DIVISION PARAMETERS OF ASPARTATE-GROWN
ESCHERICHIA COLI 15T⁻ FOLLOWING NUTRITIONAL SHIFT-UP

by

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TABLE OF CONTENTS

LIST OF FIGURES

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INTRODUCTION

One of the fundamental objectives of biological research today is a more complete understanding of the process of cell division. Because bacterial cells are the most biochemically defined and best understood cells in nature, they are frequently used as models in cell division research.

The apparent primary objective of bacterial growth and metabolism is the production of additional (daughter) cells. In bacteria this process is probably as simple as in any other cell, but even in bacteria the process is only very poorly understood. The growth processes of all bacteria seem to be similar and involve size increase to some critical point, followed by separation into two daughter cells, and subsequent size increases of the individual daughter cells until they, too, can divide. The rate at which these processes occur vary for individual species of bacteria and can be easily related to influences such as environmental conditions and culture media.

The factors that control the rate and timing of the division process, however, still require elucidation, as does the actual physiological process of division. For example, the process of septum formation, the site where it occurs, and its relationship to DNA synthesis and cell size have been studied but are not yet well defined.

The work described here deals specifically with the control of the division process during the transition from slow to fast growth rates in

the bacterium <u>Escherichia coli</u>. This study developed as an off-shoot
of a problem concerning the differential doubling time of <u>E. coli</u> 15T⁻
grown at two different aspartate concentrations. Aspartate is a poor
carbon source; that is, it is capable of supporting cell growth, but at a
slow rate. Research has shown that slow-growing cells do not readily con-
form to the models that seem adequate to describe cell growth at faster
rates (Clark, 1968; Helmstetter <u>et</u> <u>al</u>., 1968).

Exponentially growing cultures at each aspartate concentration were
subjected to a nutritional shift-up, i.e., each culture was transferred
to richer medium which supported a faster rate of growth. Cells must
undergo radical alterations in regulation during the transition period
between two balanced states of growth (Maaløe <u>et</u> <u>al</u>., 1958). By sub-
jecting both cultures to shift-up conditions, some insight into the
differential growth rate in aspartate was sought.

The results of the shift-up experiments will be discussed extensively
later, but briefly the higher aspartate concentration behaved in an
expected manner, compatible with published information by Maaløe <u>et</u> <u>al</u>.
(1958). The pre-shift rate of cell division was maintained for approximately
a generation time prior to the attainment of the faster rate.

An unexpected finding that changed the entire emphasis of this
research was the observation that cells grown in a low level of aspartate
could, immediately following nutritional shift-up, divide at a faster
rate. A careful characterization of the division response of low level
aspartate-grown cells was then undertaken in an attempt to ascertain
individual division events. It was hoped identification of division
events would 1) explain the accelerated rate of transition, 2) illuminate

events which must occur during rate transitions, and 3) further explain

rate maintenance. Specifically addressed points of the characterization

were a comparison of electronic particle counts and colony-forming units,

cell size (mass), and DNA content.

LITERATURE REVIEW

Bacterial cell division can serve as a model for differentiation, because a newly-divided cell changes in structure as well as size in the process of becoming a cell which will itself eventually divide. To understand the many hypotheses proposed for the control of the division process, the nomenclature introduced by Helmstetter et al. (1968) is helpful. Three necessary time intervals for the cell cycle were described as follows: 1) I, the time necessary to accumulate the capacity for chromosome initiation; 2) C, the chromosome replication time; and 3) D, the interval between the completion of chromosome synthesis and the physical separation of the two daughter cells. Helmstetter et al. (1968) also developed a model to interrelate these parameters in the growth of steady-state cultures. For slow-growing cells they found that I = C + D. Cells with generation times greater than 40 minutes contain a period devoid of DNA syntheses called a gap; that is, more time is required to complete the division cycle than to replicate the chromosome (Cooper and Helmstetter, 1968).

For fast growth, however, Helmstetter et al. (1968) found a different situation. Under these conditions there is no gap in chromosome synthesis; and although C was found to equal 40 minutes, cells can have generation times shorter than C + D (which is approximately 60 minutes) by having multiple replication forks. In other words, the chromosome is already partially replicated when division occurs.

The termination of chromosome replication was shown to be a prerequisite for cell division by Helmstetter and Pierucci (1968) and Clark

(1968). Dix and Helmstetter (1973) found that chromosome termination in conjunction with protein synthesis was necessary for division, while Jones and Donachie (1973) gave evidence that termination, protein synthesis, and specific RNA synthesis all had to occur prior to division.

The preceding theories must be integrated into what has been observed in the actual process of cell growth and division. Higgins and Shockman (1971) found the microscopically observable properties of dividing Gram positive cocci to be fairly well defined. Fluorescent antibody studies show that the parent streptococcus initiates cross wall and new cell wall growth along the equatorial ring; thus the new hemispheres of the daughter cells are formed back to back (Cole, 1965).

However, in the Gram negative Salmonella typhosa, Cole found fluorescent antibody label to be uniformly distributed, as if cell wall growth occurred by a process of diffuse intercalation. Approaching the problem of Gram negative growth in a different way, Donachie and Begg (1970) grew cells in a low penicillin concentration. Due to increased penicillin sensitivity at the future site of septum formation, a bulge formed which was visible throughout the cell cycle. They used this bulge as a reference marker for measuring growth of individual cells embedded in agar. Their findings indicate that in minimal media cell growth is unidirectional; however, in complex media cells grow by elongation in both directions.

Higgins and Shockman (1971) have developed a model for Gram negative cell wall growth that relies heavily upon the Donachie and Begg (1970) data. They assume cross-wall growth and the cross-wall itself to be different from cylindrical wall growth and the cylindrical portion of the cell wall. They suggest that e sites (wall cylinder elongation sites) are con-

verted to cross-wall sites (xw sites) at the end of a round of chromosome replication. In a shift-up to richer medium, either initiation of extra e sites or failure of an e site to be converted to a xw site would allow cells to increase in volume until a size characteristic of the new medium can be attained (Higgins and Shockman, 1970; Schaechter et al., 1958).

Many bacteria in nature are confronted with a continually changing nutritional supply, and their survival demands a flexibility in growth rate based on available nutrients. Artifically in the laboratory such a response can be monitored in nutritional shift experiments. The transition period between two steady-state growth rates can serve as a tool to study cell cycle control, provided growth parameters such as cell number, mass, and synthesis of macromolecules (DNA, RNA, protein) are accurately measured. The transitional events can then be related to the steady-state parameters at the beginning and end of the experiments.

The growth parameters of Salmonella typhimurium cells undergoing a nutritional shift-up from glucose salts to nutrient broth were examined by Kjeldgaard et al. (1958). They found that RNA synthesis increased immediately after the shift, followed within minutes by an increase in optical density. Approximately twenty minutes post-shift, the rate of DNA synthesis assumed this new, faster rate. Cell number, which was the last growth parameter to increase, did not reach the new media's characteristic rate until seventy minutes after the shift. The observation that DNA synthesis and cell division continue at the pre-shift rate for considerable periods of time was termed "rate maintenance."

Helmstetter et al. (1968) analyzed a nutritional shift-up by their model and were able to explain the rate maintenance observed by Kjeldgaard et al. for DNA synthesis and cell division by stating that C and D are constants in cells growing at different rates in different media. Initiator

synthesis time (I) would be less following a nutritional shift-up, but a chromosome newly initiated before the shift would require a full C + D before division.

It is apparent from the original data of Kjeldgaard et al. that there are slight deviations from rate maintenance immediately following a shift-up when cell number is measured by determination of viable counts. Helmstetter et al. (1968) suggested that a shortened D-period coupled with a temporary reduction in the rate of chromosome synthesis could produce this phenomenon.

Cooper (1969) theorized possible deviations from rate maintenance due to post-shift variations in C and D. He also measured the rate of elution of B/r cells from a membrane using the Helmstetter technique (Helmstetter, 1968). This technique capitalizes upon the ability of E. coli B/r cells to attach firmly to a membrane filter. The filter is inverted, and when the attached cells divide, the daughter cells are eluted from the membrane by the passage of pre-warmed media. In this manner, populations of a uniform age can be obtained; or, as in this example, growth rate may be determined. Cooper found that the rate at which daughter cells divided from the membrane-bound cells increased for a short time, as in the Coulter count data, and then decreased. Therefore, this deviation from rate maintenance did not appear to be an artifact of the method used to determine cell number.

Comparison of the transitional events for cultures at the two aspartate concentrations used in this study yielded a discrepancy in cell number following the shift. The high aspartate culture showed rate maintenance in cell number; however, the low level exhibited what may be an exaggerated version of the deviation from rate maintenance discussed above.

MATERIALS AND METHODS

Bacteria. Escherichia coli 15T$^-$ (555-7) described by Lark et al.
(1963) was used throughout this investigation. The organism is a quadruple
auxotroph requiring thymine, arginine, tryptophan and methionine for
growth.

Media. M9 salts medium of Kellenberger et al. (1962) was supple-
mented with various carbon sources: aspartate at final concentrations
of 0.1% and 0.5%, glucose at 0.4%, and succinate at 0.45%. Thymine (4
μg/ml), L-arginine (100 μg/ml), L-tryptophan (50 μg/ml), and L-methionine
(50 μg/ml) completed the medium.

pH determinations of media components were made on a Leeds and
Northrup 7411 pH meter. The pH of culture media (pH 7.0) did not vary
during the course of nutritional shift-up experiments.

