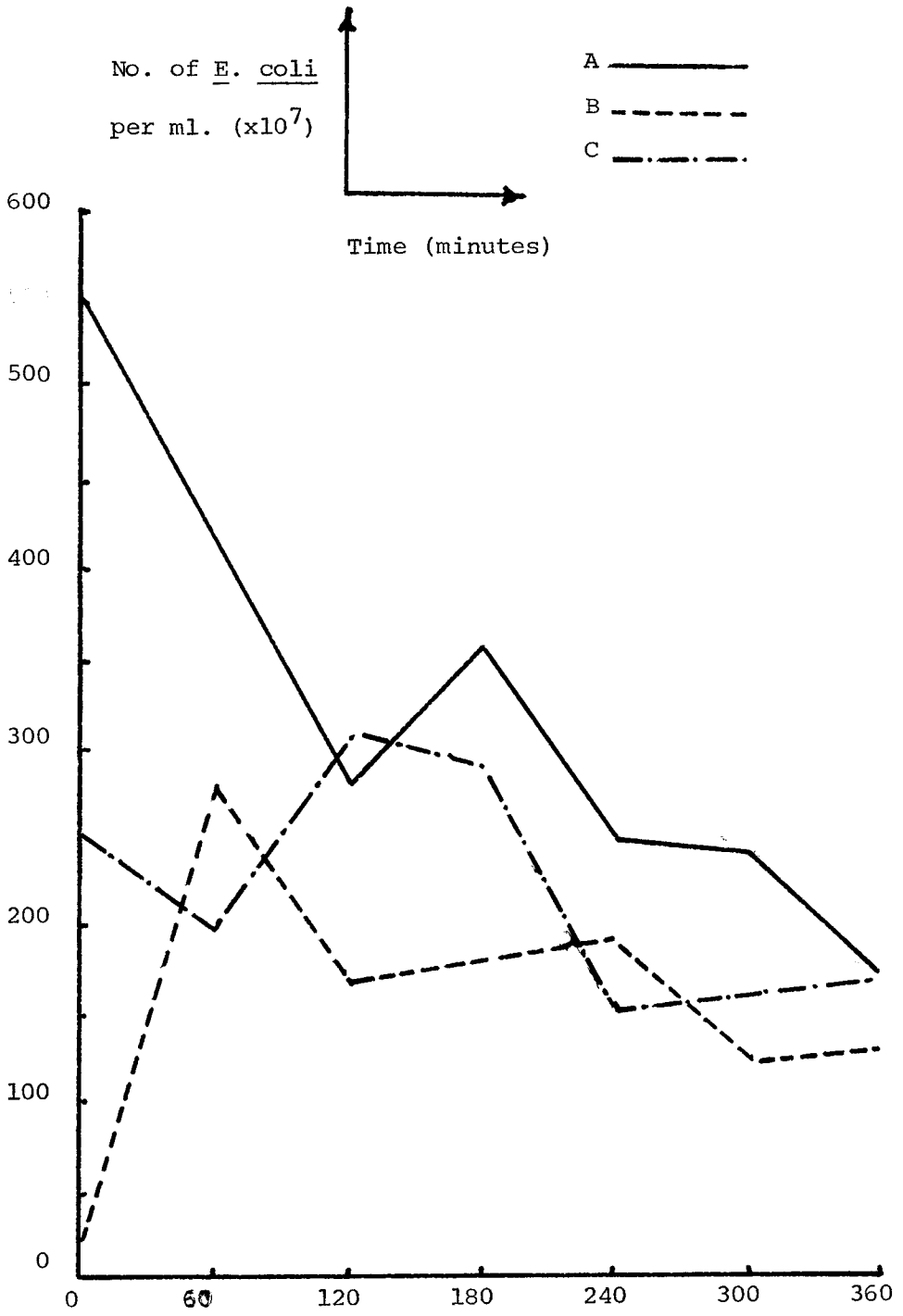

Fig. 1. Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Grape Juice.

FIGURES (CONT.)

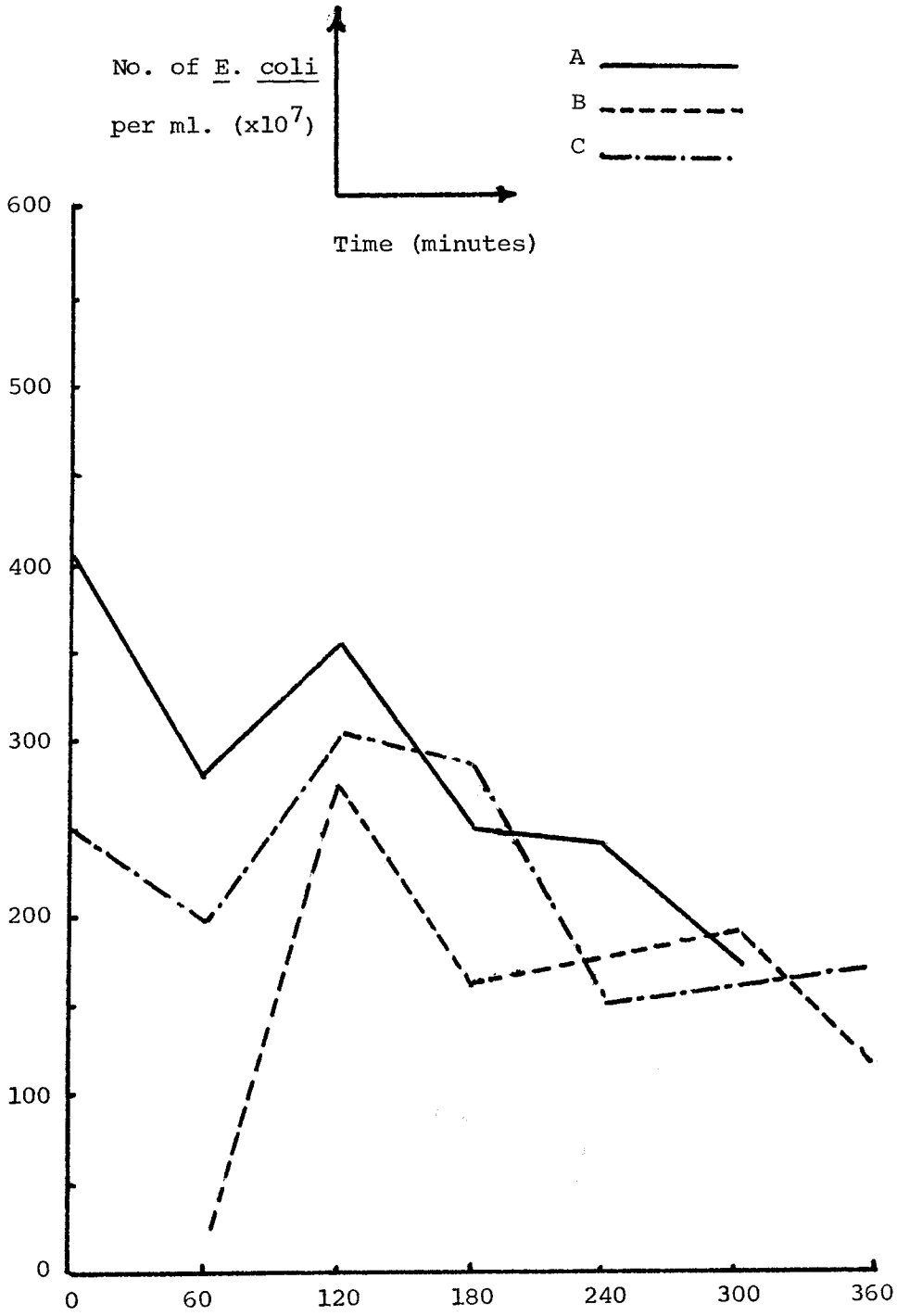

Fig. 2. Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Grape Juice (A is Advanced 1 Hour and B is Delayed 1 Hour).

FIGURES (CONT.)

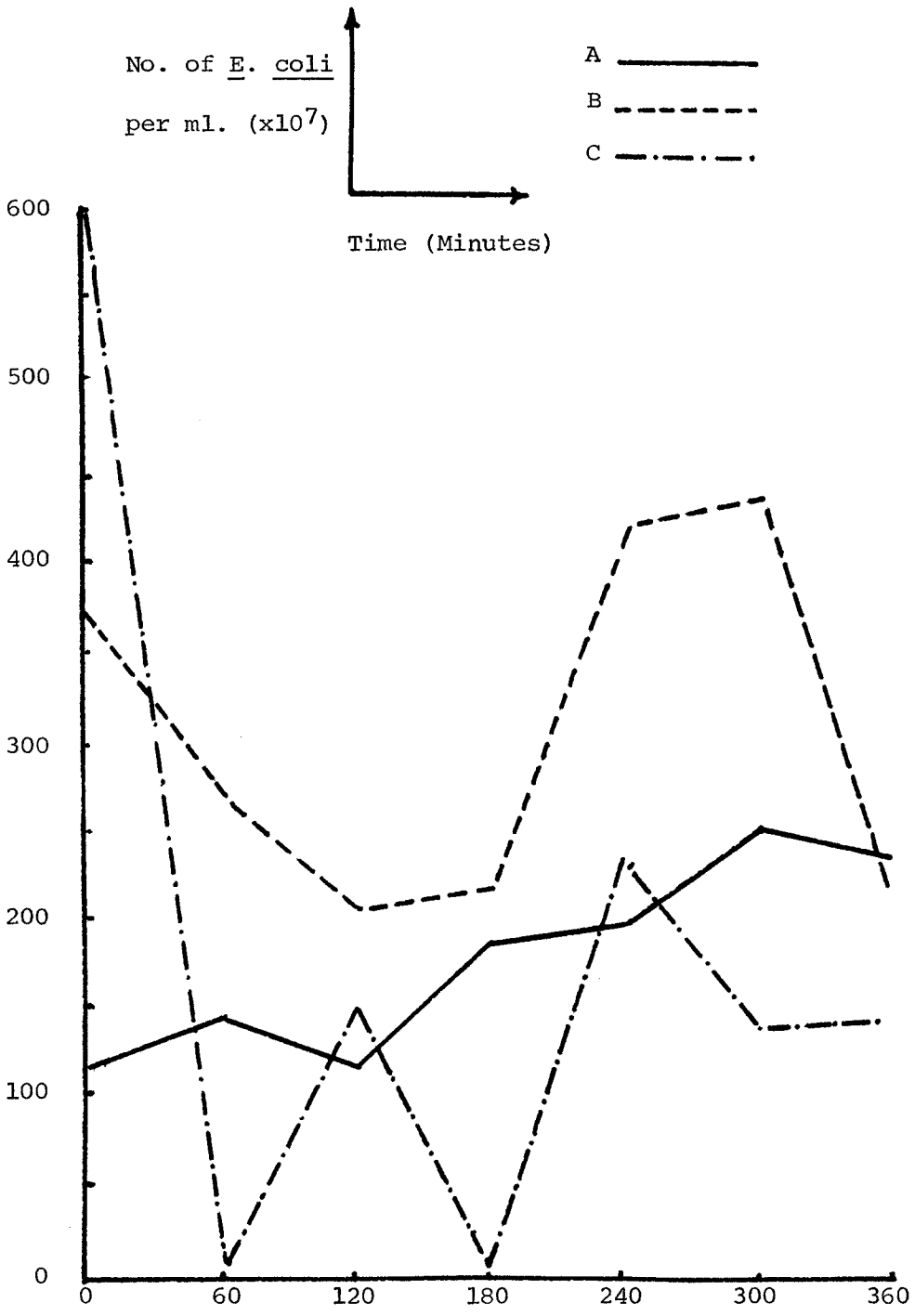

Fig. 3. Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Cranberry Juice.

FIGURES (CONT.)

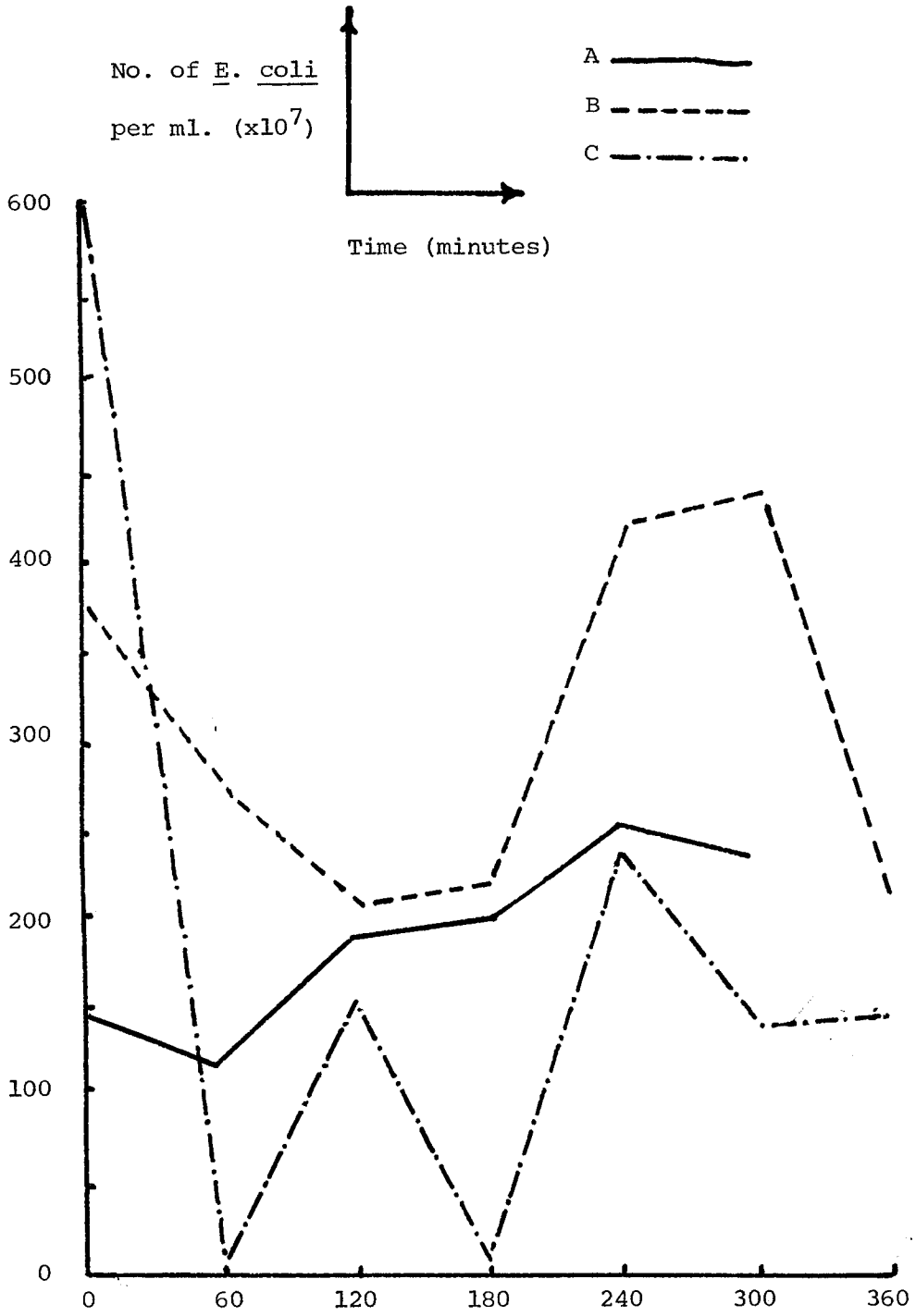

Fig. 4. Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Cranberry Juice. (A is Advanced 1 Hour).

FIGURES (CONT.)

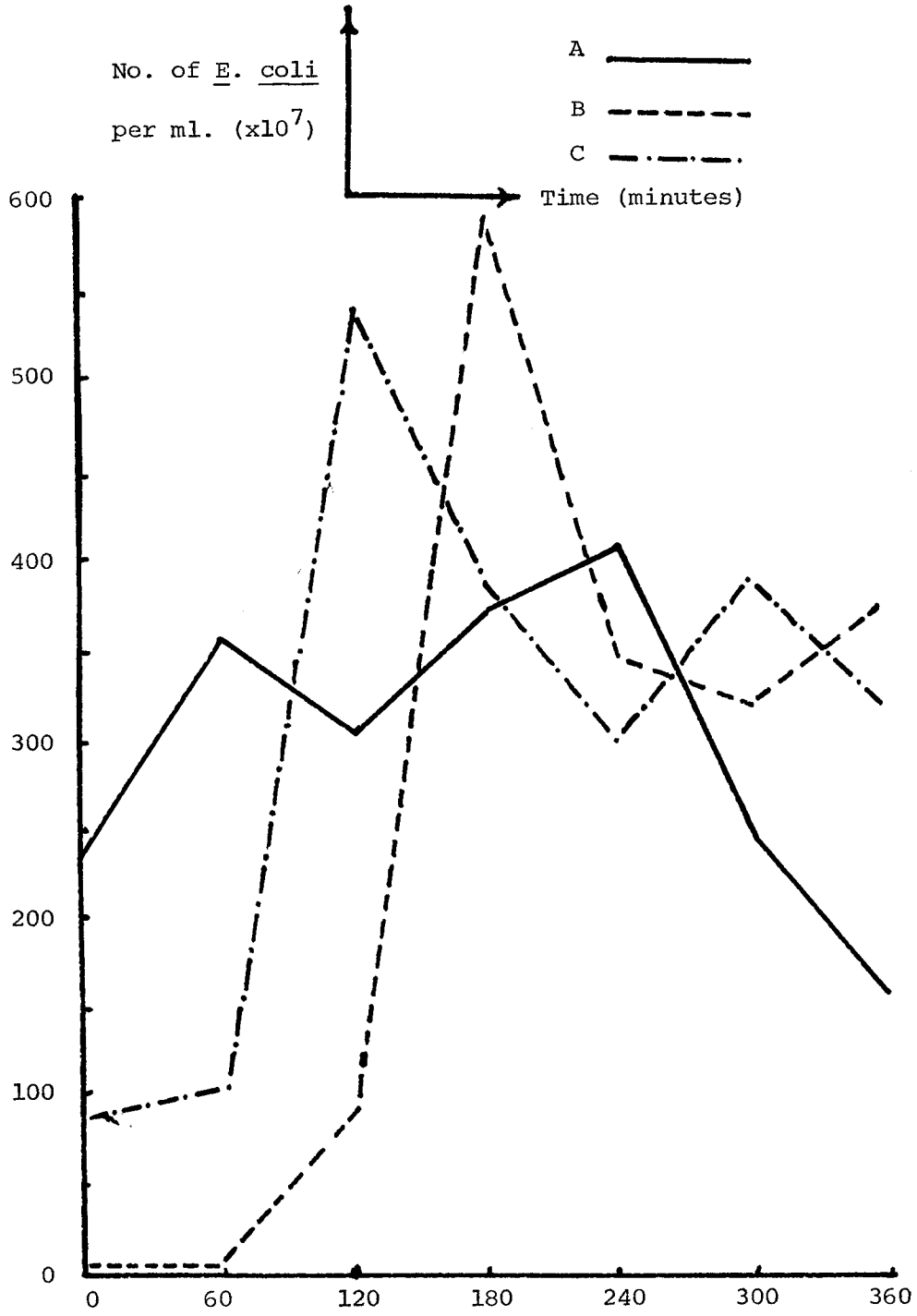

Fig. 5. Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Orange Juice.