Cell growth. Cultures were bubble-aerated at 37° C and diluted with
pre-warmed medium to maintain exponential growth at cell densities between
5×10^7 and 2×10^8 cells/ml. Table 1 lists the growth rates of E. coli
15T$^-$ in various media.

In nutritional shift-ups, an exponentially growing culture was added
directly to the richer carbon source, thus avoiding the possible perturbation
of filtration and resuspension. For example, a 0.1% aspartate culture
was added to sufficient prewarmed 40% glucose to result in a final glucose
concentration of 0.4%.

Cell number determination. 0.1 ml culture samples were diluted in
25 ml 0.85% NaCl and counted on a Model F_N Coulter counter equipped with
a 30-μm orifice. The NaCl solution was filtered once through a 0.45 μm
cellulose nitrate membrane filter before use.

TABLE 1

Growth Rates of E. coli 15T⁻ in M9 Salts Medium

Supplemented with Various Carbon Sources

Carbon Source and Concentration	Doubling Time in minutes
Aspartate, 0.1%	120
Aspartate, 0.5%	80
Glucose, 0.4%	40
Aspartate, 0.1% + Glucose, 0.4% . .	50
Succinate, 0.45%	70

The number of colony-forming units was measured by appropriately diluting samples in M9 salts, adding to 0.5% nutrient agar, and spreading on 1.5% nutrient agar. Each diluted sample was plated in triplicate, and all media was supplemented with thymine at 4 µg/ml.

Cell size determination.

I. Optical density. Samples were placed in quartz microcuvettes with a 1 cm pathlength. Optical density determinations for all experiments were made at 450 nm on a Gilford 240 spectrophotometer, with the exception of the succinate experiment (Fig. 2) which was read at 600 nm. Both 450 and 600 nm are accepted values for optical density determinations of bacteria. Culture densities were kept in the range of 0.1 and 0.4 O.D. units by dilution in the respective medium.

II. Particle size analysis. Samples containing 0.4 ml of exponentially growing cells were added to 0.1 ml of 37% formaldehyde, mixed and refrigerated at 4° C. Immediately before assay the refrigerated samples were diluted 1:1000 in 2x-filtered 0.85% NaCl. Double-filtered saline was necessary to reduce background in the particle size analysis, and was prepared by refiltering the 0.85% NaCl solution described previously (see Determination of cell number) through a fresh membrane filter.

Diluted samples were sized on a model Z_F Coulter counter fitted with a 30-µm orifice and interfaced with a Canberra 8100/e multi-channel analyzer. 256 memory channels were used, and the analyzer was calibrated with 1.011 µm polystyrene spheres (Duke Standards, Inc.) and 1.857 µm polyvinyl toluene spheres (Coulter Electronics, Inc.) also diluted in twice-filtered NaCl.

Measurement of DNA.

I. Preparation of thymine solution. A filter-sterilized, aqueous solution of thymine (pH 7.0) containing 0.0119 mCi [methyl-^{3}H]

thymine (New England Nuclear) was prepared. The thymine content was assayed by weight and extinction coefficient and found to be 410 µg/ml. $[^{3}H]$ was measured on glass fiber filters in a Beckman LS 100 liquid scintillation spectrometer. Based on our assay systems, the stock solution contained 20,492 cpm/µg thymine. This specific activity was used in DNA content calculations. The thymine solution was stored in 4 ml aliquots in sealed vials at -20° C. Single vials were thawed and refrozen a maximum of four times.

II. Experimental procedure. Cells were grown in media containing 4 µg/ml of the stock thymine solution described above as the sole source of thymine. Duplicate 0.1 ml aliquots of exponentially growing cells were pipetted into 0.3 ml ice-cold 5% trichloroacetic acid, mixed, and held on ice a minimum of 30 minutes. Then samples were filtered through 2.4 cm glass fiber filters, washed three times with water and twice with 95% ethanol, dried, and placed in vials. The samples were counted approximately 24 hours after flooding the filters with 10 ml of Permablend II scintillation fluid (Packard Instruments).

Data Analysis. In the shift-up experiments in Figs. 1-6, the $\log_{10}$ of cell number (y) and corresponding time in minutes (x) for each post-shift sample were subjected to computerized regression analysis, as were the numerical O.D./cell values (y) vs. corresponding time increments (x) in Fig. 7. The computer program used was designed such that the computer determined which data points most reasonably fit particular regression lines (Hudson, 1966). Each data set was fit with one, two, or three regression lines, and the F-test was used to determine which line provided the best fit.

RESULTS

Coulter counts and optical density.

Cultures growing in both aspartate concentrations were subjected
to nutritional shift-ups with quite dissimilar results.

Upon addition of cells to glucose, the 0.5% culture (Fig. 1) showed
the classic response of rate maintenance first described by Kjeldgaard
et al. (1958). The pre-shift division rate was maintained for approximately
a generation time (80 minutes) before assuming a growth rate characteristic
of the new medium. Also as expected, an increase in optical density was
noted within 10 minutes after the shift.

A 15T⁻ succinate culture added to glucose also gave a classic
nutritional shift-up response (Fig. 2). In both the 0.5% aspartate and
the succinate medium, 15T⁻ appears to follow the Cooper-Helmstetter (1968)
model.

Addition of the 0.1% aspartate culture to glucose gave an unexpected
result. In all experiments there was an immediate initial increase in cell
number. This rapid initial increase was maintained in many experiments,
resulting in continued rapid division and a generation time of 50 minutes
(Fig. 3); however, the rate of increase was not strictly linear. In other
experiments after the initial burst of division, the rate of division
slowed. In the most extreme cases, the slower rate paralleled the pre-
shift rate in what approximated a classic Kjeldgaard et al. (1958) shift.
By comparing the 15T⁻ 0.1% aspartate to glucose shift in Fig. 3 with the
two experiments in Fig. 4 (again 0.1% aspartate to glucose shifts), one
can see the variability in growth rate following the initial burst of
division that characterized all these experiments.

Figure 1. Nutritional shift-up response of 15T$^-$ 0.5% aspartate culture
added to glucose. At the time indicated by the arrow, the
parent culture was divided and a portion added to pre-warmed
40% glucose to achieve a final glucose concentration of 0.4%.
Cell number was measured on an electronic particle counter.
To minimize confusion on the graph, actual data points are
not given for the unshifted portion of the culture; however,
the parent culture was periodically monitored during the entire
experiment. Before the shift-up, these counts were necessary
to determine the growth rate, and following the shift, counts
were taken to check for general culture health. The log of
the optical density and log of cell number are plotted according
to the ordinate scale. All values in this and following·
figures are transposed so curves representing steady-state
growth in the pre-shift media coincide. The inset shows
lines drawn from statistical analysis of the post-shift
electronic particle counts. When tested for 1, 2, or 3 lines,
three lines were found to best fit the data. The legends
for the inset are identical to those for the main graph.

Symbols:

    ●   optical density

    O   cell number, shifted culture

 ······· parent culture

THIS BOOK
CONTAINS
NUMEROUS PAGES
WITH DIAGRAMS
THAT ARE CROOKED
COMPARED TO THE
REST OF THE
INFORMATION ON
THE PAGE.

THIS IS AS
RECEIVED FROM
CUSTOMER.

Figure axes: LOG CELL NUMBER (y-axis) vs MINUTES (x-axis). Inset graph with y-axis labeled 8, 6, 4, 2 and x-axis labeled 100, 200, 300. Main graph x-axis labeled 100, 200, 300; y-axis marked 10.
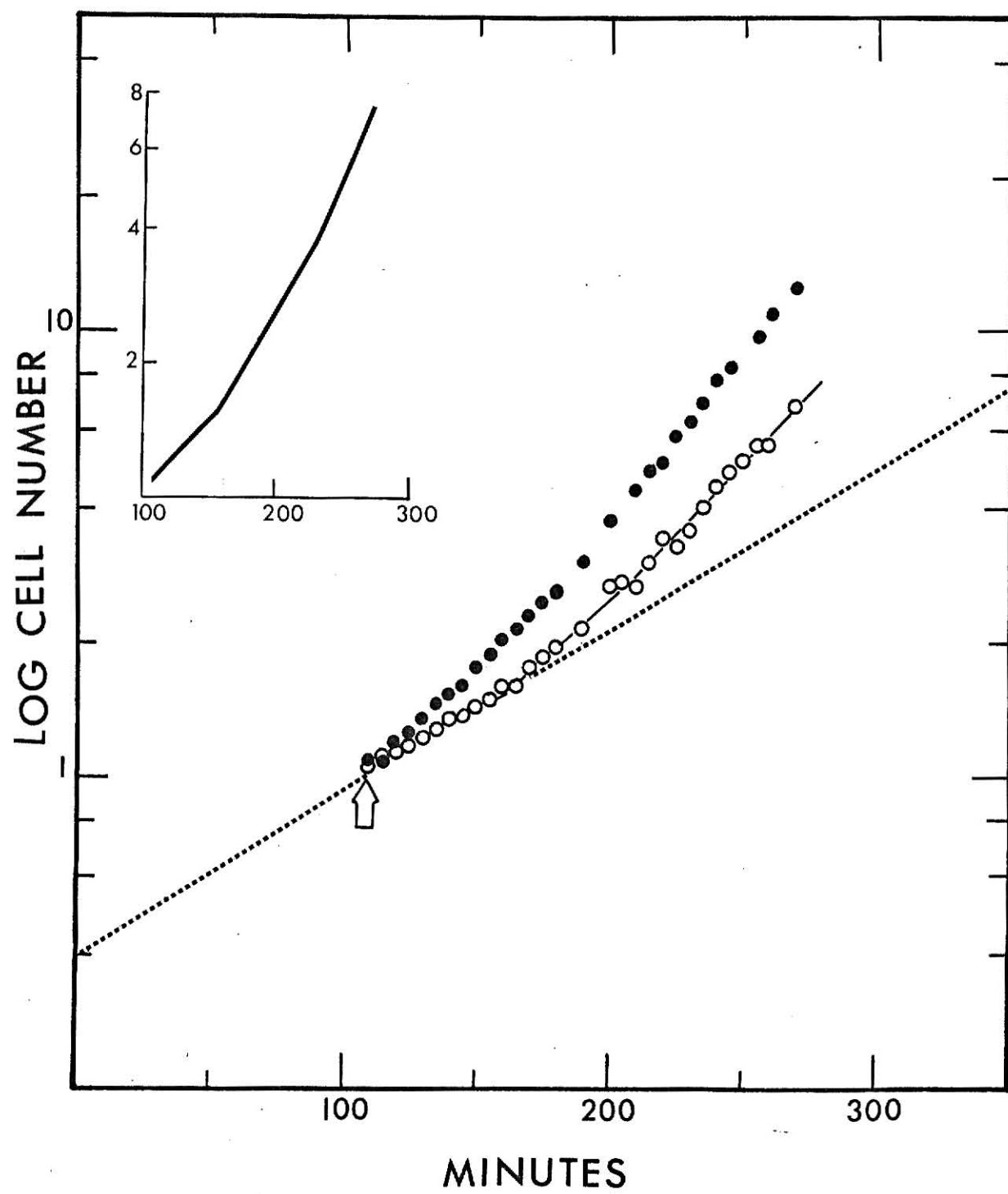
Figure 2. Nutritional shift-up response of 15T⁻ succinate culture upon
addition to glucose. The experimental procedure described
in *Materials and Methods* was followed. Statistical analysis
of the post-shift cell number data showed two lines to fit
best.