FIGURES (CONT.)

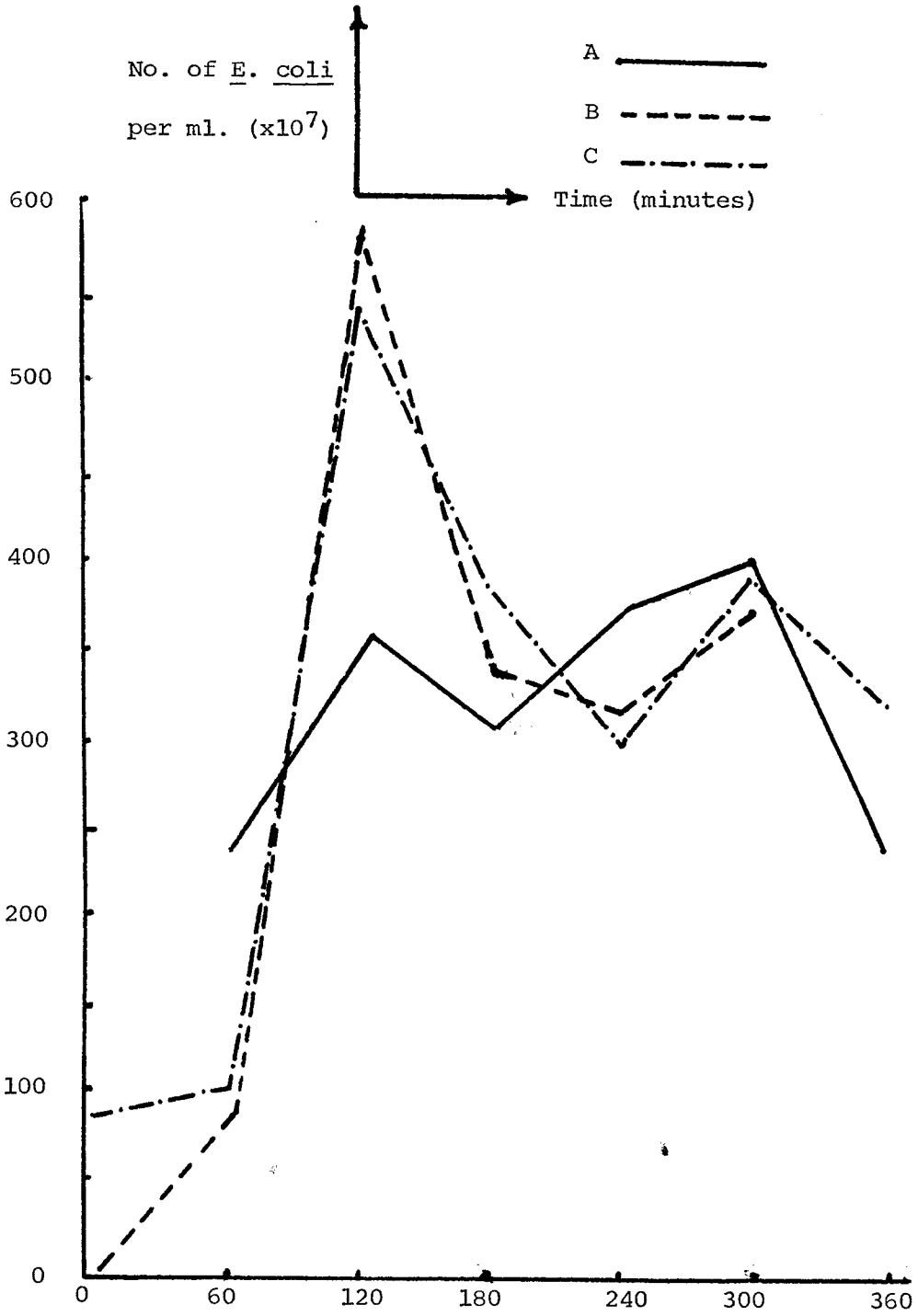

Fig. 6. Bacterial Growth in Urine/BHI Media as a Function of Time After Ingestion of Orange Juice. (A is Delayed 1 Hour and B is Advanced 1 Hour).

FIGURES (CONT.)

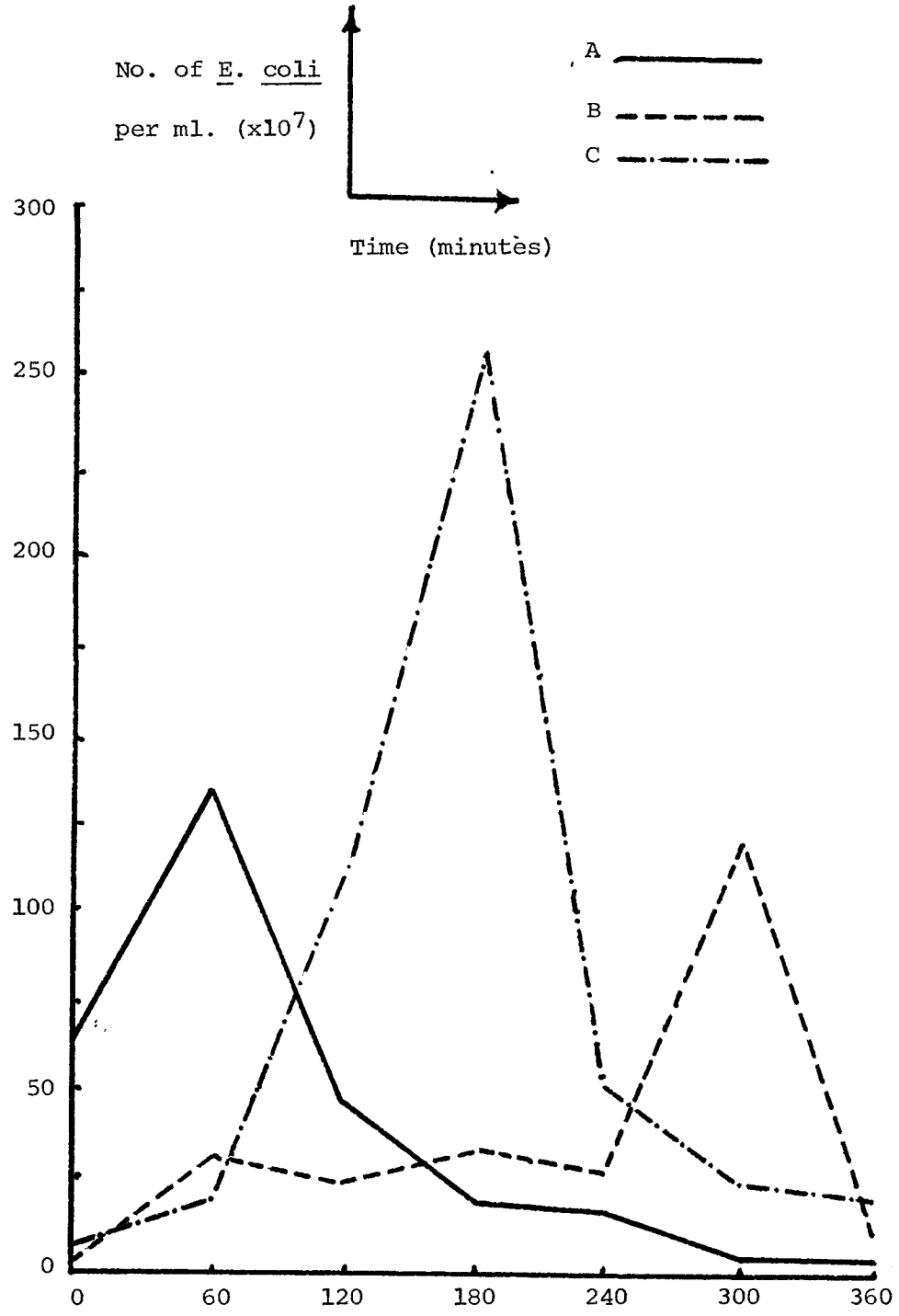

Fig. 7. Bacterial Growth in Urine Media as a Function of Time After Ingestion of Grape Juice

FIGURES (CONT.)

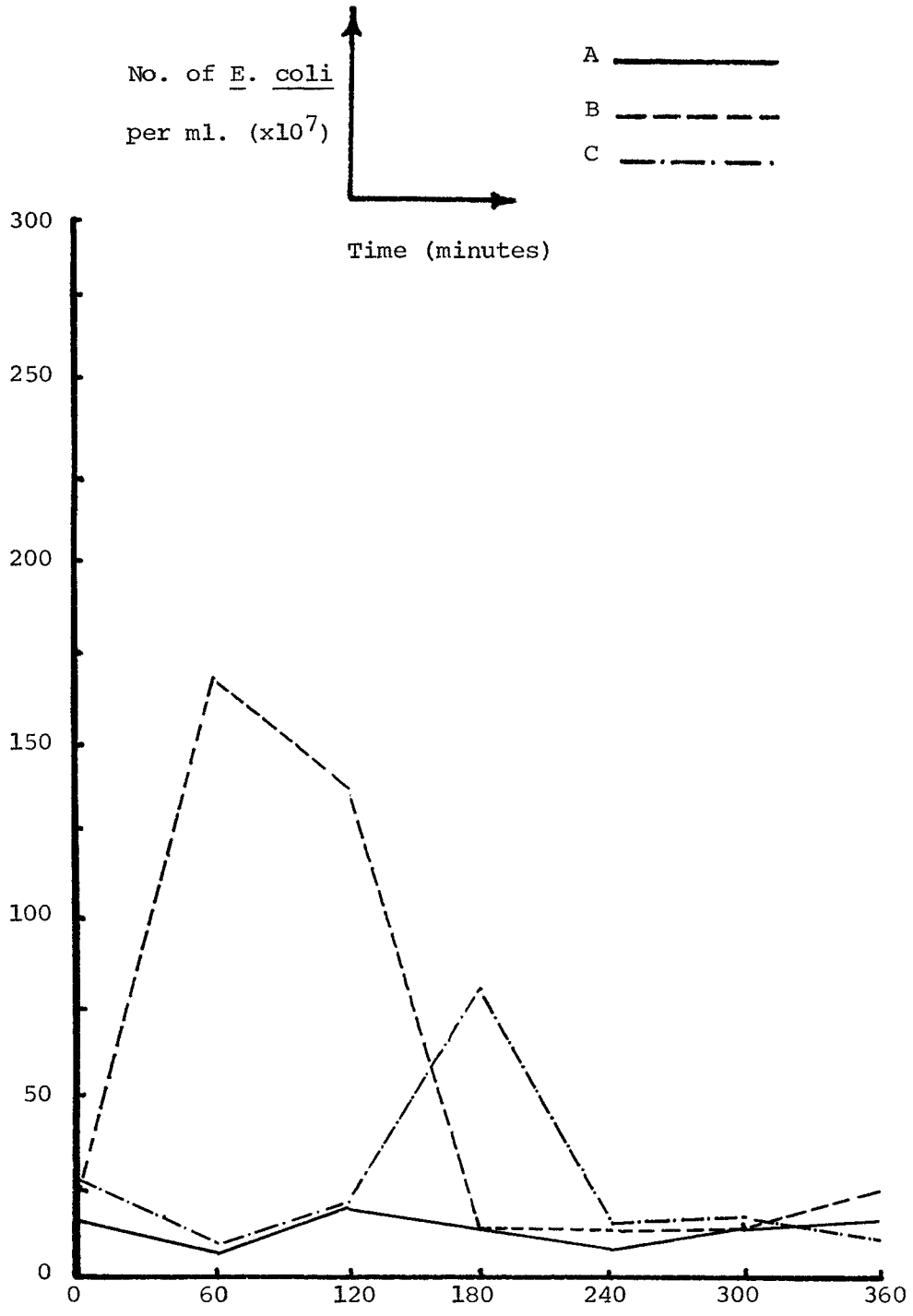

Fig. 8. Bacterial Growth in Urine Media as a Function of Time After Ingestion of Cranberry Juice.

FIGURES (CONT.)

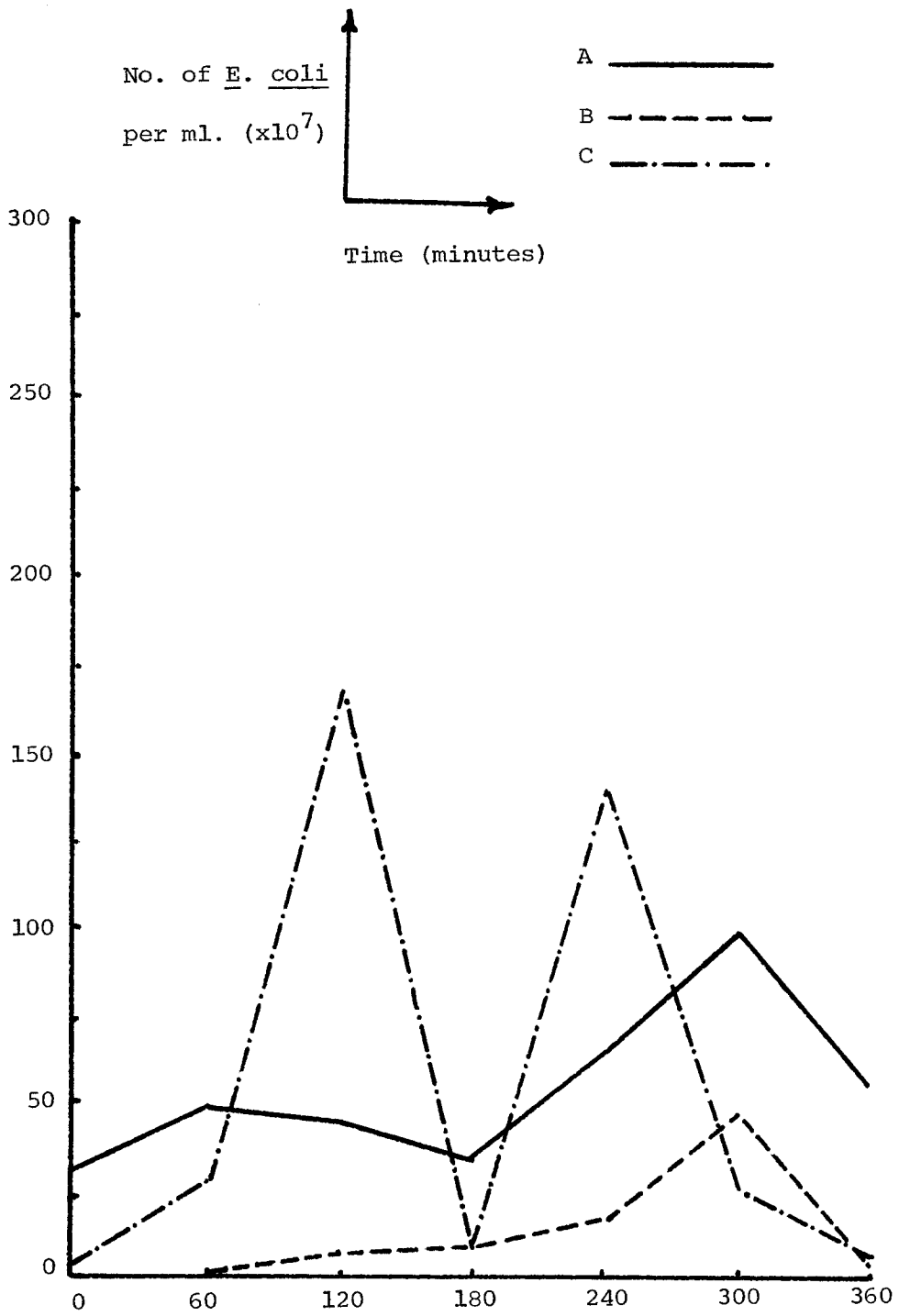

Fig. 9. Bacterial Growth in Urine Media as a Function of Time After Ingestion of Orange Juice.

FIGURES (CONT.)