Symbols:

● optical density

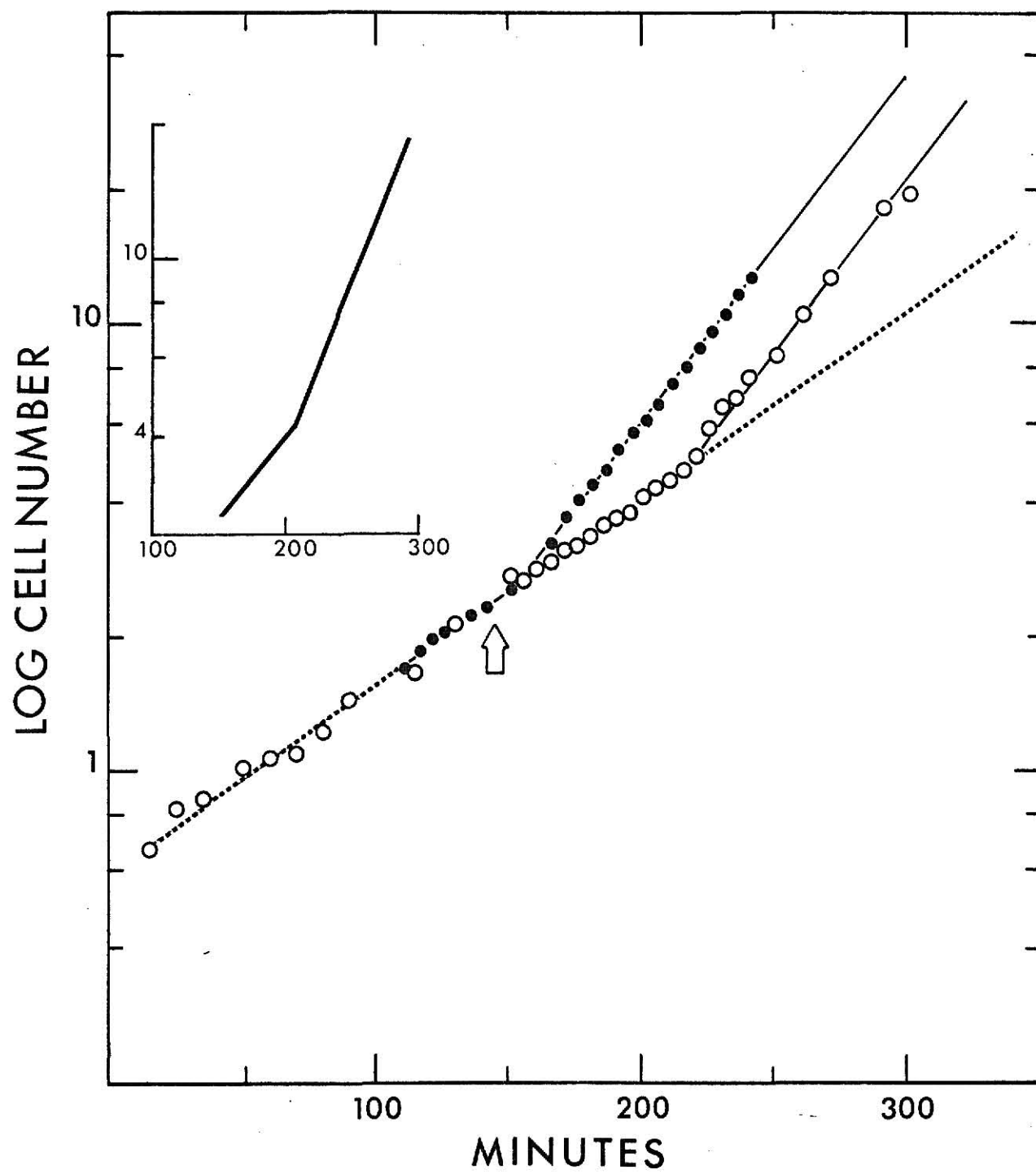○ cell number, shifted culture

▪▪▪▪▪ parent culture

Figure 3. Nutritional shift-up of 15T$^-$ in 0.1% aspartate upon addition
of glucose. The experimental procedure followed was described
in Materials and Methods. In this experiment immediately
following the shift there was a burst of division, then a
decrease almost to the pre-shift rate before the final increase.
Statistical analysis of the post-shift cell number data
showed 3 lines to fit best. Notice that the initial burst
of division followed by a decline are also apparent from the
statistical analysis.

Symbols:

● optical density

○ cell number, shifted culture

••••• parent culture
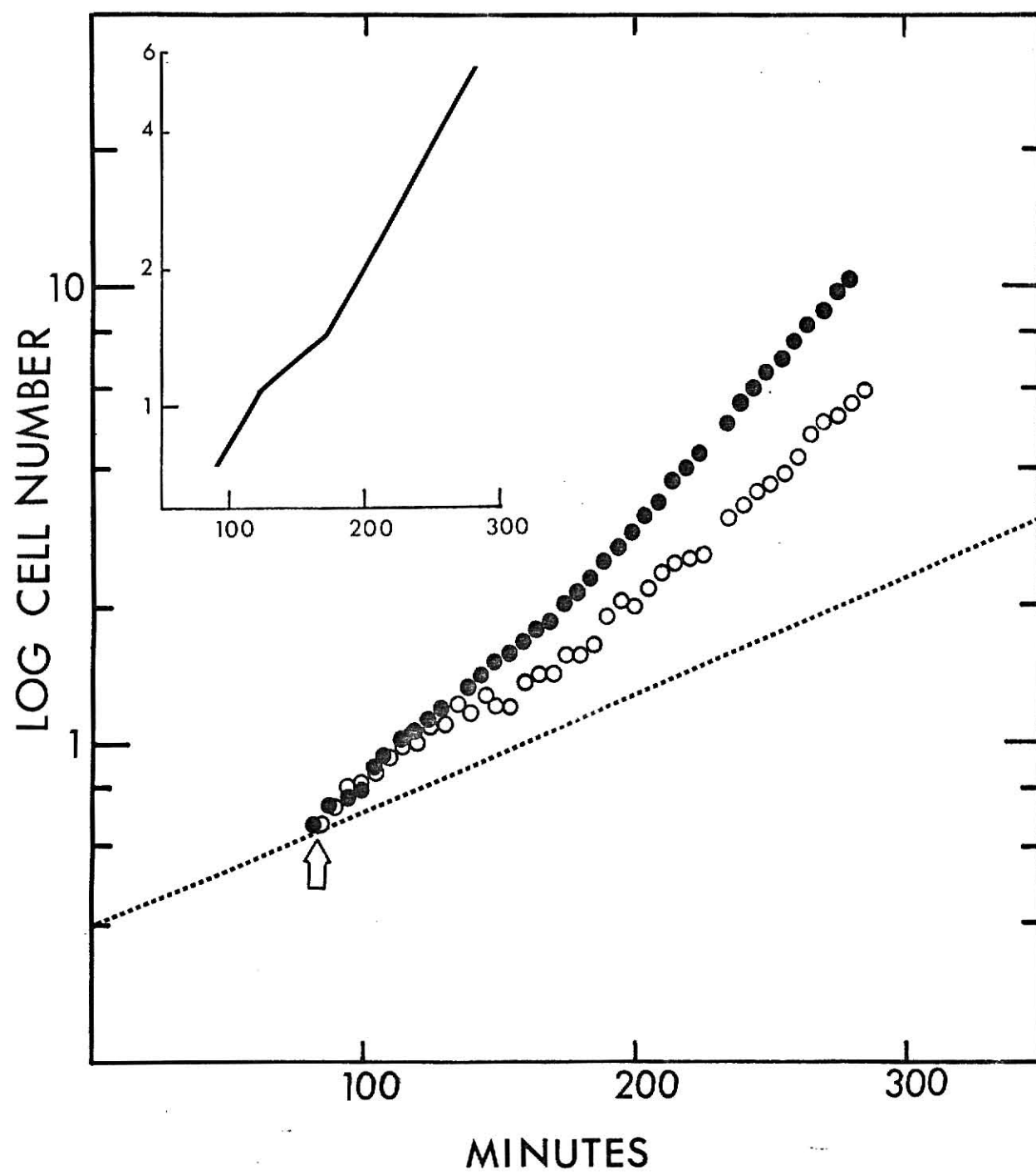
Figure 4. Effect of dilution on 15T⁻ 0.1% aspartate to glucose shift.
The culture with the highest division rate was not diluted
upon addition to glucose; the other culture was diluted 1:2
into fresh media containing glucose.