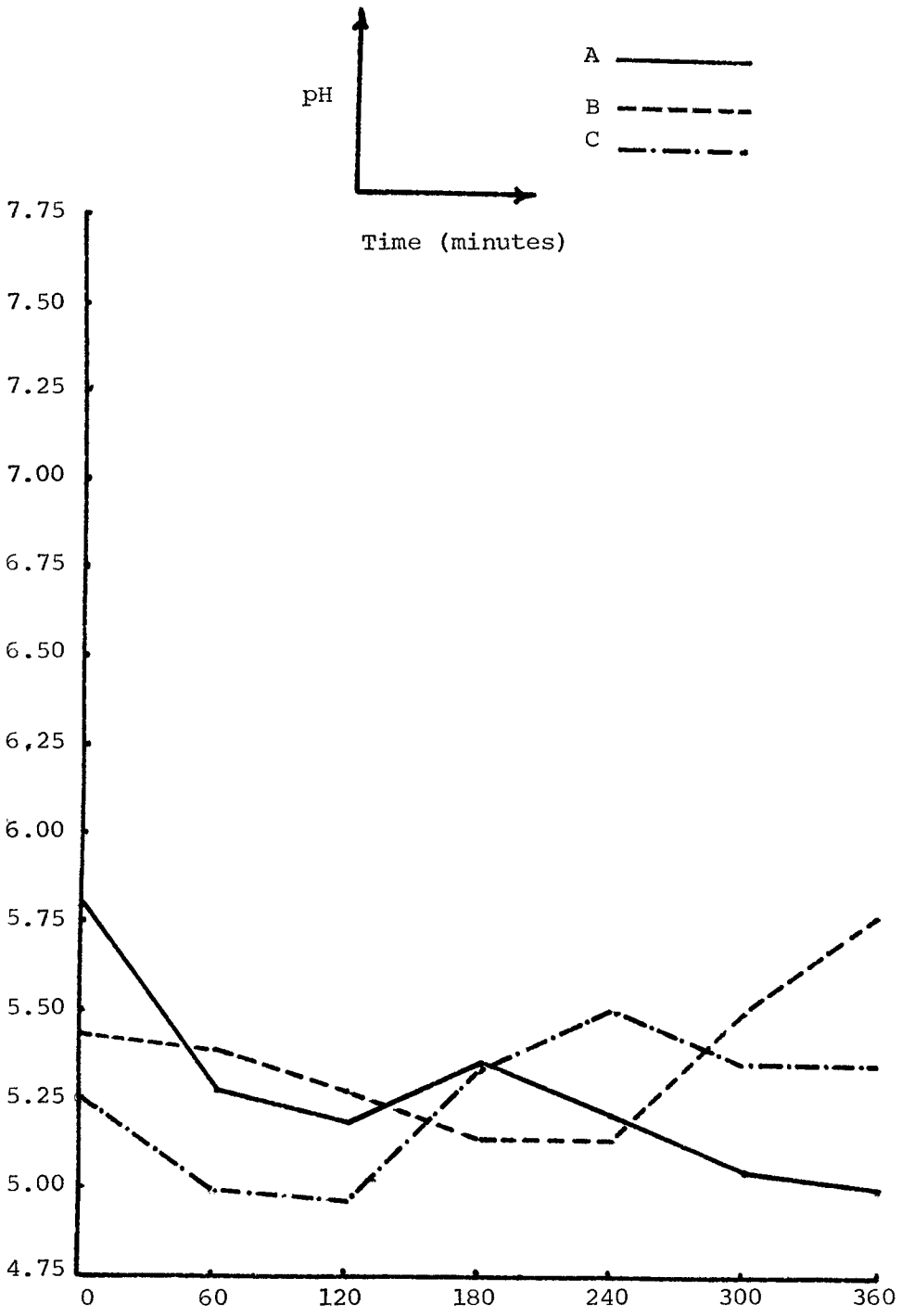

Fig. 10. pH of Urine as a Function of Time After Ingestion of Grape Juice.

FIGURES (CONT.)

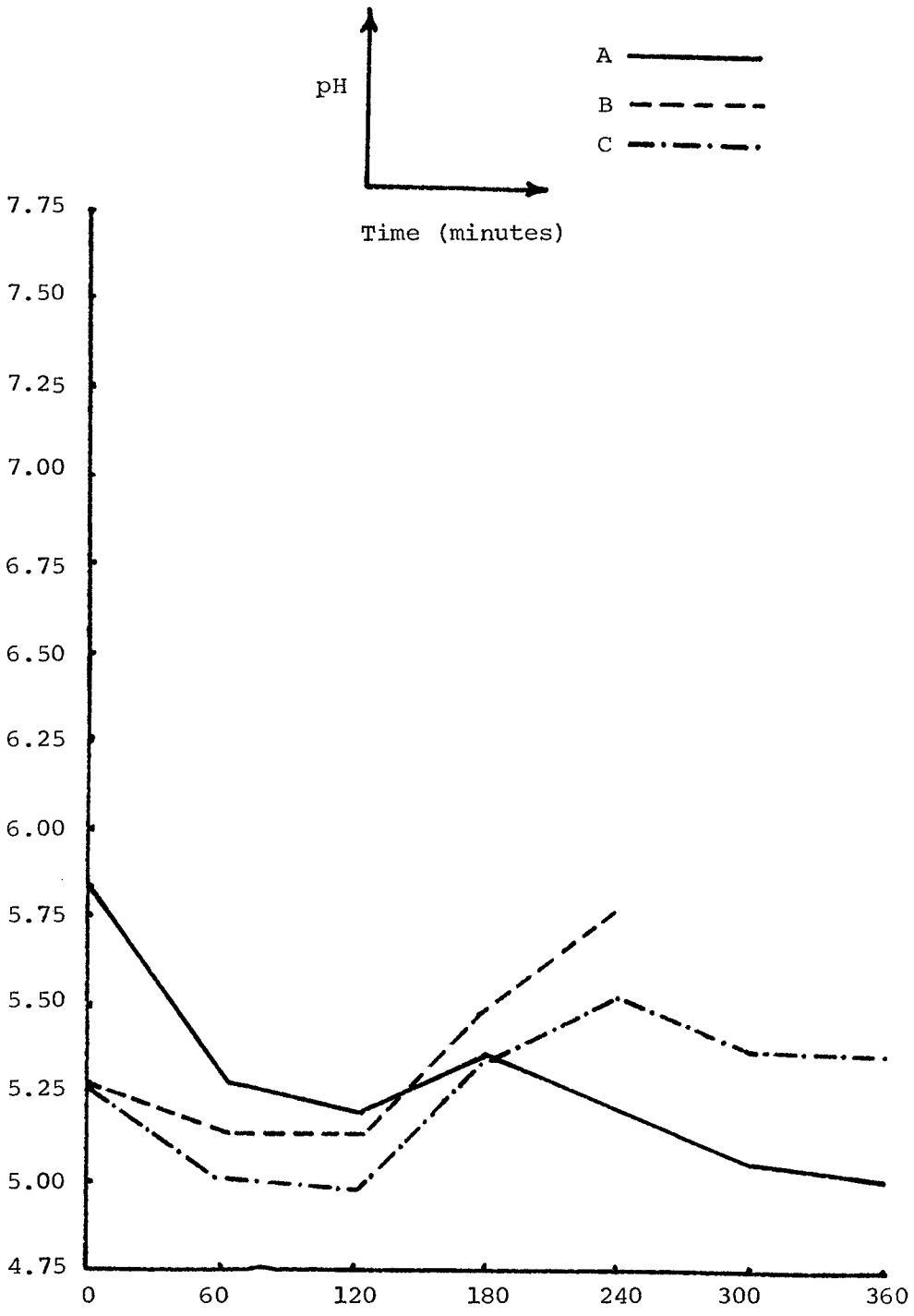

Fig. 11. pH of Urine as a Function of Time After Ingestion of Grape Juice (B is Advanced 2 Hours).

FIGURES (CONT.)

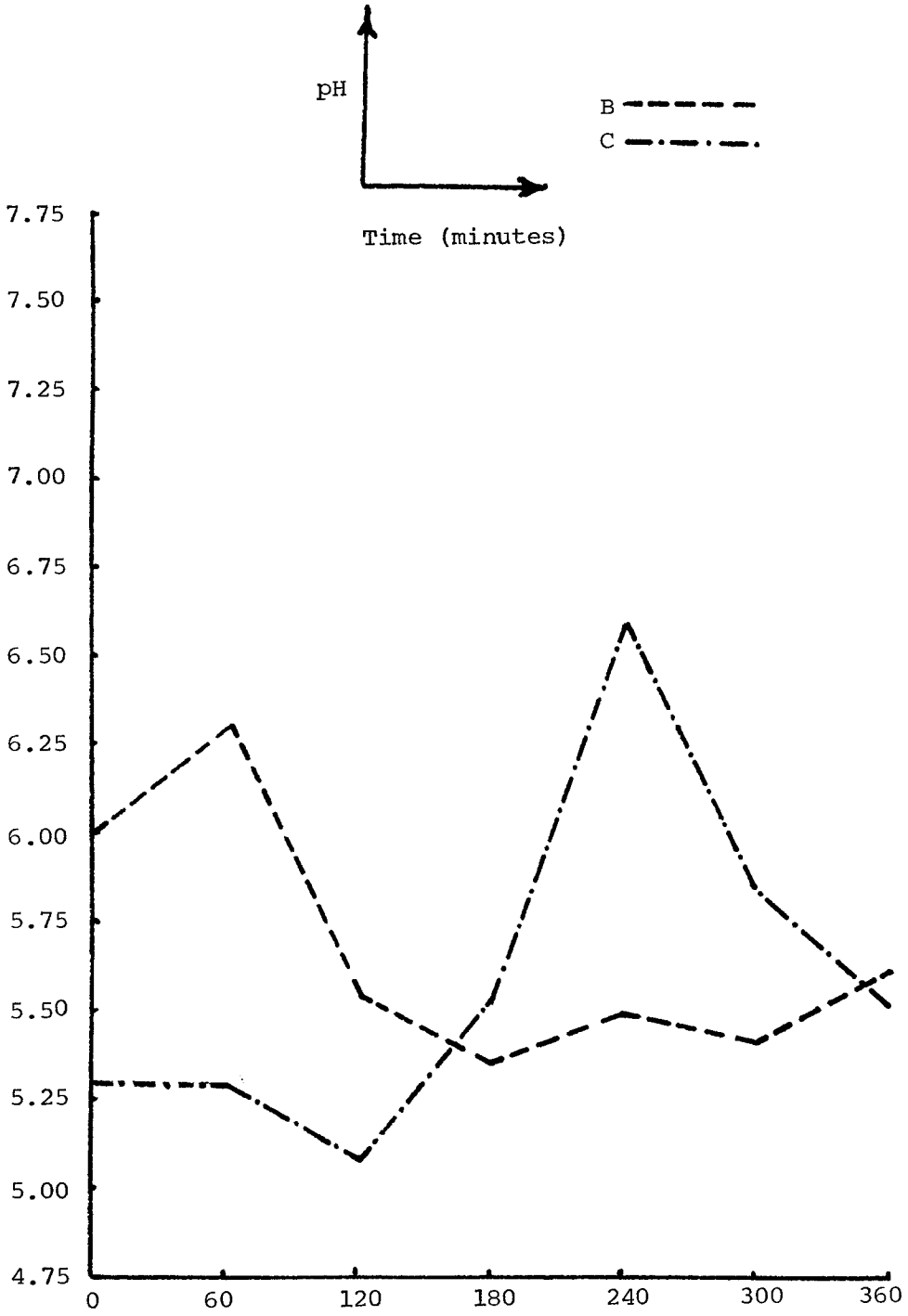

Fig. 12. pH of Urine as a Function of Time After Ingestion of Grape Juice (From Experiments in which No Growth Data were Obtained).