Symbols:

      O   undiluted culture, cell number

      △   diluted culture, cell number

The inset shows the statistical analysis for these two
cultures. Three lines were found to best fit the diluted
culture, while two fit the undiluted culture best.

Symbols:

      ▬▬   undiluted culture

      ▬▬   diluted culture

The division pattern following the shift seemed to be influenced by dilution. Fig. 4 shows a faster post-shift rate for a culture which was a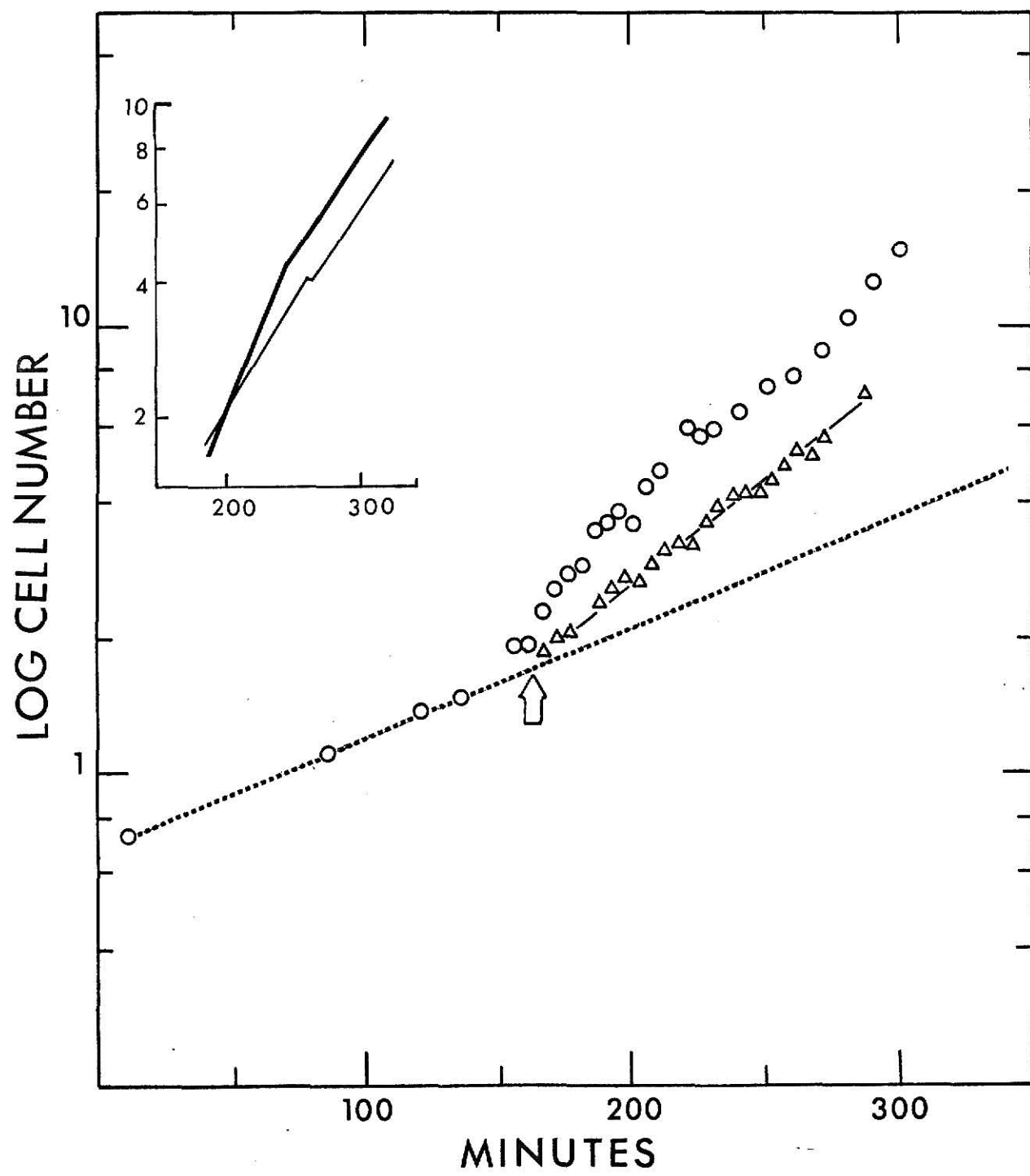dded directly to glucose with no dilution, while a slower rate occurred when a culture was simultaneously diluted 1:2 into fresh aspartate media containing glucose. This indicated that conditioned media might enhance the immediate increase in cell number, but experiments resuspending filtered cells in conditioned media, or comparing shifts in conditioned media plus glucose vs. fresh media plus glucose did not bear this out.

To find out if the rapid response to glucose was unique to 15T$^-$, we grew B/r obtained from Helmstetter in 0.1% aspartate, added glucose and again observed an immediate cell number increase (Fig. 5).

Comparison of colony-forming units to electronic particle count.

To determine if the atypical division pattern could be some artifact of counting cells electronically, 0.1% aspartate to glucose shifts with 15T$^-$ were simultaneously sampled for Coulter counts and viable counts. The viable count data for 0.1% aspartate did vary somewhat from the Coulter count data: initially the viable counts appeared to be higher, but dropped to a level slightly below the Coulter counts toward the end of the experiment (Fig. 6). The viable count vs. Coulter count data for a shifted 0.5% aspartate culture were much more compatible.

One criticism of these experiments is that by plating on nutrient agar we were in effect subjecting cells to two nutritional shifts. Therefore, preliminary experiments were conducted in which the cells were plated on minimal media containing the same concentration of glucose and aspartate as the post-shift medium. The result was nearly coincident values for viable and Coulter counts.

Optical density/cell.

Bacterial cells become larger as growth rates increase (Maaløe and

Figure 5.   Response of E. coli B/r in 0.1% aspartate following addition
            to glucose. Only cell number is shown, and it can be seen
            that B/r cells divide immediately as does 15T⁻. The inset
            shows that 3 lines best fit the data.

            Symbol:

                O   cell number, shifted culture
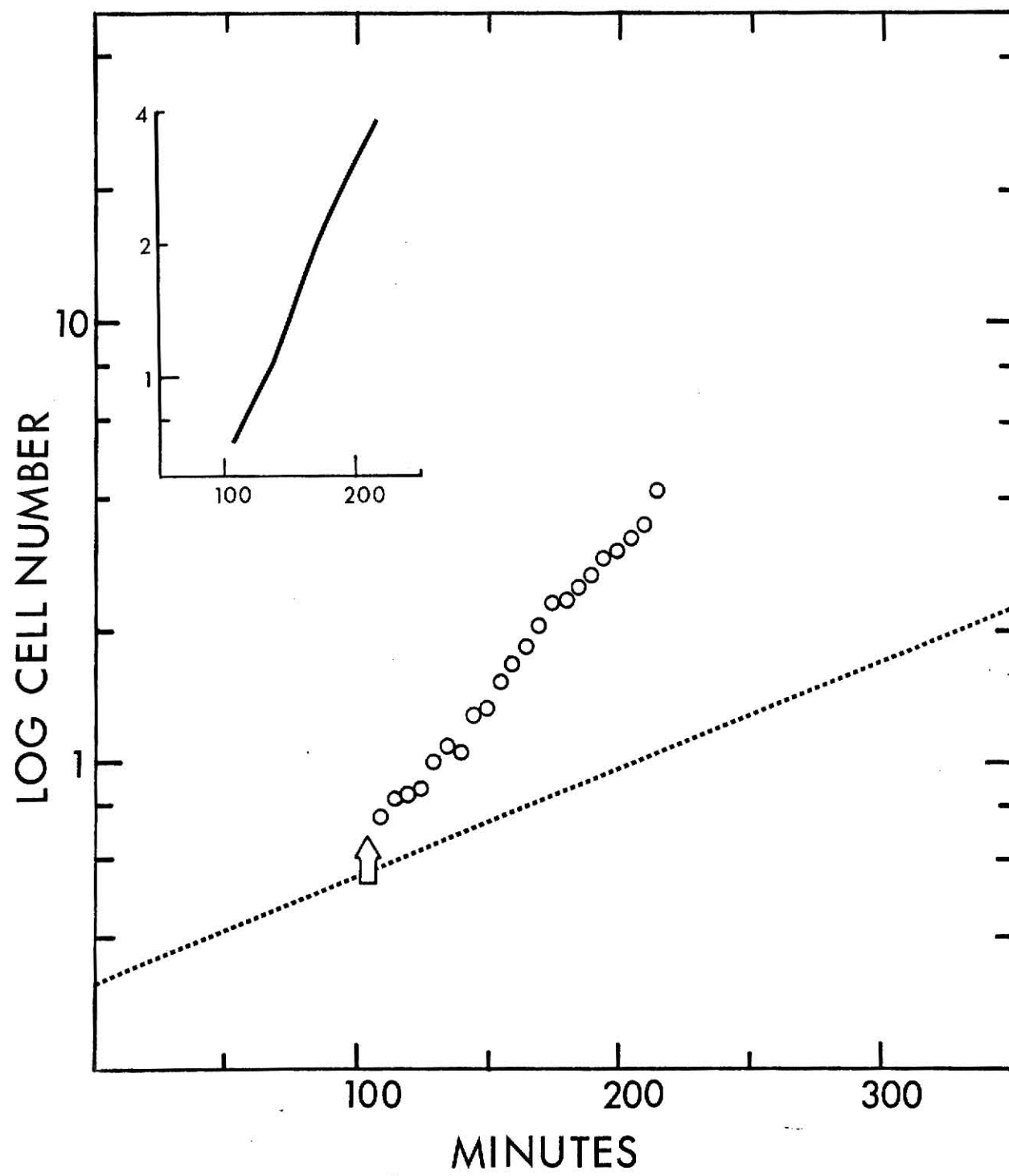
Figure 6. Comparison of colony forming units to electronic particle
counts for 0.1% aspartate and 0.5% aspartate cultures shifted
to glucose. Cultures were diluted and plated on nutrient
agar to determine colony forming units.