FIGURES (CONT.)

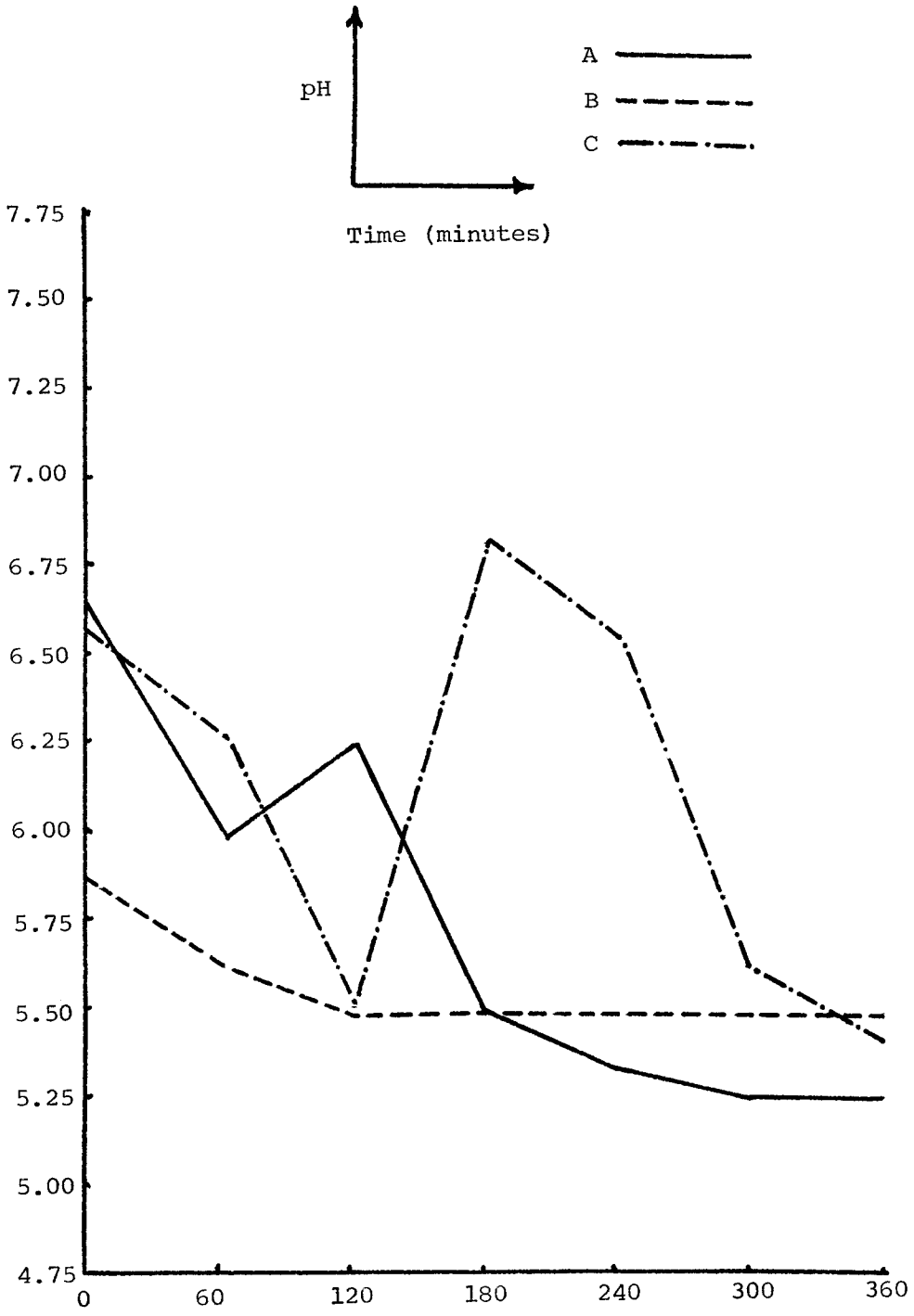

Fig. 13. pH of Urine as a Function of Time After Ingestion of Cranberry Juice.

FIGURES (CONT.)

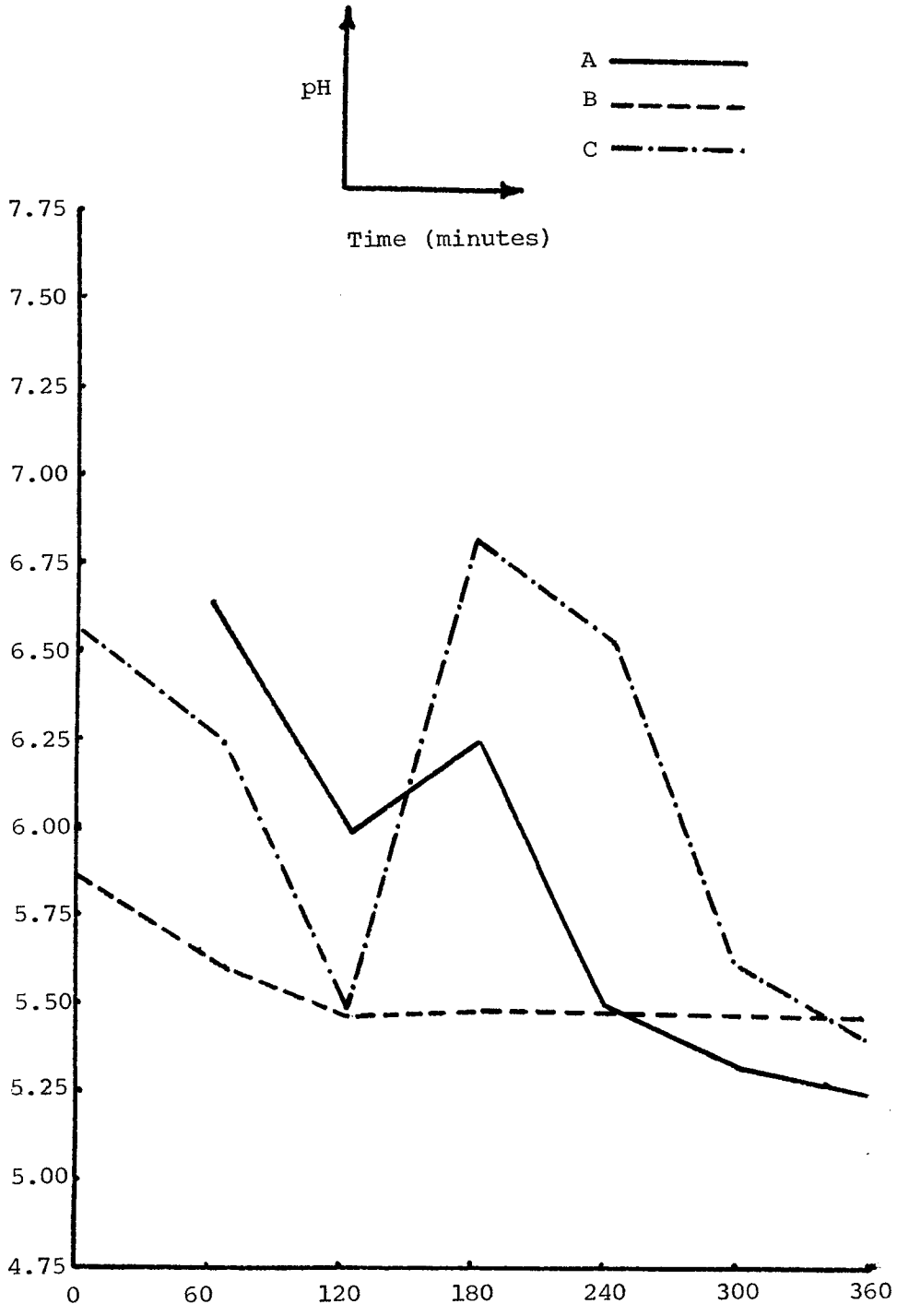

Fig. 14. pH of Urine as a Function of Time After Ingestion of Cranberry Juice (A is Delayed 1 Hour).

FIGURES (CONT.)