Symbols:

0.1% aspartate

⬡ colony forming units

O electronic particle counts

0.5% aspartate

▲ colony forming units

O electronic particle counts

The inset shows the statistical analysis of these experiments.
In both cases the viable count is represented by the fine
line, while the heavy line gives the analysis of the Coulter
count data. For both experiments, three lines best fit the
Coulter count data, while two lines fit the viable count
data best.
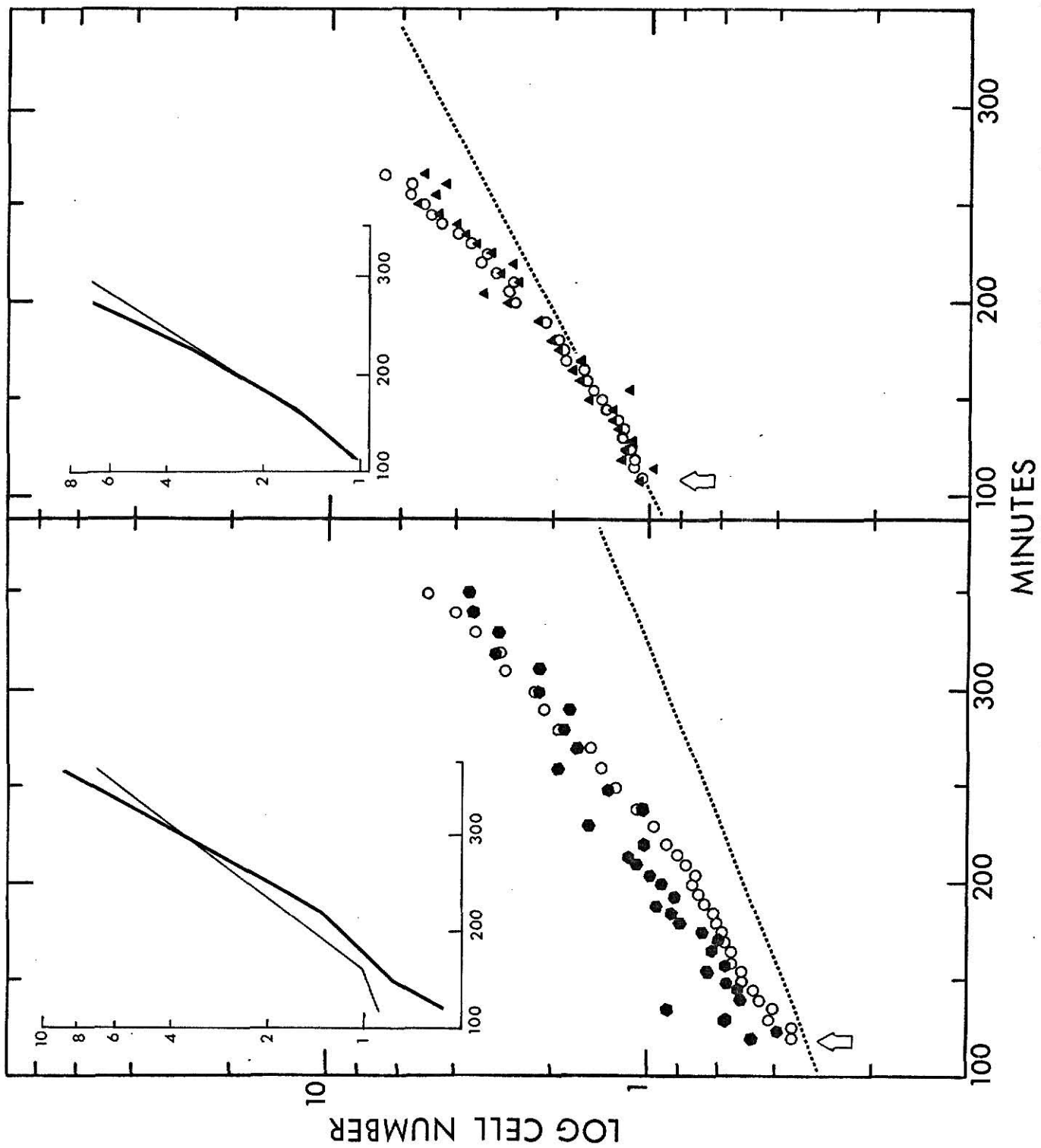
Kjeldgaard, 1966; Schaechter et al., 1958), and Donachie et al. (1968)
have attempted to explain this fact with a model relating rates of cell
doubling to the mass/DNA ratio of such cells.

The optical density of both the 0.1% (Fig. 3) and the 0.5% (Fig. 1)
aspartate cultures increased very shortly after the shift, as originally
discovered by Kjeldgaard et al. This indicates that the total mass of
the culture is increasing, however the cell number is also increasing.
In order to relate the increase in mass to individual cells, shifted cultures
were simultaneously assayed for cell number and optical density. Optical
density/cell decreased immediately after the shift, then increased rapidly
(Fig. 7).

The eventual size increase after a shift was more obvious from plots
of particle size analysis (Fig. 8) in which the peak shifts to the right
(see standards, Fig. 9B). A narrow peak, indicating a uniform size distri-
bution of cells, appeared in samples taken shortly after the shift. As the
time after the shift increased, the peak width broadened. This was in-
dicative of size diversity in the cell population. An unshifted control
culture retained a narrow peak (Fig. 9A).

DNA/cell.

The diphenylamine procedure for DNA determination (Burton, 1956)
was not sufficiently sensitive to measure DNA changes under our culture
conditions; therefore cells were grown in [^{3}H]-labeled medium to determine
relative DNA content per cell before and after glucose addition. The
results for 0.1% aspartate shifts were somewhat ambiguous; of a total of
seven experiments, five showed an increasing DNA content per cell following
the shift, while the remaining experiments showed a decrease (Fig. 10).
These results could not be correlated to the duration of the burst of
division following glucose addition.

Figure 7. O.D./cell calculated from simultaneous electronic particle
count and optical density measurement. This experiment is
typical with a drop or plateau in O.D./cell initially,
followed by a linear increase. Statistical analysis fit
two lines to the data.

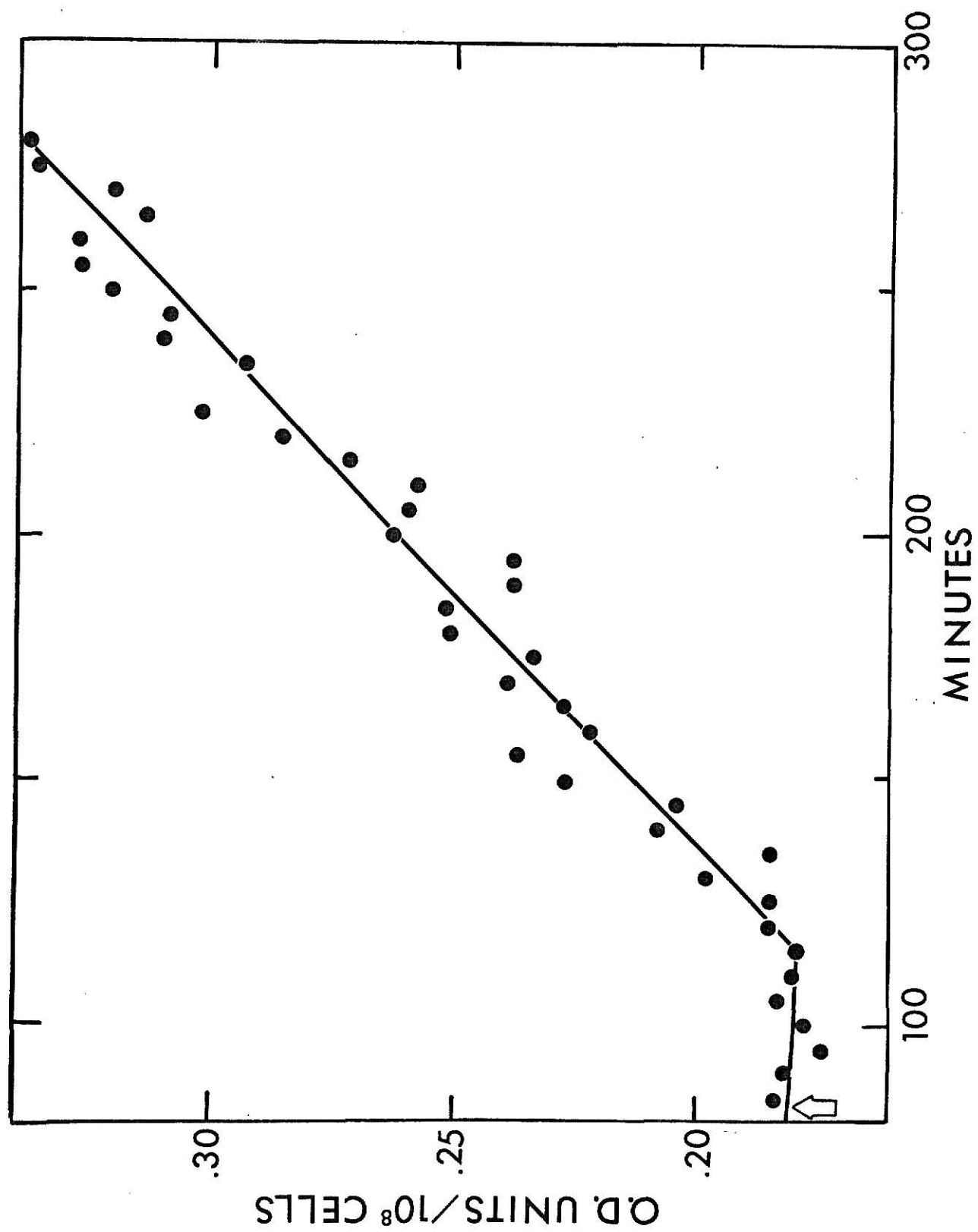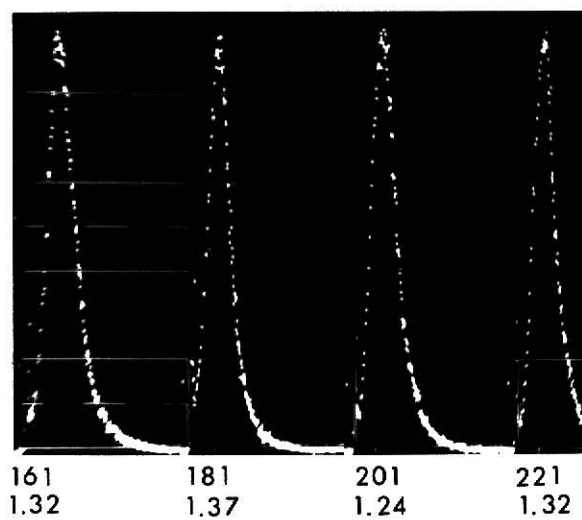Symbol:

●   optical density/cell

Figure 8.   Particle size analysis of samples taken following the
            addition of 15T⁻ 0.1% aspartate to glucose. The three
            Polaroid photographs show the oscilloscope screen of the
            analyzer which was used for particle size analysis. Data
            from four samples are shown in each photograph with the
            data from one sample occupying one quadrant. The peak
            channel can be used to determine the modal cell volume
            (see Fig. 9B). The breadth of the peak indicates the
            uniformity (narrow peak) or diversity (broad peak) of the
            population of cells that was analyzed. In the consecutive
            samples taken following addition of 15T⁻ 0.1% aspartate
            to glucose, both the peak channel and the breadth of the
            peak increase with time until approximately the 10th sample.

            Symbols:

                *   minutes after the addition to glucose

                **   volume of the modal cell in μm^3

11 *   21   41   61
.646 **   .672   .853   .904

81   101   121   141
1.01   1.01   1.11   1.14

161   181   201   221
1.32   1.37   1.24   1.32
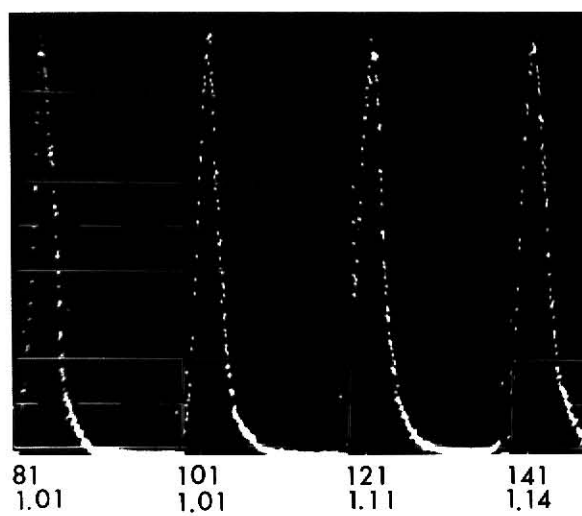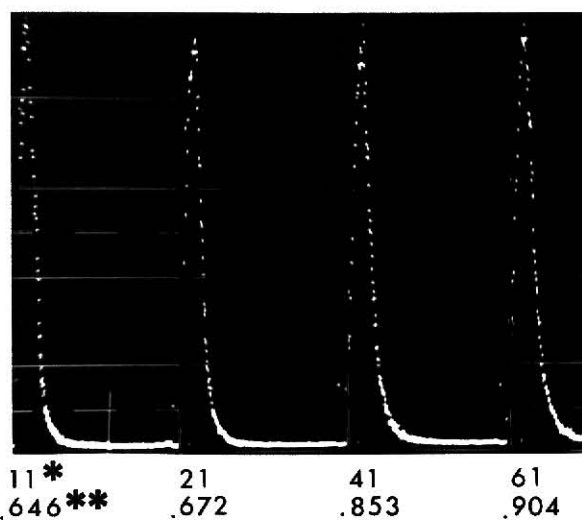
Figure 9A. The photograph (obtained as described in Fig. 8) shows the size distribution of an unshifted 15T⁻ 0.1% aspartate culture sampled at 30 minute intervals.

Figure 9B. The photograph shows the peak channels of the two particle size standards described in Materials and Methods. Both standards were contained in one sample.

Since the diameters of the spheres (shown below the peaks) are known, they can be used to calculate volumes using the formula: $1/6 \pi d^3$ = volume (d = diameter).

Another formula used is: V = KAIP (volume [V] = constant, [K] x amplification [A] x current [I] x peak channel [P]).

Once the constant is determined for the conditions and settings used, only the peak channel of a sample is needed to determine its volume.

Figure 9C. The first two quadrants represent 15T⁻ cells in 0.1% aspartate shifted to glucose, the third is a control which was not shifted.

Symbols:

    *   time in minutes

    **   volume in μm^3

A

30 *
.517 **   60   90
           .543   .569

B

1.011 μm   1.857 μm

C

1.292 **   1.163   .698
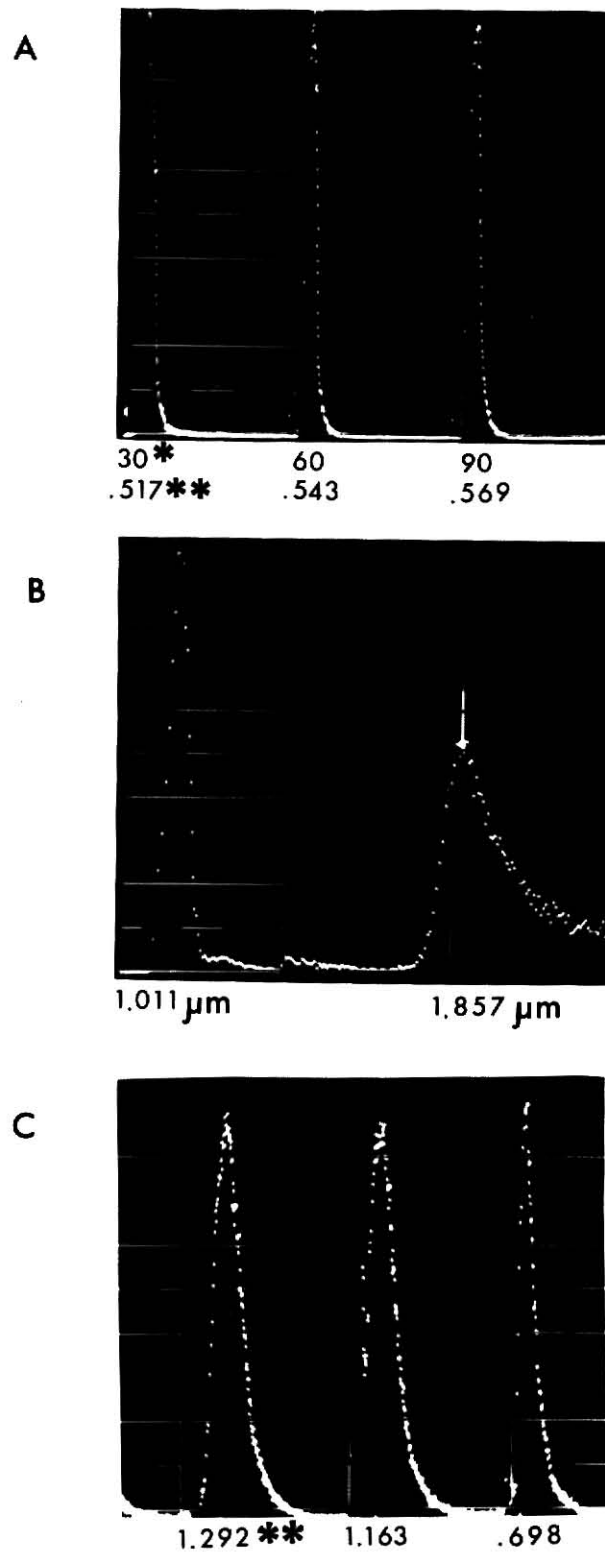
Figure 10. DNA/cell values for seven 15T⁻ 0.1% aspartate to glucose
shifts. DNA content was determined by [^{3}H] incorporation
(see Materials and Methods). Five of the seven experiments
showed DNA/cell values to increase after the shift (c, d,
e, f, and g), while two showed an eventual decrease in
DNA/cell values (a and b). Experiments c and e maintained
a rapid increase in cell number from the time of glucose
addition.