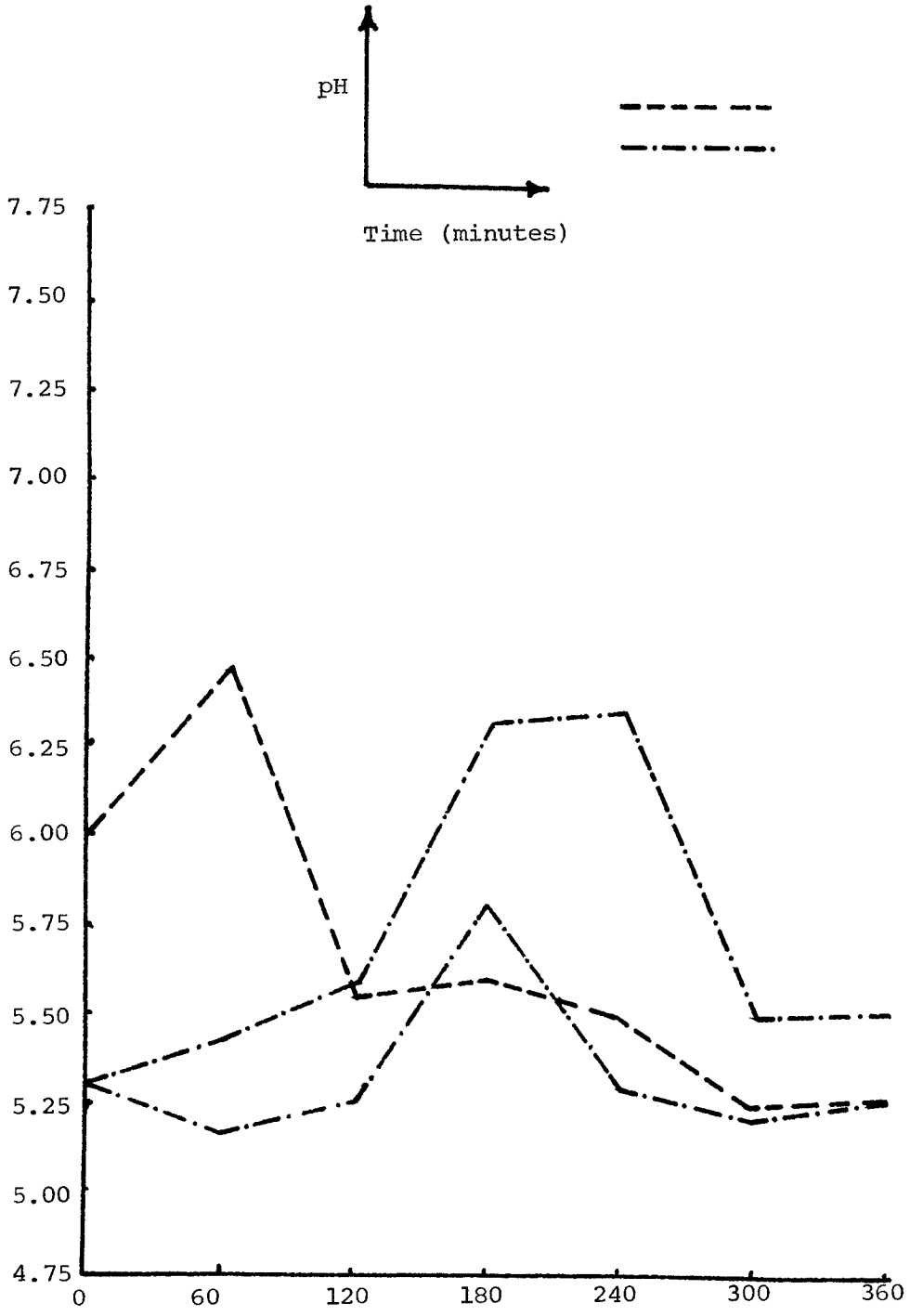

Fig. 15. pH of Urine as a Function of Time After Ingestion of Cranberry Juice (From Experiments in which No Growth Data were Obtained).

FIGURES (CONT.)

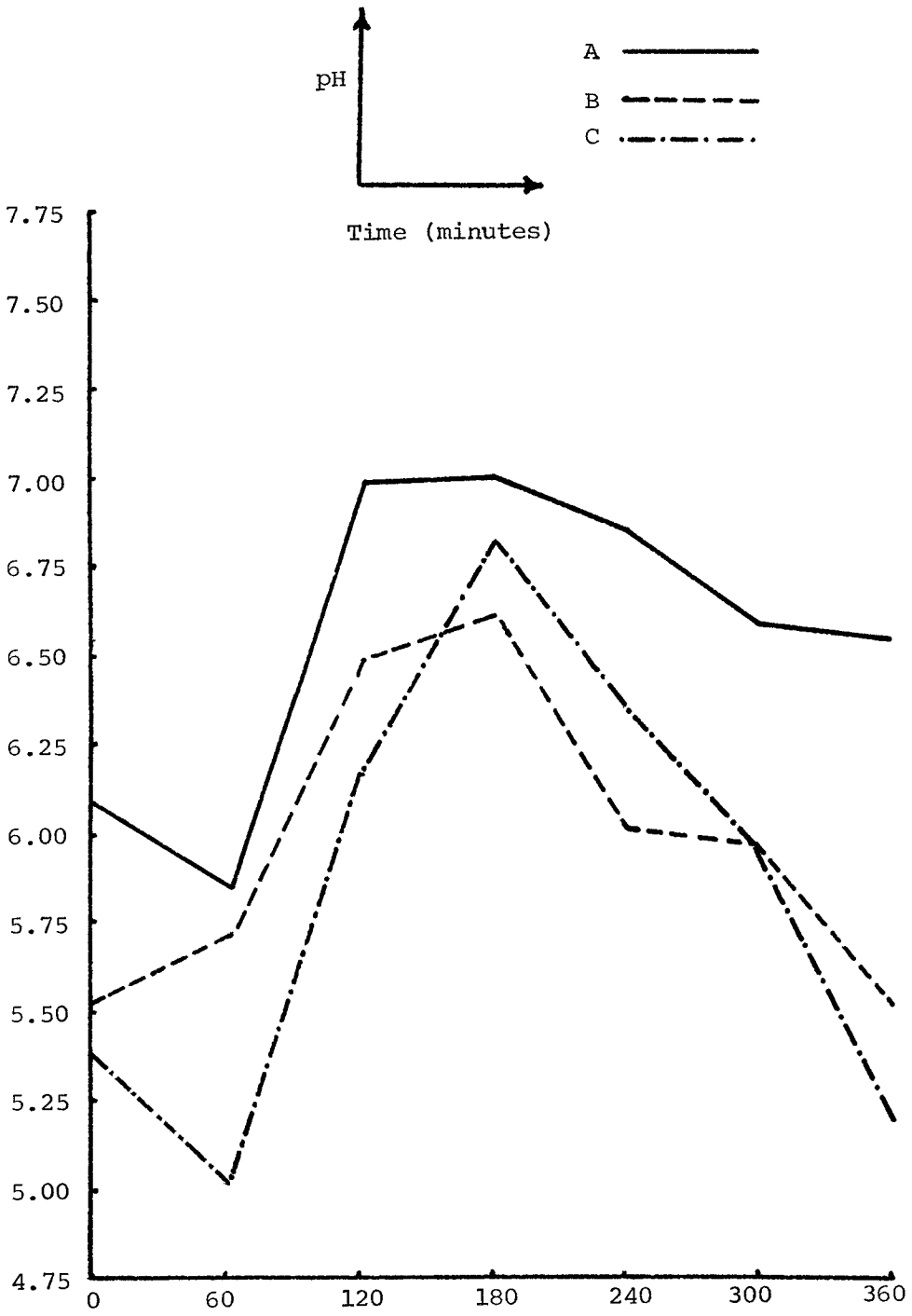

Fig. 16. pH of Urine as a Function of Time After Ingestion of Orange Juice.

FIGURES (CONT.)