Symbol:

●   DNA/cell
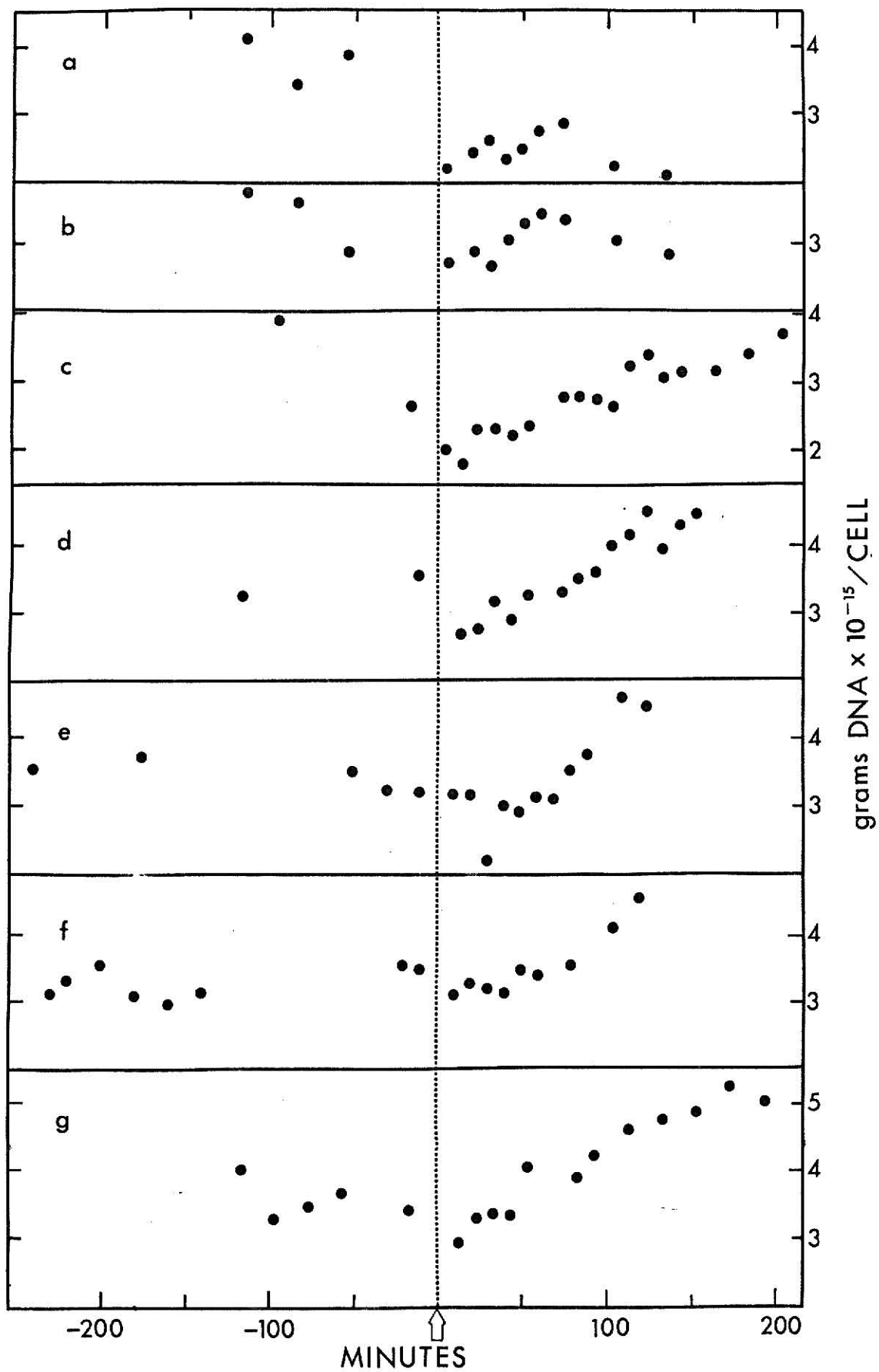
DISCUSSION

The lack of rate maintenance in 0.1% aspartate cultures is an
unusual observation, but not one totally without precedent. It appears
to be a greatly exaggerated version of the slight post-shift increase in
cell number described in the introduction (Helmstetter et al.; Cooper, 1969).
Cooper (1969) describes one theoretical shift-up situation (see curve b,
Fig. 11) in which a shortened D-period would lead to cells dividing faster
than the pre-shift rate until the change to the faster rate at 55 minutes.
A second possibility he offers is a shortened D-period followed by a
greatly reduced rate of chromosome replication. This would give a curve
composed of 4 segments (curve c, Fig. 11). Both of these models fit
data we have obtained, except that the growth rate of the shifted culture
never dropped below that of the parent culture as shown in curve c, Fig.
11.

Marr and Case (1974; personal communication, 1975) have shown similar
four-segmented growth curves by electronically determining cell number
in cultures of Salmonella typhimurium LT2 shifted from minimal to complex
media. However, the rates they obtained immediately following the shift
did not greatly exceed the pre-shift rates as ours did.

Several authors (see below) have found the concept of a fixed D-
period incompatible with their observations. By using restricting amounts
of thymine in B/r thy⁻ cultures, Meacock and Pritchard (1975) found varia-
tion in the length of the D-period in cultures with identical growth rates
but different chromosome transit times.

In a temperature-sensitive DNA mutant of Salmonella typhimurium,
Shannon and Rowbury (1972) found that the rate of cell division at the

Figure 11. Theoretical deviations from rate maintenance in a nutritional
            shift-up. From S. Cooper, 1969. This theoretical shift
            involves a culture growing at a 60 minute generation time
            shifted at time 0 to a medium capable of supporting a 30
            minute generation time. If C = 40 and D = 20 minutes in
            the original culture, and remain unchanged after the shift,
            curve a shows rate maintenance for C + D minutes before the
            new rate is assumed. Curves b and c, which are described
            in detail in the text, show deviations from rate maintenance.
            Curve b shows the effect of a decreased D-period, while
            curve c results from a decreased D-period plus a greatly
            decreased chromosome synthesis time.

            Symbols:

            ....   rate maintenance

            ━━━━   deviations from rate maintenance
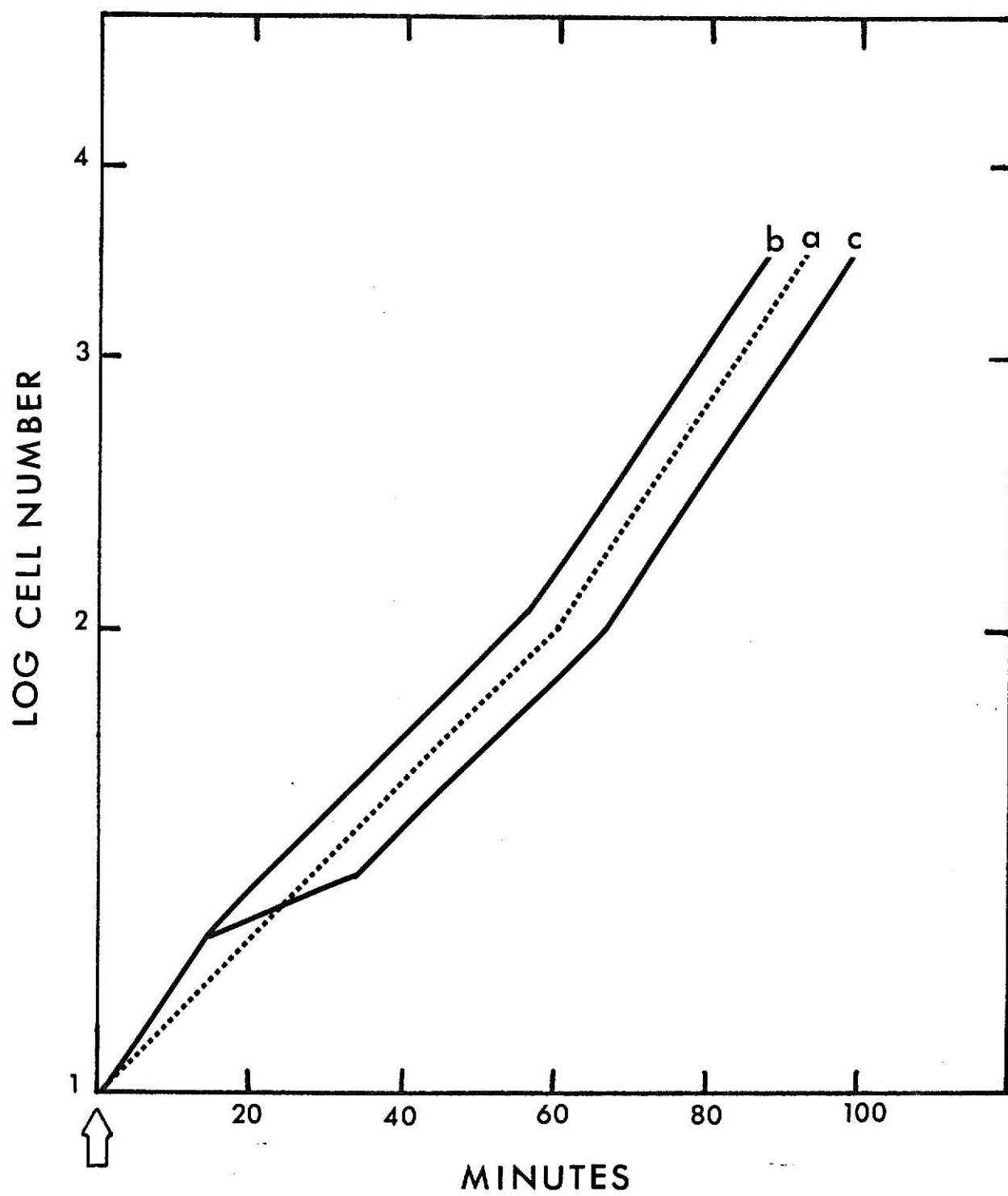
non-permissive temperature was nutrient dependent. Cultures in minimal medium showed little residual division when shifted to a non-permissive temperature. However, cultures growing in minimal media, which were simultaneously shifted to nutrient broth and to the restrictive temperature, showed extra divisions approximately 90 minutes after the shift. The authors suggested that the C + D time interval was not determined by chromosome replication and a fixed D-period between termination and division, as suggested by Cooper and Helmstetter (1968). Rather, the time attributed to C + D was instead the time for septum formation and associated division events.