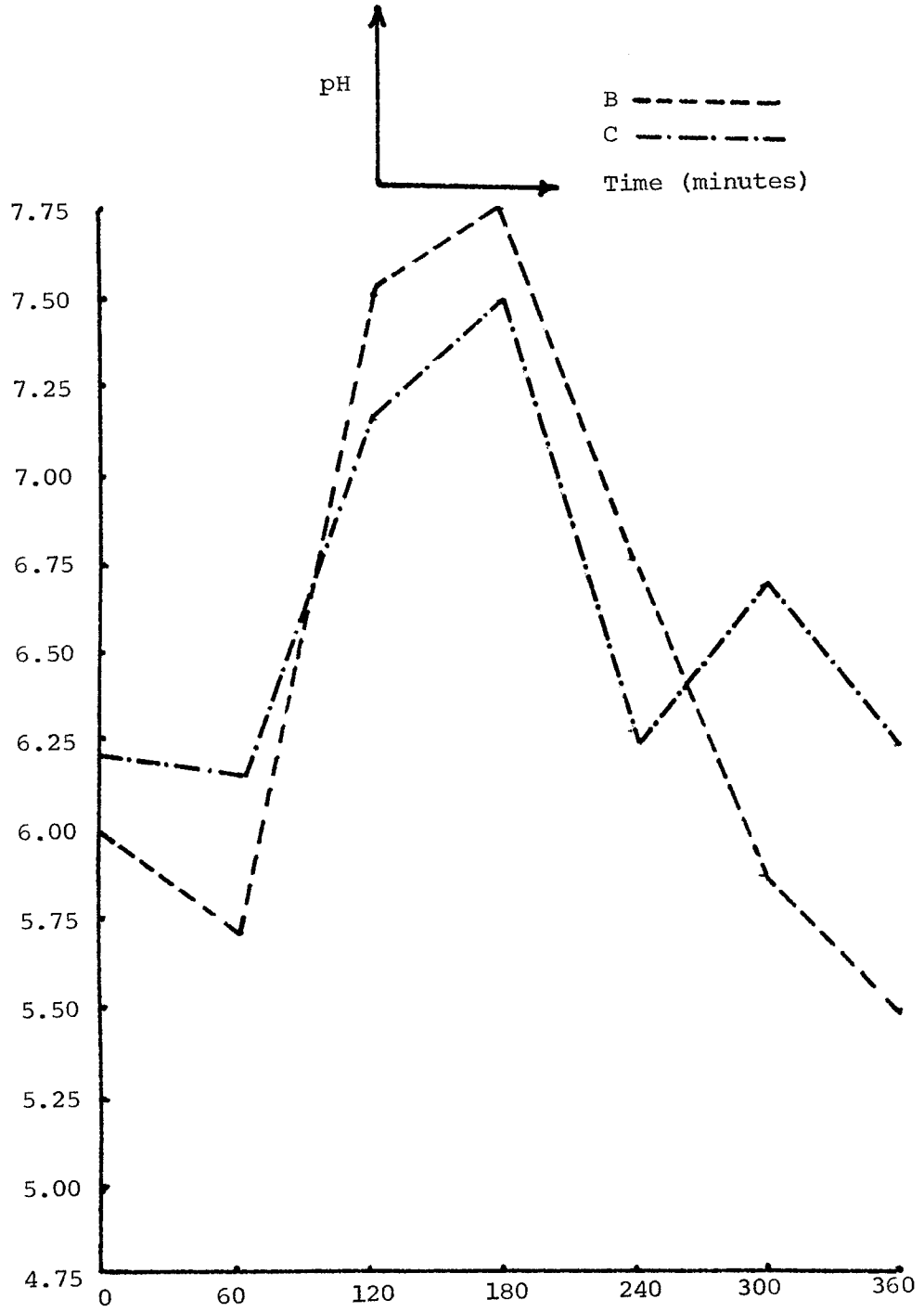

Fig. 17. pH of Urine as a Function of Time After Ingestion of Orange Juice(From Experiments in which No Growth Data were Obtained).

FIGURES (CONT.)

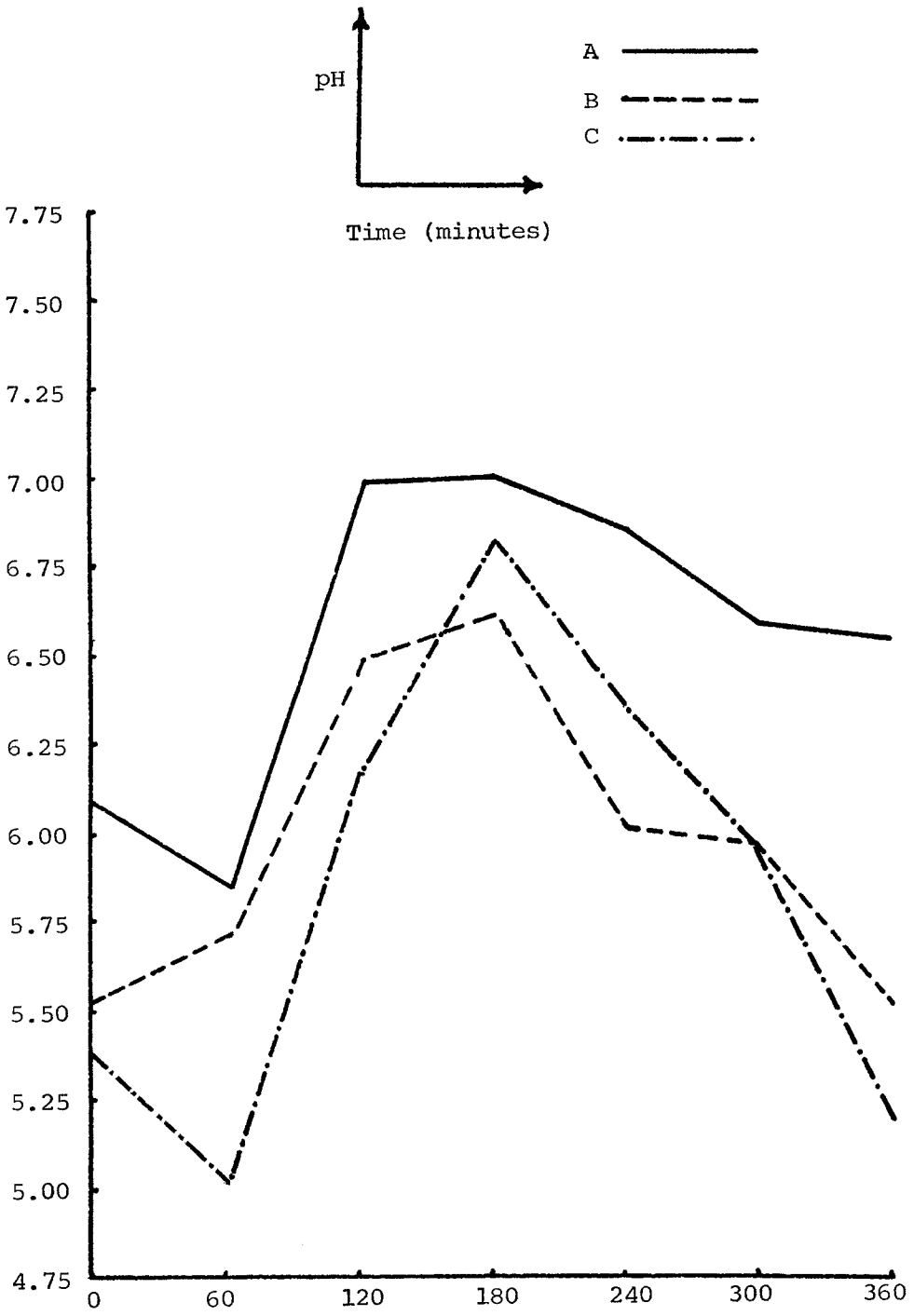

Fig. 16. pH of Urine as a Function of Time After Ingestion of Orange Juice.

FIGURES (CONT.)

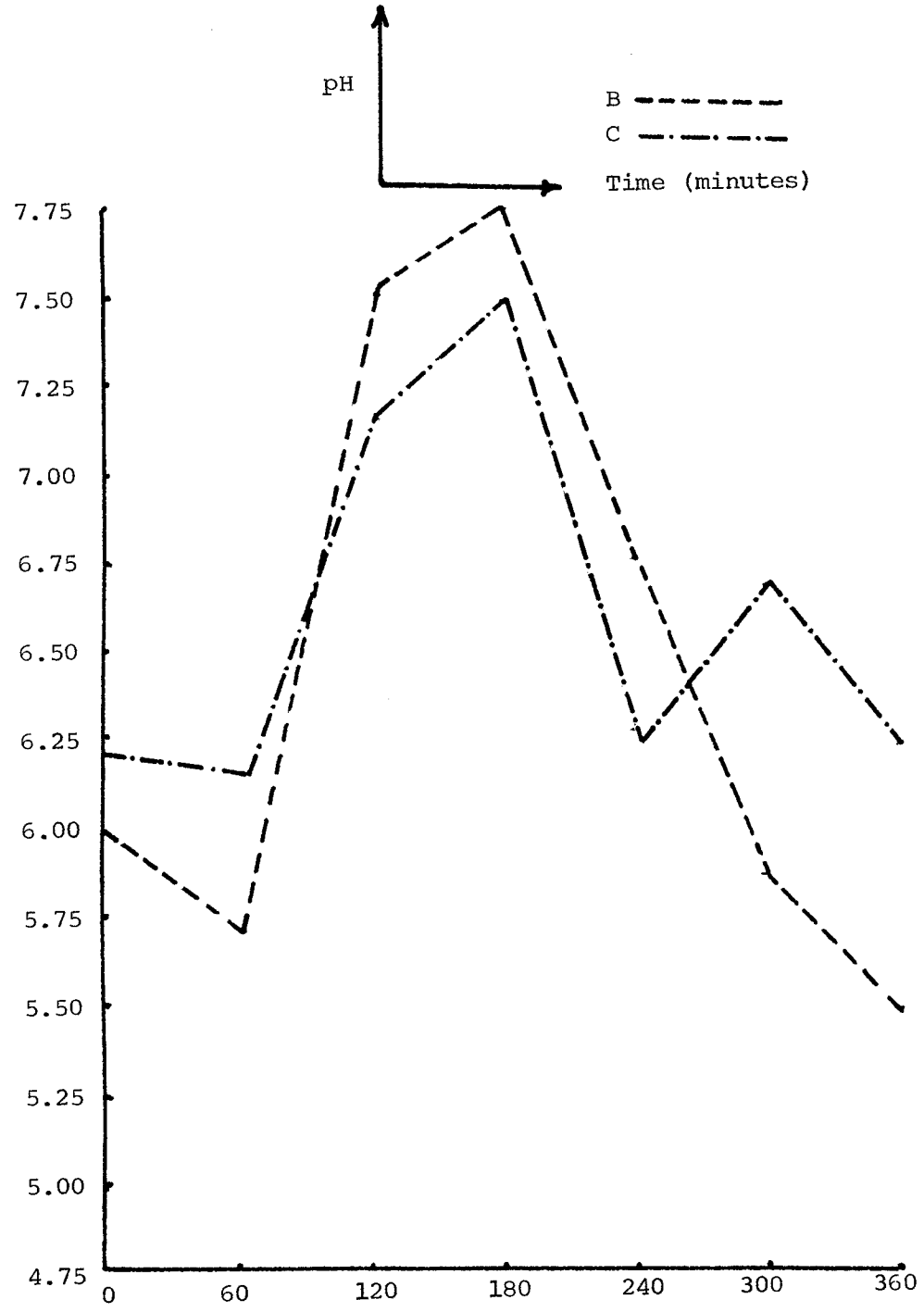

Fig. 17. pH of Urine as a Function of Time After Ingestion of Orange Juice (From Experiments in which No Growth Data were Obtained).

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