Pardee (1974) has revised the original Cooper-Helmstetter model to include protein synthesis occurring concurrently with C (P_C) and at the termination of DNA synthesis (P_D). These protein synthesis requirements have been described by several authors, among them Inouye and Pardee (1970), Dix and Helmstetter (1973), and Jones and Donachie (1973). The original D period is divided into D' and T, with T being the time required for physical separation of two daughter cells after they have become physiologically independent. D' is the remaining interval between C and T.

Original Scheme   I   →   C → D

Division

Present Scheme   I   →   C → D' T

P_C P_D
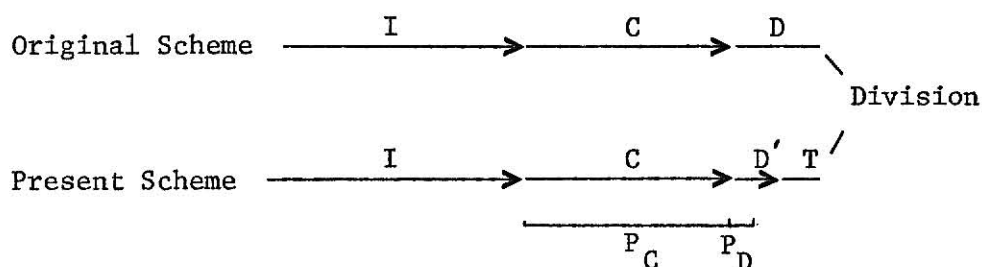
Perhaps our experimental conditions provide whatever component physiologically separated cells need to rapidly become physical entities, thus greatly reducing or eliminating time T. The decrease in the division rate occasionally observed following the rapid burst of synthesis might be due

to rate maintenance in cells too young to divide at the time of the shift.
We offer this as an alternative to Cooper's (1969) suggestion of a greatly
reduced rate of DNA synthesis as an explanation of Figure 11, curve c.
Present experimentation in our laboratory, using the sucrose gradient
method of Gudas and Pardee (1974) to synchronize 15T⁻ cultures, may enable
us to define cell size limitations for the rapid post-shift increase. Pre-
liminary results have shown a rapid division response upon glucose addition
to plateau-level cells; however, a delayed response resulted when glucose
was added during the division phase.

Since we have found B/r responds like 15T⁻ to glucose addition (Fig. 5),
the Helmstetter synchrony method (1968), discussed in the Literature Review,
would allow us to determine the age of cells that can immediately divide
upon glucose addition with a greater accuracy than the sucrose gradient
technique. Cells synchronized by the sucrose gradient method are exposed
to possible perturbation during the centrifugation process due to temperature
changes and the exposure to sucrose itself.

Because the fast-shift response could possibly be a synchronous
division of all cells sufficiently matured to divide, Wu and Pardee's
membrane-morphology-control (memorcon) hypothesis (1973) was of interest.
This hypothesis was developed to explain the synchronous divisions that
result when exponential cultures of E. coli 15T⁻ or B/r swell due to heat
shock or osmotic change. They suggested that the stretching of membrane
might dissociate multi-enzyme complexes necessary for septum formation,
thus giving all the cells the membrane configuration of a newly-divided cell.
The separated enzymes would function independently in membrane longitudinal
growth until they were again in close contact, at which time the culture
would synchronously divide.

Using particle-size analysis, we have attempted to relate Wu and

Pardee's division model (1973) to our work. This experiment involved adding formaldehyde to two cell samples: one immediately before and the other immediately after addition of glucose. Particle size analysis revealed that both samples containing glucose were composed of larger cells than the control (Fig. 9, photograph C), and the broader peak width of the glucose samples also indicates a greater size distribution.

If further experimentation confirms this initial observation that cells swell upon addition to glucose, we cannot readily explain why our cells immediately divide, while Wu and Pardee (1973) observed a delay before synchronous division. Their shortest heat treatment (6 minutes) resulted in the shortest delay of division (approximately 10-15 minutes). This could imply that some necessary component is denatured and the delay occurs while the component is again synthesized; therefore, the shorter the heat treatment, the less time necessary for repair. However, since they also observed a delay in osmotically-swollen cells, perhaps their alternative explanation is more plausible: i.e., that division is triggered by some cytoplasmic component which leaks out of swollen cells. This might somehow tie in with the dilution difference noted in Fig. 4.

Turning from this discussion of cell cycle and possible membrane involvement, another factor in our nutritional shifts is the use of aspartate for the initial carbon source. Although several papers deal with dicarboxylic acid transport in general in E. coli (Kay and Kornberg, 1969; Kay and Kornberg, 1971; and Lo and Sanwal, 1975), only Kay (1971) deals specifically with aspartate transport. Kay studied aspartate transport mutants derived from E. coli K-12 and found evidence of two aspartate transport systems: one system (dct) which transports a wide variety of C_4 dicarboxylic acids, and a high affinity (ast) system specific for aspartate. This system permits the transport of very low levels of exogenous aspartate and can serve

as a scavenging system at near starvation levels. The <u>ast</u> system can also function anaplerotically to resupply the TCA cycle with dicarboxylic acids.

Although specific experiments on aspartate transport in our 15T$^-$ cultures were not a part of this study, preliminary experiments using [^{3}H]-labeled aspartate and [^{14}C]-labeled glucose seemed to indicate that aspartate uptake remained constant in 0.1% aspartate after glucose addition and increased in 0.5% cultures. Whether this result relates to the different division response in the two media or to some specific transport phenomenon was not determined. This problem and also the ability of 15T$^-$ to grow at two generation times on a single carbon source appear to be complex areas requiring further investigation.

In summary, the cell number response of slow-growing cells (0.1% aspartate) to nutritional shift-up differs markedly from the rate maintenance response observed in faster-growing cells. Colony-forming units were found to be comparable to electronically-determined cell number values. Although the amount of DNA/cell was detemmined for both aspartate concentrations following a nutritional shift-up to glucose, the amount of DNA did not appear to be a factor in the low aspartate-level response. There did, however, seem to be an immediate size increase in response to glucose addition. The results <u>in toto</u> suggest this system to be an excellent one for further investigation of cell division and its control.

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DIVISION PARAMETERS OF ASPARTATE-GROWN

ESCHERICHIA COLI 15T⁻ FOLLOWING NUTRITIONAL SHIFT-UP

by

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AN ABSTRACT OF A MASTER'S THESIS

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Escherichia coli 15T⁻ (555-7) has a doubling time of 120 minutes when grown in appropriately supplemented M9 medium containing 0.1% aspartate as the carbon source. In the same medium containing 0.5% aspartate, the doubling time is reduced to 80 minutes. To study control of the division cycle in slow-growing cells, cultures growing at both aspartate concentrations were submitted to nutritional shift-ups. Log-phase, bubble-aerated cultures were added to glucose (final concentration 0.4%), and cell number determinations were made at five-minute intervals on a Model F_N Coulter counter.

In cells growing in 0.5% aspartate, the pre-glucose rate of doubling was maintained for at least a generation, after which an abrupt shift to a new faster rate was observed. These results are consistent with observations initially made by Kjeldgaard, Maaløe, and Schaechter.

In cells growing in 0.1% aspartate, a new faster rate of doubling was observed immediately upon addition to glucose. The number of colony-forming units was found to be comparable to the electronic particle count. Optical density and O.D./cell determinations provided an approximation of individual cell size, and the modal cell volume and size distribution of cells were determined by particle size analysis. The 0.1% aspartate-grown cells increased in volume following addition to glucose, but no correlation between DNA/cell and nutritional shift-up response was found.